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**Idiopathic REM sleep behavior disorder
and olfactory dysfunction in Parkinson's disease
and premotor stages.
MRI and neuropsychological studies**

Anna Campabadal Delgado



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**Idiopathic REM sleep behavior disorder
and olfactory dysfunction in
Parkinson's disease and premotor stages.
MRI and neuropsychological studies**

Thesis presented by:

Anna Campabadal Delgado

to obtain the degree of doctor from the University of Barcelona in accordance with the
requirements of the international PhD diploma

Supervised by:

Dr. Carme Junqué Plaja and Dr. Bàrbara Segura Fàbregas

Medical Psychology Unit, Department of Medicine
Medicine and Translational Research Doctoral Program
University of Barcelona

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*'The magic is only in what books say, how they stitched the patches
of the universe together into one garment for us'.*

Ray Bradbury, Fahrenheit 451

A la meva família, pels valors.

Al Rubén, pel camí.

Barcelona, 12th June 2020

Dr. Carme Junqué Plaja and Dr. Bàrbara Segura Fàbregas, professors at the University of Barcelona,

CERTIFY that they have guided and supervised the doctoral thesis entitled 'Idiopathic REM sleep behavior disorder and olfactory dysfunction in Parkinson's disease and premotor stages. MRI and neuropsychological studies' presented by Anna Campabadal Delgado. They hereby assert that this thesis fulfils the requirements to present her defense to be awarded the title of doctor.

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Dr Bàrbara Segura

This thesis has been undertaken at the CJNeurolab, Neuropsychology team. Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS).

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FOREWORD

This thesis, presented for the degree of Doctor by the University of Barcelona, is the result of different studies carried out over a four-year period at the Medical Psychology Unit, Department of Medicine, of the University of Barcelona. The present work has been financially supported by a fellowship from the University of Barcelona (APIF 2017-2018). In addition, the studies were sponsored by the Spanish Ministry of Economy and Competitiveness (PSI2013-41393-P, PSI2017-86930-P), by the Generalitat de Catalunya (2014SGR98, 2018SGR748) and by the Fundació La Marató de TV3, Catalunya (20142310, PI043296).

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1. **Campabadal, A.**, Segura, B., Junque, C., Serradell, M., Abos, A., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Compta, Y., Bargallo, N., Iranzo, A., Cortical gray matter and hippocampal atrophy in idiopathic Rapid Eye Movement sleep behavior disorder. *Front Neurol* **10**, 312 (2019) IF(2019): 2.889.
2. **Campabadal, A.**, Abos, A., Segura, B., Serradell, M., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Compta, Y., Bargallo, N., Junque, C., Iranzo, A., Disruption of posterior brain functional connectivity and its relation to cognitive impairment in idiopathic REM sleep behavior disorder. *Neuroimage Clinical* **25**, 102138 (2020). IF(2019): 4.350.
3. **Campabadal, A.**, Segura, B., Baggio, H. C., Abos, A., Uribe, C., Garcia-Diaz, A. I., Marti, M.J., Valldeoriola, F., Compta, Y., Bargallo, N., Junque, C., Diagnostic Accuracy, Item Analysis and Age Effects of the UPSIT Spanish Version in Parkinson's Disease. *Arch. Clin. Neuropsychol* **acy053** (2019). IF(2019): 2.154.
4. **Campabadal, A.**, Segura, B., Junque, C., Serradell, M., Abos, A., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Bargallo, N., Iranzo, A., Comparing the accuracy and neuroanatomical correlates of the UPSIT-40 and the Sniffin' Sticks test in REM sleep behavior disorder. *Park. Relat. Disord* **65**, 197-202 (2019). IF(2019): 3.926
5. **Campabadal, A.***, Inganzo, A.* Segura, B., Junque, C., Serradell, M., Abos, A., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Compta, Y., Bargallo, N., Iranzo, A., Cortical Gray Matter progression in Idiopathic REM sleep behavior disorder and its relation to cognitive decline. *Neuroimage Clinical*. IF(2019): 4.350. *Under review*
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Related Academic Work

List of additional publications of the candidate that are not included in the thesis. These papers are the result of collaborative work with other projects during the time of the thesis:

1. **Campabadal, A.**, Junque, C., Dominguez, P., Baggio, H.C., Abos, A., Uribe, C., Marti, M.J., Compta, Y., Valldeoriola, F., Bargallo, N., Segura, B., Brain atrophy and cognitive dysfunction-related quality of life in Parkinson's disease. *Submitted*.
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3. Inganzo, A., Sala-Llonch, R., Segura, B., Erostarbe, H., Abos, A., **Campabadal, A.**, Uribe, C., Baggio, H.C., Compta, Y., Marti, M.J., Valldeoriola, F., Junque, C., Multimodal hierarchical cluster analysis identifies brain atrophy patterns in Parkinson's disease. *Under review*
4. Abos, A., Baggio, H.C., Segura, B., **Campabadal, A.**, Uribe, C., Giraldo, D.M., Perez-Soriano, A., Muñoz, E., Compta, Y., Junque, C., Marti, M.J., Differentiation of multiple system atrophy from Parkinson's disease by structural connectivity derived from probabilistic tractography. *Sci Rep* **9**, 16488 (2019). IF(2019): 3.998.
5. Abos, A., Segura, B., Baggio, H.C., **Campabadal, A.**, Uribe, C., Garrido, A., Camara, A., Muñoz, E., Valldeoriola, F., Marti, M.J., Junque, C., Compta, Y., Disrupted structural connectivity of fronto-deep gray matter pathways in Progressive Supranuclear Palsy. *Neuroimage Clinical* **23**, 101899 (2019). IF(2019): 4.350.
6. Baggio, H.C., Abos, A., Segura, B., **Campabadal, A.**, Uribe, C., Giraldo, D., Perez-Soriano, A., Munoz, E., Compta, Y., Junque, C., Marti, M.J., Cerebellar resting-state functional connectivity in Parkinson's disease and multiple system atrophy: characterization of abnormalities and potential for differential diagnosis at the single-patient level. *Neuroimage Clinical* **22**, 101720 (2019). IF(2019): 4.350.
7. Uribe, C., Segura, B., Baggio, H. C., Abos, A., Garcia-Diaz, A.I., **Campabadal, A.**, Marti, M. J., Valldeoriola, F., Compta, Y., Bargallo, N., Junque, C., Progression of Parkinson's disease patients subtypes based on cortical thinning: 4-year follow-up. *Park. Relat. Disord* **64**, 286-292 (2019). IF(2019): 3.926
8. Uribe, C., Segura, B., Baggio, H.C., **Campabadal, A.**, Abos, A., Compta, Y., Marti, M.J., Valldeoriola, F., Bargallo, N., Junque, C., Differential Progression of Regional Hippocampal Atrophy in Aging and Parkinson's Disease. *Front. Aging Neurosci* **10**, 325 (2018). IF(2018): 3.633.
9. Baggio, H.C., Abos, A., Segura, B., **Campabadal, A.**, Garcia-Diaz, A., Uribe, C., Compta, Y., Marti, M.J., Valldeoriola, F., Junque, C., Statistical inference in brain graphs using threshold-free network-based statistics. *Hum. Brain Mapp* **39**, 2289–2302 (2018). IF(2018): 4.554.

10. Uribe, C., Segura, B., Baggio, H.C., Abos, A., Garcia-Diaz, A.I., **Campabadal, A.**, Marti, M.J., Valdeoriola, F., Compta, Y., Bargallo, N., Junque, C., Gray/White matter contrast in Parkinson's disease. *Front. Aging Neurosci*, **10** (2018). IF(2018): 3.633.
11. Uribe, C., Segura, B., Baggio, H.C., Abos, A., Garcia-Diaz, A.I., **Campabadal, A.**, Marti, M.J., Valdeoriola, F., Compta, Y., Tolosa, E., Junque, C., Cortical atrophy patterns in early Parkinson's disease patients using hierarchical cluster analysis. *Park. Relat. Disord* **50**, 3–9 (2018). IF(2018): 4.360.
12. Garcia-Diaz, A.I., Segura, B., Baggio, H.C., Uribe, C., **Campabadal, A.**, Abos, A., Marti, M.J., Valdeoriola, F., Compta, Y., Bargallo, N., Junque, C., Cortical thinning correlates of changes in visuospatial and visuoperceptual performance in Parkinson's disease: A 4-year follow-up. *Park. Relat. Disord* **46**, 62–68 (2018). IF(2018): 4.360.
13. Garcia-Diaz, A.I., Segura, B., Baggio, H.C., Marti, M.J., Valdeoriola, F., Compta, Y., Bargallo, N., Uribe, C., **Campabadal, A.**, Abos, A., Junque, C., Structural Brain Correlations of Visuospatial and Visuoperceptual Tests in Parkinson's Disease. *J. Int. Neuropsychol. Soc* **24**, 33–44 (2018). IF(2018): 3.098.

* These authors contributed equally to the work.

GLOSSARY OF ABBREVIATIONS

BG: basal ganglia	RAVLT: Rey Auditory Verbal Learning Test
BNT: Boston Naming Test	
CTh: cortical thickness	RBD: REM sleep behavior disorder
DLB: dementia with Lewy Bodies	REM: rapid eye movement
DTI: diffusion tensor imaging	RWA: REM sleep without atonia
FFEE: executive functions	SDMT: Symbol Digits Modalities Test
fMRI: functional MRI	SN: substantia nigra
FRT: Facial Recognition Test	SPECT: single photon emission computed tomography
GM: gray matter	Stroop: Stroop Color-Word test
HC: healthy controls	TMT: Trail Making Test
IRBD: idiopathic/isolated RBD	UPDRS: Unified Parkinson Disease Rating Scale
JLO: Judgment of Line Orientation test	UPSIT: University of Pennsylvania Smell Identification Test
MCI: mild cognitive impairment	WM: white matter
MRI: Magnetic Resonance Imaging	VBM: voxel-based morphometry
MMSE: Mini-Mental State Examination	VC: visuoconstruction
MSA: multiple system atrophy	VFD: Visual Form Discrimination Test
PD: Parkinson's disease	VP: visuoperception
PDD: Parkinson's disease with dementia	VS: visuospatial
PDRP: PD related pattern	
PET: positron emission tomography	
pRBD: probable RBD	

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CHAPTER 1

Introduction

Population ageing is occurring throughout the world. Over the coming decades, the number of older persons is expected to grow even faster. According to data from the World Population Aging 2019, the number of people aged 65 or over is projected to increase from 6% today to 16% by 2050 (United Nations, Department of Economic and Social Affairs, 2019). Since advancing age is the foremost risk factor for the emergence of most neurodegenerative disorders, population aging is having a profound impact on the emergence of dementia. With this demographical change new health challenges, have appeared. Detecting those individuals that are in premorbid stages of the disease has become a priority in the scientific community and health system, as it gives the possibility of studying disease-related risk factors, run clinical trials, and initiate treatments prior to the disease manifestation. In this context, the implementation of biomarkers has proven to be useful in different ways. Biomarkers can estimate the risk of eventually developing a certain disease in a given individual, can help in early detection, give us information about a patient's sensitivity to specific treatments, and can measure disease progression. Therefore, the development of novel preclinical and clinical markers could have a significant impact on human health and socioeconomics.

This dissertation investigates relevant biomarkers for Synucleinopathies (also known as alpha-Synucleinopathies), a group of neurodegenerative disorders characterized by the abnormal accumulation of aggregates of alpha-synuclein in neurons, and in some cases, in glial cells. Specifically, this Doctoral Thesis is focused on Rapid-eye-movement (REM) sleep behavior disorder (RBD) and olfactory dysfunction as biomarkers of Synucleinopathies. To address these topics, the introduction is organized into two main sections. In the first section of the introduction, I will give an overview of RBD, including its clinical features, diagnostic criteria, and the terminology relating to RBD. I will briefly introduce the known and presumed neural circuits involved in normal REM sleep. To then discuss the RBD's association with neurodegenerative disorders. I will finally review previous literature focused on RBD's neuropsychological impairment and on RBD-related changes in brain structure and function, as well as neuroanatomical and neurofunctional bases underlying RBD's clinical and cognitive characteristics. In the second section of the introduction, I will present the olfactory system functioning and assessment, to further describe olfactory dysfunction as a preclinical manifestation and as a clinical feature of Parkinson's Disease.

1.1. REM sleep behavior disorder

1.1.1. Clinical features, diagnosis and epidemiology of RBD

In 1986 Schenck et al., gave the first description of Rapid-eye-movement sleep behavior disorder, which was branded as a new category of parasomnia (Schenck et al., 1986). RBD was defined as a parasomnia characterized by loss of normal skeletal muscle atonia during REM sleep with prominent motor activity and dreaming. Muscle atonia during REM sleep provokes dream-enacting behavior which are episodes where patients display movements during their sleep that presumably mirror the content of their dreams. Unpleasant dreams are common, with threatening situations where the patient is being attacked or chased (Schenck and Mahowald, 2002). These abnormal motor behaviors range from simple jerking and twitching to complex behaviors such as talking, biting, punching, or jumping from bed (Schenck et al., 1986). For this reason, RBD can cause repeated injuries to self and bed partners and thus (Schenck et al., 1993), is commonly associated with major quality-of-life burdens (Rolinski et al., 2014).

The dream-enacting behaviors are associated with excessive electromyographic (EMG) activity during REM sleep that is detected with a polysomnographic study (PSG). Particularly, excessive tonic or phasic chin EMG activity or excessive limb EMG twitching during REM sleep manifest is required for diagnosis. As mentioned above, in 1986 Schenck et al., gave the first description of RBD, (Schenck et al., 1986) but it was not until 1990 that “The International Classification of Sleep Disorders” first recognized RBD as a diagnosis (Diagnostic Classification Steering Committee, Thorpy MJ, 1990). These criteria have been revised and modified over time, changing the main focus from clinical history to video PSG with movement and/or vocalizations and REM sleep without atonia (RWA). To such an extent that PSG is nowadays mandatory for RBD diagnosis, and RWA during REM sleep represents the hallmark of RBD. Currently, three different RBD diagnostic criteria are accepted: 1) The International Classification of Sleep Disorders, Third Edition, 2) the American Academy of Sleep Medicine, and 3) the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (**Panel 1.1 Diagnostic criteria for RBD** and **Panel 1.2. Differential diagnosis**).

Panel 1.1 Diagnostic criteria for RBD

The International Classification of Sleep Disorders, Third Edition (ICSD-3 criteria)

RBD Diagnostic Criteria: A–D must be met

- A Repeated episodes of sleep-related vocalization and/or complex motor behaviors.
- B These behaviors are documented by polysomnography to occur during REM sleep or, based on clinical history of dream enactment, are presumed to occur during REM sleep.
- C Polysomnographic recording demonstrates REM sleep without atonia (RWA)
- D The disturbance is not better explained by another sleep disorder, mental disorder, medication or substance use

The American Academy of Sleep Medicine (AASM)

Scoring Polysomnographic Features of RBD:

- A Score in accordance with the following definitions
 - Sustained muscle activity (tonic activity) in REM sleep:*
An epoch of REM sleep with at least 50% of the duration of the epoch having a chin EMG amplitude greater than the minimum amplitude demonstrated in NREM sleep.
 - Excessive transient muscle activity (phasic activity) in REM sleep:*
In a 30s epoch of REM sleep divided into ten sequential 3s mini-epochs, at least five (50%) of the mini-epochs contain bursts of transient muscle activity. In RBD, excessive transient muscle activity bursts are 0.1–5.0s in duration and at least four times as high in amplitude as the background electromyography activity.
- B The polysomnographic characteristics of RBD are characterized by EITHER or BOTH of the following features: N1, N2, N3.
 - 1. Sustained muscle activity in REM sleep in the chin EMG.
 - 2. Excessive transient muscle activity during REM in the chin or limb EMG.

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)

Core Features of RBD Criteria:

- A Repeated episodes of arousal during sleep associated with vocalization and/or complex motor behaviors.
- B These behaviors arise during REM sleep and therefore usually occur more than 90 min after sleep onset, are more frequent during the later portions of the sleep period and uncommonly occur during daytime naps.
- C Upon awakening from these episodes, the individual is completely awake, alert and not confused or disoriented.
- D Either of the following:
 - 1. REM sleep without atonia in PSG recording
 - 2. A history suggestive of RBD and an established Synucleinopathy diagnosis (e.g. Parkinson's disease, multiple system atrophy)

Panel 1.2 Differential Diagnosis of RBD

- A NREM sleep parasomnias (sleep terrors, confusional arousals and sleepwalking)
- B Nightmares
- C Obstructive sleep apnea
- D Rhythmic Movement Disorder
- E Periodic limb movement disorder
- F Epileptic nocturnal seizures

There are other instruments that allow assessing RBD severity and that might be helpful for research purposes. Such instruments are self-administered questionnaires. Nonetheless, bearing in mind some patients may be not able to recall their sleep behavior, it is highly recommended having spouses and/or caregivers answers. **Table 1** shows widely used questionnaires for the assessment of RBD severity. Since RBD diagnostic criteria require PSG, these questionnaires can be used only to identify probable RBD (pRBD).

Instruments for the assessment of RBD severity	Sensitivity	Specificity
Innsbruck Sleep Behavior Disorder Inventory (RBD-I) ^a	91.4	85.7
REM Sleep Behavior Disorder Screening Questionnaire (RBDSQ) ^b	96.0	56.0
REM Sleep Behavior Questionnaire (RBDQ-HK) ^c	82.2	86.9
REM Sleep Behavior Disorder Severity Scale (RBDSS) ^d	-	-
A single-question screen for REM sleep behavior disorder (RBD1Q) ^e	93.8	87.2
Mayo Sleep Questionnaire (MSQ) ^f	97.5	74.2

Table 1. Instruments for assessing RBD severity. ^a(Frauscher et al., 2012); ^b(Stiasny-Kolster et al., 2007); ^c(Li et al., 2010); ^d(Sixel-Döring et al., 2011a); ^e(Postuma et al., 2012b); ^f(Boeve et al., 2011).

Epidemiology and Demography in RBD

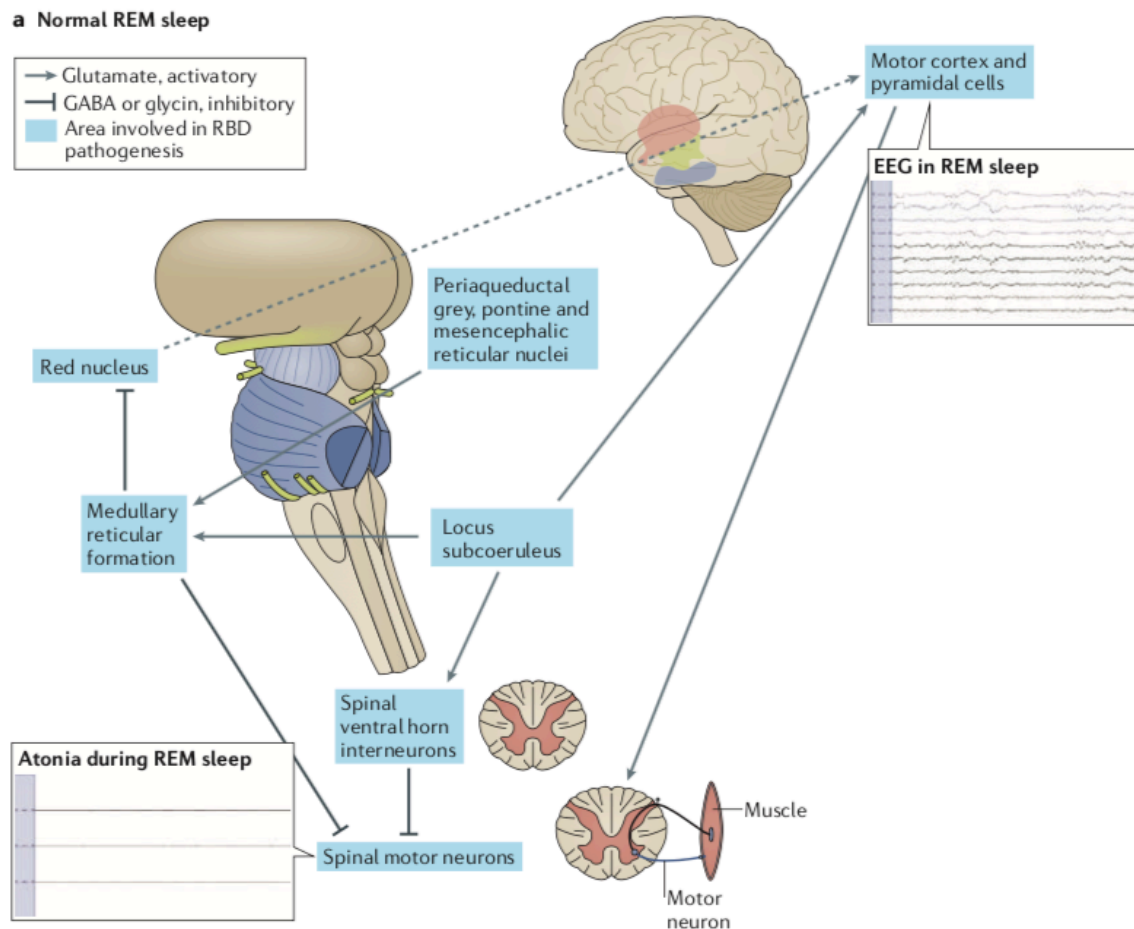
In a population-based cohort, the estimated prevalence of RBD was 1.06% when analyzing RBD's screening questionnaires and PSG data from 1997 participants from the general population (Haba-Rubio et al., 2018). RBD is usually diagnosed in individuals older than 50 years (Olson et al., 2000; Schenck et al., 1993) and historically, it has been more frequently reported in men. In a preliminary study with a cohort of 96 RBD patients, male predominance was clear, with 87.5% of patients being male (Schenck et al., 1993). Further clinical cohorts highlighted the striking men predominance in RBD (Iranzo et al.,

2016; Li et al., 2017; Schenck and Mahowald, 2002), although when assessed in general population no significant difference between men and women were found (Haba-Rubio et al., 2018). It has been suggested, that discrepancy in gender prevalence with clinical cohorts might be explained by the fact that RBD is under-recognized in women (Bodkin and Schenck, 2009), probably because female patients with RBD have less aggressive dream-enacting behaviors (Bjørnarå et al., 2013; Zhou et al., 2015), and thus they seek less sleep medical consultation.

REM sleep atonia pathway

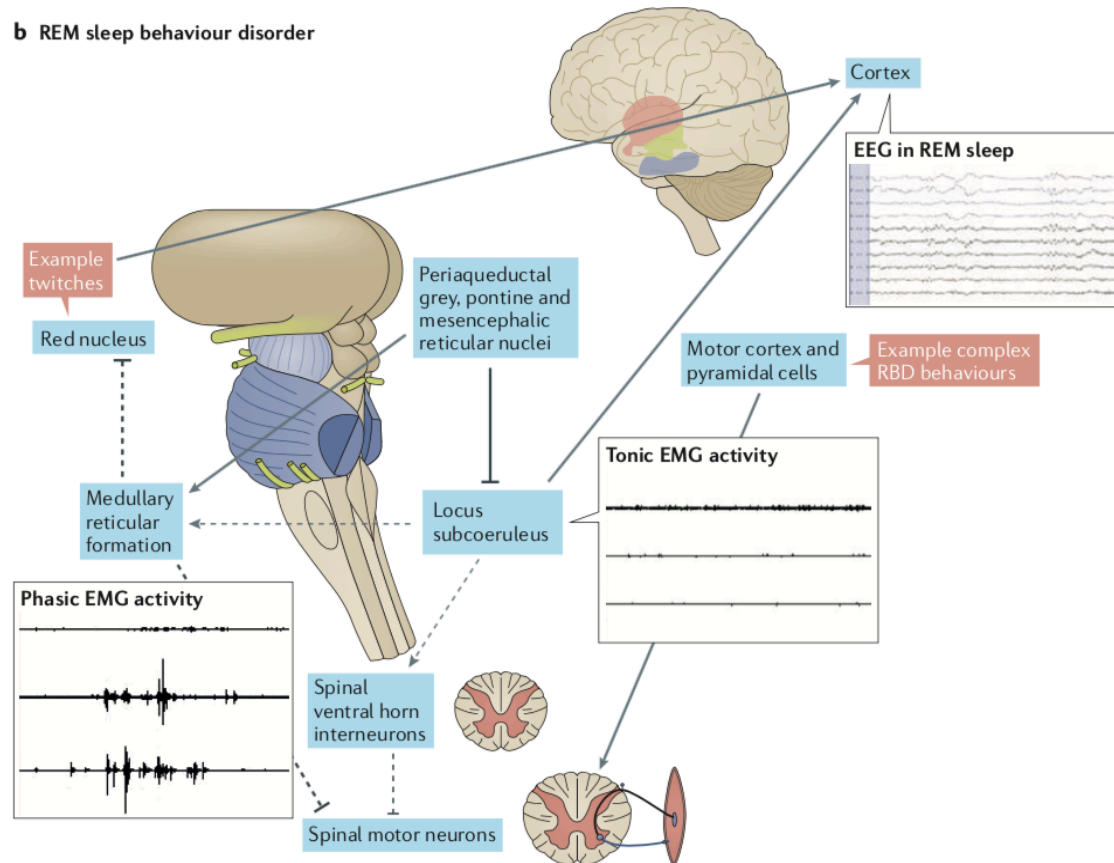
The suspected pathophysiology of RBD relies on an underlying dysfunction of the lower brainstem nuclei that modulate REM sleep muscle tone and their anatomic connections (Peever et al., 2014). Neurodegenerative lesions along the REM atonia pathway encompassing the locus coeruleus/subcoeruleus complex, the medullary reticular formation, and the spinal ventral horn interneurons might be responsible for RBD symptoms. Indeed, the coeruleus/subcoeruleus complex is affected in patients with clinically isolated RBD (Ehrminger et al., 2016), and neuronal loss in such region strongly correlated with REM sleep without atonia in patients with Parkinson's disease (PD) with RBD, but not in PD without RBD (García-Lorenzo et al., 2013), suggesting that neurodegeneration in this region might be underlying RBD symptomatology. On the other hand, the fact that RBD behaviors resemble voluntary movements during wakefulness, along with the concurrent ability of patients to speak, suggest cortical activation during REM sleep (Peever et al., 2014). **Panel 2.1** and **2.2** depicts and summarizes the main brain circuits responsible for normal REM sleep and for RBD (pictures and text are extracted from the review of Högl, Stefani, & Videnovic, 2018). The research focused on neuroimaging will be thoroughly addressed later on in the subsection **1.1.4 Brain imaging in IRBD**.

Panel 2.1 The main brain circuits responsible for REM sleep atonia, tonic and phasic motor activity in normal REM sleep.



A. “During wakefulness and slow-wave sleep, GABAergic neurons localized in the periaqueductal grey, pontine nuclei and mesencephalic reticular nuclei inhibit rapid eye movement (REM)-active neurons localized in the subcoeruleus nucleus. During REM sleep, this inhibition is absent. Descending activating projections from the pedunculopontine nucleus (not shown) to the subcoeruleus nucleus might also promote REM sleep. Motor activity is suppressed by inhibitory projections to spinal motor neurons, mediated by REM-active glutamatergic neurons in the locus subcoeruleus. Moreover, the medullary reticular formation circuit of the subcoeruleus nucleus inhibits the red nucleus, which is implicated in the generation of muscle twitches. Re-entering afferent signals from twitching limbs activate the sensory and motor cortex (dashed arrow), but this pathway is also inhibited by the same mechanism that causes inhibition of twitches during normal REM sleep, resulting in atonia”. Text and images extracted from Högl, Stefani, & Videnovic, 2018.

Panel 2.2 The main brain circuits responsible for REM sleep atonia, tonic and phasic motor activity in RBD.



B. “In REM sleep behavior disorder (RBD), degeneration of the subcoeruleus nucleus circuits that project to the medullary reticular formation and to spinal ventral horn interneurons (dashed arrows) releases the inhibition of spinal motor neurons, which results in characteristic motor behaviors (red boxes): complex movements and simple twitches or jerks during REM sleep. Blue boxes indicate areas involved in muscle atonia during physiological REM sleep. Tonic and phasic EMG (electromyography) traces depict activity in the mentalis (top row), left flexor digitorum superficialis (center row) and right flexor digitorum superficialis (bottom row) muscles. Dashed arrows represent pathways compromised in RBD”. Text and images extracted from Högl, Stefani, & Videnovic, 2018.

Secondary vs idiopathic RBD

When RBD has a primary known cause, we use the term “secondary” RBD. Different etiologies can be under this disorder, such as narcolepsy, medication intake (i.e. antidepressants), brain injuries, autoimmune diseases, or some neurodegenerative disorders, namely PD, PD with dementia (PDD), dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). On the other hand, RBD can be also manifested clinically in isolation. Traditionally, when RBD was presented alone in the absence of any other diseases, it was called “idiopathic” RBD (IRBD), a term that has been used for decades until now. Nevertheless, as it will be presented in the next subsection, there is strong evidence demonstrating that most IRBD patients will eventually convert to an overt alpha-Synucleinopathy, namely PD, DLB, and MSA. Thus, some authors have found the term “idiopathic” no longer appropriate, and “isolated” RBD has been put forward instead as a new term to design this disorder (Högl et al., 2018). For these authors, “isolated” RBD reflects the fact that these patients, even after long-standing “isolated” RBD, present biomarkers of alpha-Synucleinopathy (Iranzo et al., 2017b), meaning that although only IRBD is clinically manifested, neurodegeneration is already present. The change of designation did not affect the abbreviation that has been used for decades (so IRBD remains IRBD). For the sake of clarity, in the current Doctoral thesis the IRBD abbreviation will be used to refer to the stage of clinically isolated RBD, including indistinctly the traditional “idiopathic” and the new “isolated” RBD terms, since indeed, they are referring to the same diagnosis (**Panel 3** includes terminology relating to REM sleep behavior disorder).

Panel 3 Terminology relating to REM sleep behavior disorder

- ◆ **Idiopathic/Isolated RBD (IRBD):** IRBD is diagnosed when RBD occurs in the absence of comorbidities or neurological provoking factors. It has been recognized as an early manifestation of alpha-Synucleinopathy disorders, often Parkinson disease (PD) and dementia with Lewy bodies (DLB), and multiple system atrophy. (see **Figure 1**)
- ◆ **Prodromal RBD:** This concept was first proposed by Sixel-Döring, et al. (2016), when they demonstrated that REM behavioral events are precursors of RBD. In Högl et al. (2018) prodromal RBD is defined as “a stage of the disease in which symptoms and signs of evolving RBD are present but do not yet meet established diagnostic criteria for RBD”. (see **Figure 1**)
- ◆ **Probable RBD (pRBD):** questionnaire-based detection of RBD.
- ◆ **Prodromal PD:** “the stage wherein early symptoms or signs of PD neurodegeneration are present, but classic clinical diagnosis based on fully evolved motor parkinsonism is not yet possible” (Berg, et al., 2015).
- ◆ **REM sleep behavior disorder (RBD):** RBD is a REM sleep parasomnia characterized by a loss of atonia during REM sleep and dream-enacting behaviors such as vocalizations and jerks. RBD can be idiopathic or symptomatic of a neurological disorder (Schenck, et al 2002).
- ◆ **REM sleep behavioral events (RBE):** “motor behaviors (minor and violent) and/or vocalizations with a purposeful component, seemingly expressive of a subject's mentation during REM sleep phases” (Sixel-Döring, et al. 2014) (see **Figure 1**)
- ◆ **REM sleep without atonia (RWA):** *The American academy of sleep medicine in the international classification of sleep disorders, 3rd Edition* defines the RSWA as “the finding of increased electromyography activity during REM sleep, recorded from the mental, submental and various limb muscles”.
- ◆ **Secondary/symptomatic RBD:** this term is used when RBD has a recognized primary cause, such as medication intake, brain injuries, autoimmune disease or when is associated with neurodegenerative disorders, namely PD, PDD, DLB and MSA.

1.1.2. RBD and Neurodegenerative Diseases

The next subsection will further review RBD in the context of neurodegenerative disorders, with a special focus on RBD as a prodromal stage of Synucleinopathies (known as idiopathic/isolated RBD, IRBD).

IRBD as a Prodromal stage of alpha-Synucleinopathies

Clinically isolated RBD was first associated with parkinsonism by the researchers who described for the first time IRBD term (Schenck et al., 1986) when after a mean interval of 3.7 years from IRBD's diagnosis, 38% of their patients developed an overt parkinsonian disorder (Schenck et al., 1996). From then on, the study of IRBD as a prodromal stage of Synucleinopathies has expanded across different interdisciplinary fields. To such an extent that the scientific community has recognized IRBD as the strongest and most specific clinical predictor of alpha-Synucleinopathies available (Postuma et al., 2012a). For instance, in the Movement Disorder Society (MDS) PD prodromal criteria, idiopathic RBD had a predictive value >10x higher than any other clinical marker (Berg et al., 2015). Owing to a recent large multicenter study (n=1280 patients from 24 centers) there is now strong evidence of phenoconversion rates in these patients. In particular, the overall conversion rate from IRBD to an overt neurodegenerative syndrome was 6.3% per year, with 73.5% converting after 12-year follow-up. As for disease classifications, 199 (56.5%) developed parkinsonism as the first disease manifestation [of whom 16 (4.5%) were diagnosed with probable MSA], and 153 (43.5%) developed dementia first (Postuma et al., 2019).

In this framework, it has been proposed that IRBD should be included within the clinical manifestations of Lewy body spectrum (Högl et al., 2018). This idea is supported by the high conversion ratio and the fact that even when patients remain idiopathic after long-standing IRBD, biomarkers of prodromal PD are present, evidencing underlying neurodegenerative disease (Iranzo et al., 2017b). In agreement with the idea of Lewy body spectrum, Sixel-Döring, et al., put forward the concept of prodromal RBD, when they demonstrated that REM behavioral events are precursors of RBD. (Sixel-Döring et al., 2016) (see **Panel 3 Terminology**). Therefore, it is hypothesized that neurophysiological and behavioral findings on PSG and EMG progress along a continuum over time, initially patients enter prodromal RBD stages, progressing into clinically isolated RBD, and

eventually converting to an overt Synucleinopathy. (**Figure 1** depicts the Lewy body spectrum as proposed in Högl, et al., 2018).

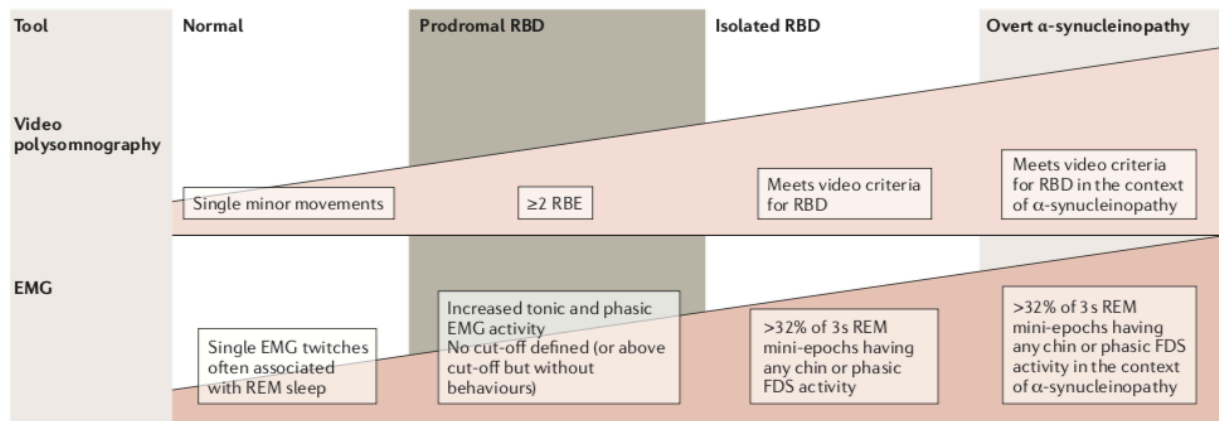


Figure 1. From prodromal RBD to Synucleinopathy. Neurophysiological and behavioral findings on polysomnography and EMG (electromyography) progress along a continuum over time. From initially normal findings, patients enter a prodromal stage of REM sleep behavior disorder (RBD) that progresses into isolated RBD, and eventually into RBD with overt alpha-Synucleinopathy. FDS, flexor digitorum superficialis; RBEs, REM sleep behavioral events. Extracted from Högl et al. (2018).

Biomarkers of degeneration in IRBD

The first intriguing question here was whether specific markers could help to identify those patients at higher risk of phenoconversion (see **Table 2 Biomarkers in IRBD**). In this sense, several studies addressing this topic, have detected potential predictors such as motor signs (Postuma et al., 2012d; Postuma et al., 2015c), hyposmia (Mahlknecht et al., 2015; Postuma et al., 2015c), cognitive dysfunction (Génier Marchand et al., 2017; Youn et al., 2016), color perception impairment (Postuma et al., 2011; Postuma et al., 2015c), abnormal DAT uptake (Iranzo et al., 2017a, 2010b; Li et al., 2017), and EEG abnormalities (Iranzo et al., 2010a; Nepozitek et al., 2019; Rodrigues Brazète et al., 2016). It is worth noting that some of these predictors increase the risk of conversion in IRBD patients. In Postuma et al.'s multicenter study, the rate of phenoconversion was significantly higher with abnormal quantitative motor testing [hazard ratio (HR) = 3.16], objective motor examination (HR = 3.03), olfactory deficit (HR = 2.62), mild cognitive impairment (HR = 1.91– 2.37), erectile dysfunction (HR = 2.13), motor symptoms (HR = 2.11), an abnormal DAT scan (HR = 1.98), color perception (HR = 1.69), constipation (HR = 1.67), REM atonia loss (HR = 1.54), and age (HR = 1.54). (Postuma et al., 2019). The authors reported abnormal motor testing, olfaction, and cognition as the best predictors

of phenoconversion. Moreover, and in agreement with previous studies (Génier Marchand et al., 2018; Génier Marchand et al., 2017), they found out that cognition was the only measure that differentiates at baseline, those patients converting to primary dementia versus parkinsonism (see further details on this topic in *Cognitive profile of IRBD patients that will eventually evolve to DLB* in section **1.1.3. Neuropsychological impairment in IRBD**).

A recent longitudinal work (Fereshtehnejad et al., 2019) traced the evolution of early motor and non-motor signs from IRBD stages to the development of overt neurodegenerative disease (**Figure 2A–C** illustrates the combined standardized trajectories for all markers included in Fereshtehnejad, et al. 2019). They estimated that olfactory dysfunction is the earliest event, beginning at least 20 years before parkinsonism diagnosis, and then it declines with a slow trend until the time of diagnosis. Orthostatic blood pressure drops appear also very early in the disease course, followed by constipation and erectile dysfunction, which start to become abnormal 10–15 years prior to phenoconversion. Color vision abnormalities appear at a similar time, early as 16 years, particularly in those destined to develop dementia first. These non-motor prodromal features all manifest years before observable motor signs. Within 7 to 9 years before parkinsonism is diagnosed, the first motor manifestations appear, along with slight urinary dysfunction and subtle cognitive decline (**Figure 2D** schematic summary). Most manifestations progress linearly, except motor abnormalities, which exhibit a more rapid progression during the last 2 years prior to phenoconversion. The authors suggested that their findings agree with Braak model of PD (Braak et al., 2003), since hyposmia was found to appear earlier in the disease followed by autonomic manifestations, and finally motor and cognitive abnormalities. Nevertheless, the fact that cognitive impairment appears so early in the disease, even before motor onset, might highlight the existence of an IRBD subgroup with early cortical involvement.

Table 2. Biomarkers in IRBD.

<i>Earlier signs in IRBD (>15-10 years before parkinsonism diagnosis) (see Figure 2)</i>
Olfactory dysfunction ^a
Orthostatic blood pressure ^a
Erectile dysfunction ^a
<i>Biomarkers that predict short-term (2-5 years) risk of alpha-Synucleinopathy</i>
Subtle signs of parkinsonism ^b
Olfactory dysfunction ^b
Impaired color perception ^b
Reduced nigrostriatal DA binding in the striatum ^c
Impaired cognition ^d
EEG abnormalities ^e
<i>Biomarkers that predict dementia vs parkinsonism first</i>
Impaired cognition ^f
<i>Biomarkers that progress over time</i>
Signs of parkinsonism ^g
Neuropsychological deficits ^h
Tonic and phasic muscle activity during REM sleep ⁱ
Striatal dopaminergic uptake ^j
<i>Biomarkers that remain stable over time</i>
Olfaction ^k
Hyperechogenicity of the substantia nigra ^l

^a(Fereshtehnejad et al., 2019); ^b(Mahlknecht et al., 2015; Postuma et al., 2011; Postuma et al., 2015c); ^c(Iranzo et al., 2017a, 2010b; Li et al., 2017); ^d (Génier Marchand et al., 2017; Youn et al., 2016); ^e(Iranzo et al., 2010a; Rodrigues Brazète et al., 2016); ^f(Génier Marchand et al., 2018; Génier Marchand et al., 2017); ^g(Postuma et al., 2012d); ^h(Terzaghi et al., 2013; Youn et al., 2016); ⁱ(Iranzo et al., 2009); ^j(Iranzo et al., 2011); ^k(Iranzo et al., 2013; Mahlkecht et al., 2015; Postuma et al., 2011); ^l(Iranzo et al., 2014b). The classification is based on Pérez-Carbonell and Iranzo in Rapid-Eye-Movement Sleep Behavior Disorder. Chapter 4 Clinical aspects of Idiopathic RBD (page 45). <https://doi.org/10.1007/978-3-319-90152-7>.

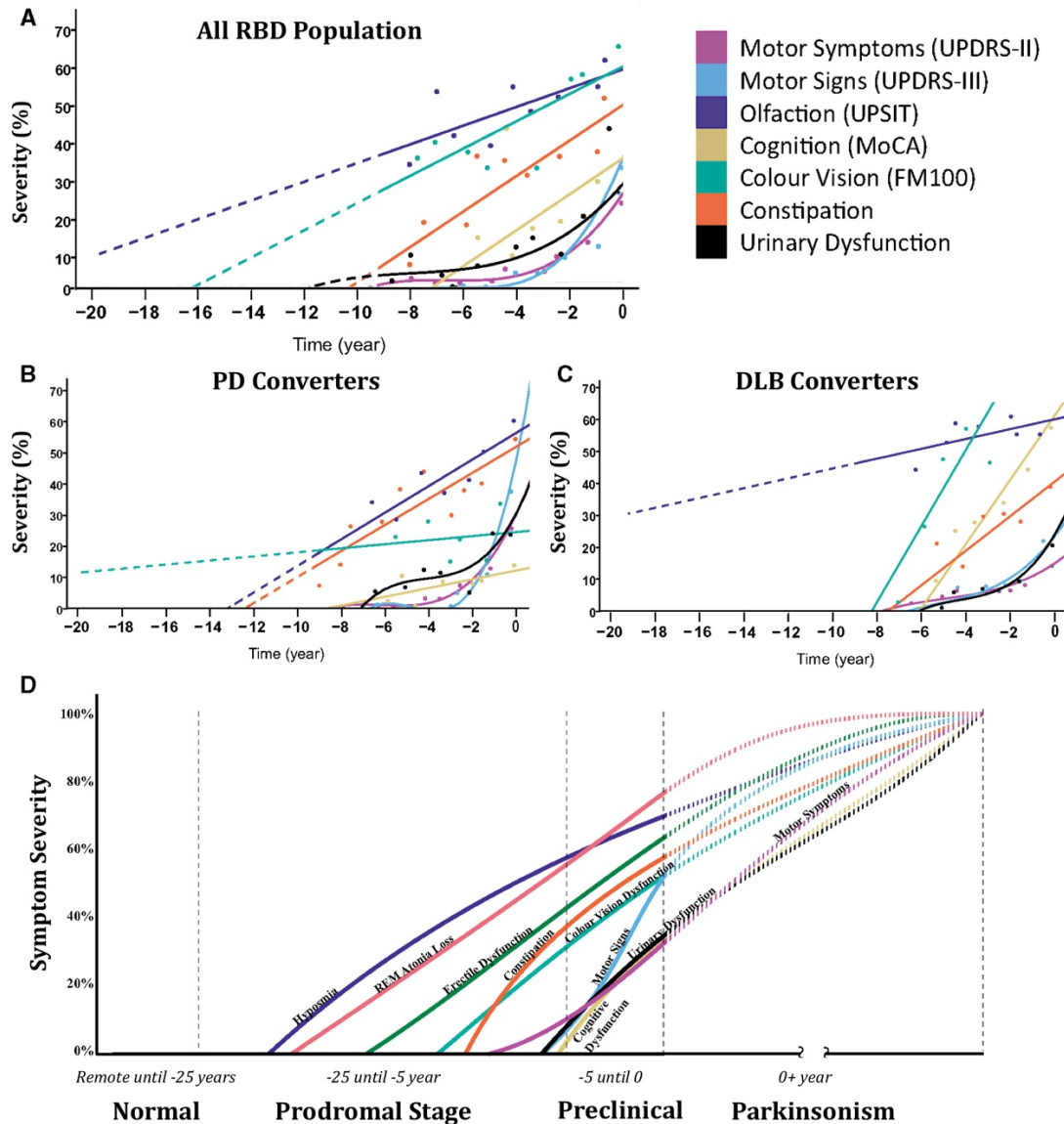


Figure 2. Progression trajectory of motor and non-motor manifestations from prodromal stages to phenoconversion. **A.** Combined progression trajectory of motor and non-motor manifestations from prodromal stages to phenoconversion based on actual measurements in the entire study population; **B.** PD-converters; **C.** DLB-converters; (Note that the dashed lines represent expected imputed trends, while the solid lines and the dots demonstrate actual observations); and **D.** Schematic model for progression of motor and non-motor manifestations throughout the prodromal, phenoconversion and advanced stages of patients with idiopathic RBD converting to PD. This schematic illustrates the approximate trajectories of the major motor and non-motor manifestations as patients progress from normal, through IRBD, to advanced PD. This is based on control data, patients with IRBD tracked through time, and patients with advanced PD who also have RBD. The predicted progressions after phenoconversion were dotted due to the uncertainty of real symptomatic severity without the influence of dopaminergic treatment. Olfaction is generally the first manifestation to become abnormal and reaches near maximum loss at the time of phenoconversion. We scaled down both cognitive and color vision dysfunction slopes by half to avoid the bias driven by DLB subjects (the estimated maximum symptoms were based on PD-RBD subjects without dementia at baseline). Autonomic features are similarly present early, and

approximate 50–70% maximal values at phenoconversion. REM atonic loss and color vision loss have patterns similar to autonomic loss. By contrast, motor and cognitive abnormalities start relatively late, and are at only 20–30% maximal values at the time of phenoconversion (text and figures extracted from Fereshtehnejad et al. 2019). Abbreviations: DLB, dementia with Lewy bodies; IRBD, idiopathic RBD; PD, Parkinson's disease; PD-RBD, PD patients with RBD; RBD, REM sleep behavior disorder.

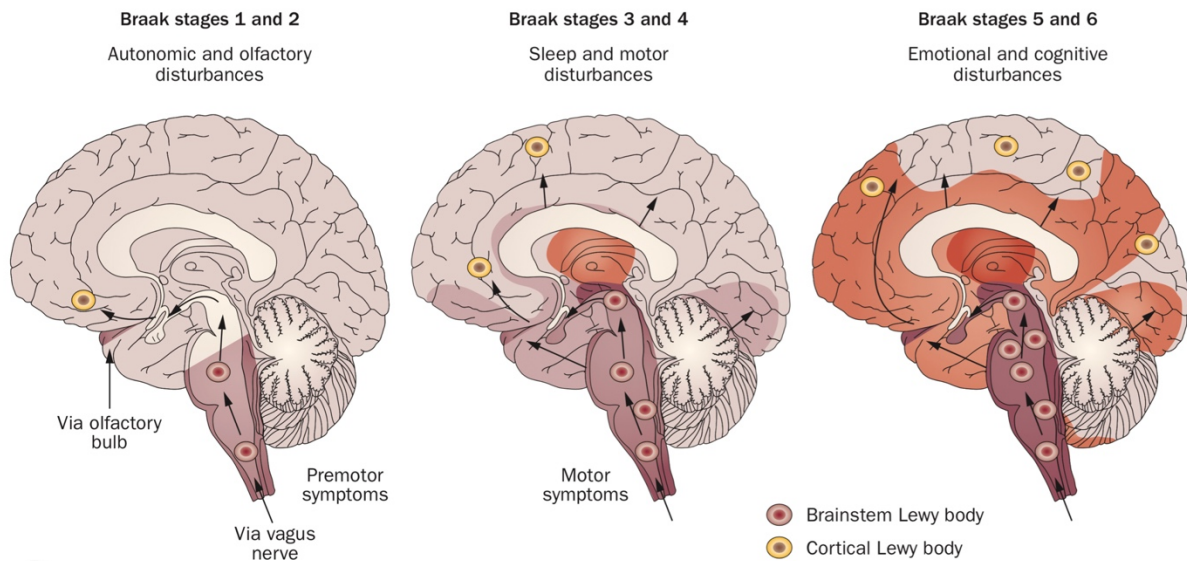
Neuropathology of IRBD

The strong association of RBD with the Synucleinopathies is supported by neuropathological findings. In the first attempt to examine brain pathology in an IRBD patient, Lewy body pathology in the brainstem and severe neuronal loss in the substantia nigra (SN) and coeruleus/subcoeruleus complex was identified, leading to the conclusion that brainstem degeneration might explain RBD (Uchiyama et al., 1995). However, despite brainstem-predominant Lewy pathology, only mild degeneration of the SN, coeruleus/subcoeruleus complex, and raphe nuclei were identified in another work, thus calling into question the role of these nuclei in RBD (Boeve et al., 2007). Neuronal loss and Lewy body pathology were also present in the peripheral autonomic nervous system, olfactory bulb, amygdala, and sparing cortex, after 10 years of IRBD course (Iranzo et al., 2014a). In a multicentric study where clinical and neuropathological features were analyzed on 172 autopsied RBD (with or without coexisting neurologic disorders), 93% showed PD or DLB (79%) or MSA (11%) pathological findings (Boeve et al., 2013).

RBD in the context of the Braak Staging System for PD

The Braak staging system for PD characterizes alpha-synuclein pathology progression in the brain within 6 stages. It is based on the topographical and temporal progression of alpha-synuclein from the brainstem and olfactory regions to cortical areas, spreading to anteromedial temporal mesocortex and lastly to the neocortex (Braak et al., 2003) (**Figure 3A** depicts and briefly introduces Braak Staging system for PD). The scientific community proposed that clinically isolated RBD might probably correspond to the early stages of Braak scheme since they are supposed to involve the medullary and pontine circuits that precisely control REM sleep atonia (**Panel 2.1** and **2.2.**). Nevertheless, it is still unclear at which Braak stage occurs RBD (see **Figure 3B**). Previous works hypothesized that RBD patients with olfactory impairment might present with Braak stage 2 (Stiasny-Kolster et al., 2005), which is also supported by abnormalities in the coeruleus/subcoeruleus complex (Ehrminger et al., 2016; Uchiyama et al., 1995).

A



B

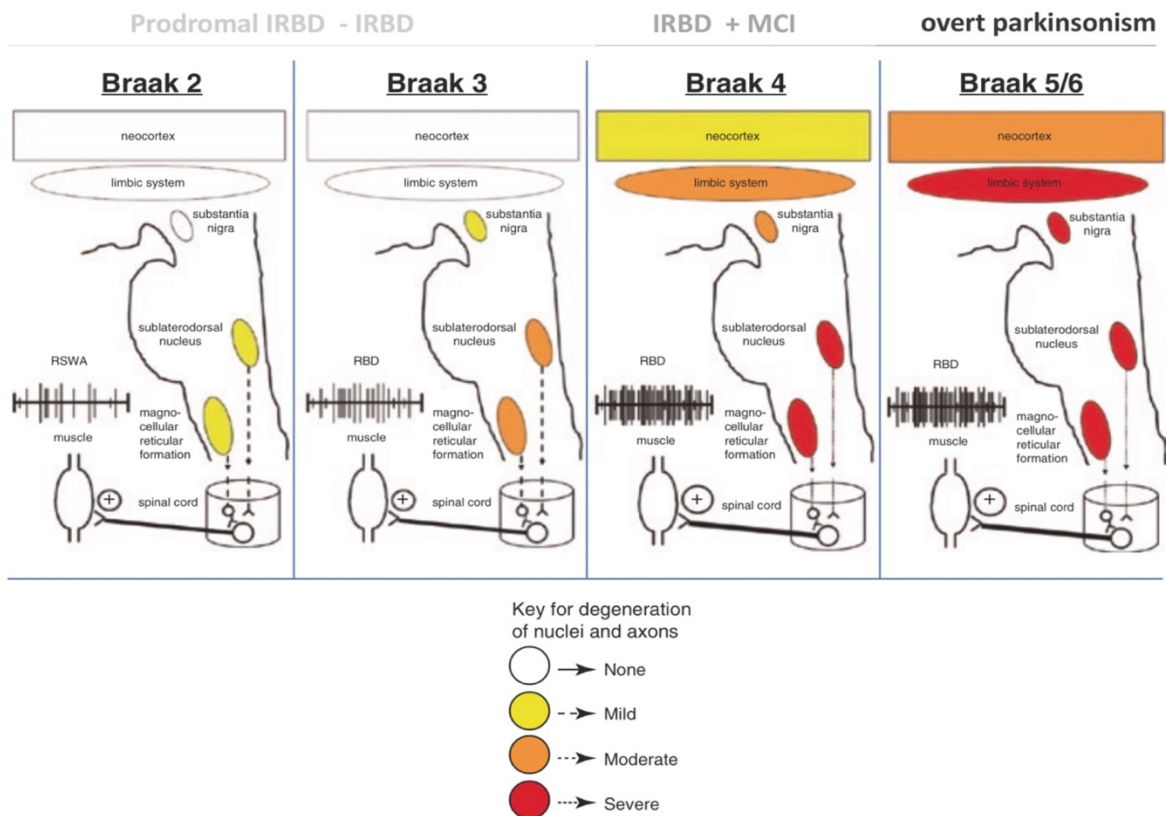


Figure 3. Braak staging system for PD. **A.** Progression of PD-related intraneuronal pathology according to Braak staging system for PD. α -Synuclein-related pathology is possibly initiated in the periphery via input from the olfactory epithelium or vagal inputs from the stomach, perhaps involving xenobiotic factors. Early stages of the disease encompass structures in the lower brainstem and the olfactory system with non-motor manifestations, such as hyposmia and RBD. Motor symptoms are often evident in III-IV stages with involvement of structures such as the

Substantia nigra. Thereafter, in IV-V stages the anteromedial temporal mesocortex is involved. From there, the neocortex succumbs in later stages, beginning with sensory association and prefrontal areas, and followed by premotor areas, primary sensory and motor fields. The red shading represents the pattern of pathology. Figure extracted from Doty, 2012; B. Electrophysiologic-neurodegenerative correlations according to the Braak staging system. Schematic of the key brainstem nuclei—the sublaterodorsal nucleus and magnocellular reticular formation—and their corresponding degrees of degeneration associated with REM sleep without atonia according to Braak Stages 2–5/6. Note that overt parkinsonism and/or cognitive impairment would not be expected until at least Stage 4, but RSWA (Stage 2) and RBD (Stage 2 or 3) would occur earlier in the course. This temporal sequence of degenerative changes could explain why RBD precedes parkinsonism and dementia in many patients with Lewy body pathology. Adapted from Bradley F. Boeve in Rapid-Eye-Movement Sleep Behavior Disorder. Chapter 6 REM sleep Behavior Disorder Associated with Dementia with Lewy Bodies (page 67). <https://doi.org/10.1007/978-3-319-90152-7>. Abbreviations: RSWA, REM sleep without atonia.

Recently, in an attempt to study in-vivo staging of pathology in IRBD using five imaging modalities, Knudsen et al., (Knudsen et al., 2018) identified a caudorostral gradient of dysfunction, with pathology in the sympathetic and parasympathetic nervous system and in the locus coeruleus cells equal to that found in PD, whereas 71% of IRBD patients had normal nigrostriatal dopaminergic innervation. They concluded that this pattern supports the hypothesis that alpha-synuclein initial target is peripheral autonomic nerves and then spreads rostrally to the brainstem before involving the dopaminergic system, thus being compatible with Braak's staging system for PD.

On the other hand, since neuropsychological impairment is often found in some IRBD patients (see **1.1.3 Neuropsychological impairment in IRBD**), it could be speculated that patients presenting cognitive dysfunction are in more advanced stages, where mesocortex or even neocortex is already involved. The intriguing question here is why some IRBD patients present cognitive impairment even before presenting an overt parkinsonism. Bearing in mind, that these patients are at higher risk to evolve to an overt dementia, other classification systems such as the Unified staging system for Lewy Body (Beach et al., 2009a) might explain different evolution among those IRBD evolving to PD from those evolving to DLB. This system supports the idea that in early stages only the olfactory bulb is affected, and from here, two different progressions are possible, the brainstem predominant and the limbic predominant. Clearly, there is a need for further neuropathological studies in IRBD to elucidate this issue.

Genetics of IRBD

Genetic studies of RBD are still scarce. Until now, GBA mutations have shown the strongest evidence in RBD (Barber et al., 2017; Gan-Or et al., 2015), whereas pathologic mutations in LRRK2 are not present in IRBD (Barber et al., 2017; Fernández-Santiago et al., 2016), which agrees with the idea that LRRK2 patients usually present a more benign PD course (Puschmann, 2013), contrary to patients with RBD. **Figure 4** depicts those genes that may have a role in RBD and that are implicated in PD, DLB, atypical Parkinsonism, and MSA.

	Parkinson's disease	Autosomal recessive PD and atypical Parkinsonism	Dementia with Lewy bodies	Multiple system atrophy
High	GBA LRRK2 SNCA VPS35 GWAS loci: GBA , RAB29-NUCKS1, SIPA1L2, ACMSD-TMEM163, STK39, MCCC1, TMEM175- GAK , DGKQ, BST1, FAM47E- SCARB2 , SNCA , HLA-DQB1, GPNMB, FGF20, INPP5F, MIR4697, CCDC62, GCH1, VPS13C, BCKDK-STX1B, MAPT , SREBF1-RAI1, RIT2, DDRGK1	PARK2 (Parkin) PINK1 PARK7 (DJ-1) ATP13A2 SYNJ1 PLA2G6 ATP6AP2 DNAJC6	GBA APOE SNCA SCARB2	
Moderate	CHCHD2 DNAJC13 RIC3 SMPD1 Chr. 22q11.2 deletion GWAS loci: USP25, NMD3, ITGA8	VPS13C RAB39B FBXO7	MAPT	COQ2 FBXO47 ELOVL7 EDN1 MAPT GBA
Low	TMEM230 EIFG1 PARK10 MC1R UCHL1 GIGYF2		APP PSEN1 PSEN2	SLC1A4 SQSTM1 EIF4EBP1 IL1A IL1B IL8 ICAM1 TNF SERPINA1

Figure 4. RBD's genetics. Genes implicated in Parkinson's disease, atypical Parkinsonism, dementia with Lewy bodies, and multiple system atrophy are organized according to the level of confidence in their involvement in these diseases. These genes were identified in case-control studies, with candidate gene approach, genome-wide association studies, and family-based studies. Genes in red are those that may have a role in REM sleep behavior disorder; however, note that the role of many of the other genes in RBD has not been studied yet. Extracted from Gan-Or and Rouleau in Rapid-Eye-Movement Sleep Behavior Disorder. Chapter 41 Genetics of REM sleep Behavior Disorder (page 591). <https://doi.org/10.1007/978-3-319-90152-7>.

RBD in alpha-Synucleinopathies

RBD does not always precede the motor onset of Synucleinopathies. Patients with an established parkinsonism disorder may also begin to suffer comorbid RBD. RBD associated with DLB has an important role because of its high prevalence (>70%) (Ferman et al., 2011). RBD in DLB may occur either in advance or simultaneously with the onset of DLB. Indeed, it is currently one of the core features for the diagnosis of DLB, together with the presence of fluctuating cognition, visual hallucinations, and parkinsonism (McKeith et al., 2017). Regarding MSA, the prevalence of PSG-confirmed RBD was up to 88% in these patients (Palma et al., 2015) with no differences between Parkinson and cerebellar type (Cohen De Cock et al., 2011; Muntean et al., 2013).

According to what we know about Braak staging system for PD, one would expect that RBD would be present in most newly PD patients. Nevertheless, a meta-analysis focused on the prevalence of RBD in PD de novo patients demonstrated an overall mean prevalence of 23.6% (range 4.3%-69.4%) (Zhang et al., 2017), moreover, RBD is not restricted to early stages of PD, as it can occur at any time during the course of the disease. In a cross-sectional cohort, RBD prevalence was 46%, and it increased with age and disease duration (Sixel-Döring et al., 2011b). PD patients with RBD showed more severe cognitive (Arnulf, 2012; Jozwiak et al., 2017) and motor impairment, more frequent hallucinations (Arnulf, 2012), and larger atrophy in temporal regions than those without RBD (Rahayel et al., 2019). In keeping with the idea of worse outcome in PD with RBD, presence of RBD is a risk factor for dementia (Anang et al., 2014; Nomura et al., 2013) and is associated with faster motor progression in these patients (Pagano et al., 2018).

Summary

RBD is a parasomnia present in 1% of the general population characterized by loss of atonia and dream-enacting behavior during REM sleep. RBD may be presented as a secondary symptom of other neurological disorders or clinically in isolation (IRBD). IRBD is considered a prodromal stage of alpha-Synucleinopathies, with almost 75% of patients converting after 12 years of the disease. Motor manifestations, hyposmia, and cognitive impairment are the best predictors of phenoconversion in IRBD. Mechanisms underpinning this disorder may involve the lower brainstem nuclei that modulate REM sleep muscle tone and their anatomic connections. Indeed, the strong association of RBD with the Synucleinopathies is supported by the neuropathological findings in the brainstem, which in turn supports the idea that IRBD might probably correspond to the early stages of Braak scheme for PD progression.

1.1.3. Neuropsychological impairment in IRBD

With the increasing incidence of neurodegenerative disorders in the population, the study of the cognitive impairment associated with these diseases gained important attention on the neuroscientific community. Initially, many efforts were made to detect subtle changes underlying the early stages of Alzheimer's disease (AD). Within this framework, appeared the term Mild cognitive impairment (MCI) that was proposed as a transitional stage between normal cognitive function and clinically probable AD dementia. In this first attempt to characterize MCI, memory decline was the hallmark of the diagnostic criteria. This memory impairment was greater than expected for an individual's age and education and was not sufficiently severe to warrant a diagnosis of probable AD dementia (Petersen et al., 2001). Shortly thereafter, in an effort to recognize the heterogeneity of its clinical presentation, a new look to these criteria was taken. Although memory was still essential for the diagnosis, the decline of other cognitive domains was also considered. Hence, based on the results of neuropsychological testing, different MCI subtypes were proposed: amnesic and non-amnesic MCI, and single or multiple domain MCI (Winblad et al., 2004).

Later on, the MDS PD-MCI (PD with MCI) task force published the MCI diagnostic criteria adapted for PD patients. In keeping with the traditional MCI, PD-MCI enables to identify patients at increased risk of developing dementia. These criteria also allow characterizing the PD-MCI subtype according to diverse cognitive domains. Contrary to the classical MCI, in PD-MCI the non-amnesic, single-domain impairment is the most common subtype (Litvan et al., 2012).

Under these circumstances, a burgeoning interest in characterizing the cognitive profile of prodromal alpha-Synucleinopathies appeared, that is to say of IRBD condition. Actually, a fairly extensive literature on neuropsychological performance in IRBD is currently available. Studies reporting the presence of MCI, state that it is frequently identified in 15-50% of these patients. (Bezdicek et al., 2018; Gagnon et al., 2009; Galbiati et al., 2019a; Génier Marchand et al., 2017; Marcone et al., 2019; Rahayel et al., 2018b; Terzaghi et al., 2019, 2013). **Table 3** lists MCI percentage according to different works. The frequencies reported per subtype are 24-56% for non-amnesic single-domain (Gagnon et al., 2009; Galbiati et al., 2019a; Terzaghi et al., 2013), 19%-43% for amnesic multiple-domain (Gagnon et al., 2009; Galbiati et al., 2019a; Terzaghi et al., 2013), and 12.5-35% for

amnesic MCI (Gagnon et al., 2009; Galbiati et al., 2019a; Terzaghi et al., 2013) (see **Figure 5**). The objective of this section is to comprehensively describe preliminary investigations on the understanding of IRBD's cognitive profile and to provide an overview of longitudinal research aiming at identifying those cognitive domains that commonly decline throughout the disease. **Table 4** at the end of this section includes the main findings in IRBD cognition. To further review neuropsychological tests and batteries refer to Lezak, et. al 2012 (Lezak, 2012). The substrates underlying cognitive impairment in these patients will be further addressed in the next section (**1.1.4. Brain Imaging in IRBD**), where brain structural and functional changes in IRBD will be thoroughly reviewed.

Reference	IRBD sample size	IRBD with MCI, (n) %
Gagnon et al., 2009	32	(16)50%
Terzaghi et al., 2013	20	(7)35%
Génier Marchand et al., 2017	76	(41)54%
Marcone et al., 2019	38	(13)34%
Bezdicek et al., 2018	60	(9)15%
Rahayel et al., 2018b	52	(17)33%
Terzaghi et al., 2019	63	(19)30%
Galbiati et al., 2019a	80	(29)36%

Table 3. Percentage of IRBD patients with MCI according to different studies. Abbreviations: IRBD, Idiopathic REM sleep behavior disorder; MCI, mild cognitive impairment; n, sample size.

Memory

Both in aging and neurodegenerative diseases it is essential to assess learning and memory performance, up to the point that the identification of memory-specific profile of an individual helps in the differential diagnosis of some neurodegenerative disorders (Levy and Chelune, 2007; Salmon and Bondi, 2009). Within this framework, knowing the memory pattern of IRBD is attracting considerable attention.

On one hand, verbal memory impairment has been consistently reported in IRBD (Fantini et al., 2011; Gagnon et al., 2009; Her et al., 2019; Li et al., 2018; Massicotte-Marquez et al., 2008; Shin et al., 2019; Terzaghi et al., 2013, 2008). In particular, relative to healthy individuals, IRBD showed impairment in immediate memory (Gagnon et al., 2009; Massicotte-Marquez et al., 2008; Shin et al., 2019), learning (Gagnon et al., 2009;

Massicotte-Marquez et al., 2008), delayed recall (Gagnon et al., 2009; Li et al., 2018; Marques et al., 2010; Massicotte-Marquez et al., 2008; Shin et al., 2019; Terzaghi et al., 2013) and recognition (Massicotte-Marquez et al., 2008; Shin et al., 2019). Besides, memory test with higher executive demand were also able to identify differences between IRBD and healthy controls (HC). For instance, patients showed lower performance than controls in logical memory (Fantini et al., 2011; Terzaghi et al., 2013, 2008) and prospective memory tests (Bezdicek et al., 2018; Li et al., 2018; Marcone et al., 2019).

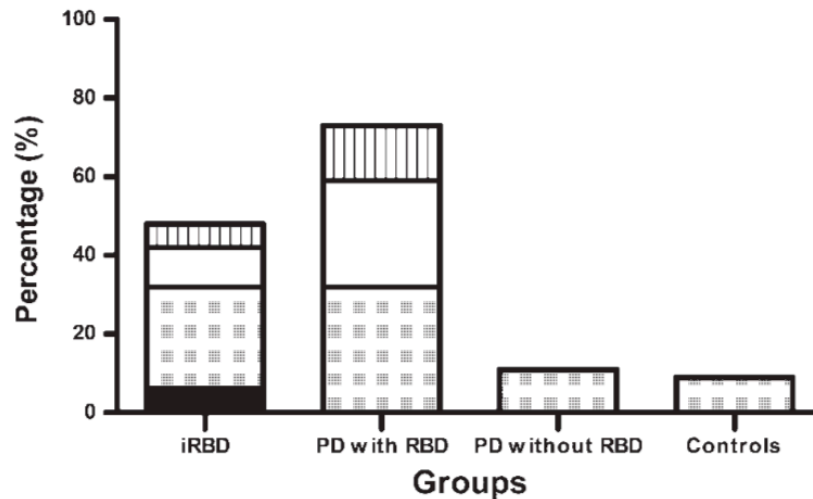


Figure 5. Percentage and subtypes of mild cognitive impairment (MCI) in idiopathic rapid eye movement sleep behavior disorder (IRBD) patients, Parkinson’s disease (PD) with RBD patients, PD without RBD patients, and controls. Striped area represents non-amnesic MCI multiple domain, white area represents amnesic MCI multiple domain, gray area represents non-amnesic MCI single domain, and black area represents amnesic MCI single domain. (Gagnon et al. 2009).

On the other hand, visual memory is still poorly investigated. Relative to healthy individuals IRBD patients showed impaired performance in Rey–Osterrieth’s complex figure (ROCF) delayed recall (Terzaghi et al., 2013, 2008) and in a visual-short term memory experimental task (Rolinski et al., 2016b). Visual memory assessed with recall of ROCF, remains unclear since other authors did not find significant results (Fantini et al., 2011; Her et al., 2019). The main issue of assessing visual memory through ROCF is that the recall success partly depends on the strategy used in copying the complex figure, as well as VS/VP functioning, thus memory output is somehow related to the individual’s copy performance. To clarify this point, it would be appropriate to control ROCF memory scores by patient’s copy performance.

With few exceptions, previous literature outlines learning and memory deficits in IRBD patients, with several works focused on verbal, but only a few on visual memory.

Executive functions and Attention

There is prolific literature focused on the study of attention and executive functions in IRBD patients. Verbal fluency is one of the most affected subdomains, with a particular impairment in semantic fluency compared to healthy individuals (Gagnon et al., 2009; Lerche et al., 2018; Massicotte-Marquez et al., 2008). Although these findings may be reflecting slowed thinking and executive dysfunction, the fact that semantic is more frequently impaired than phonemic fluency (Gagnon et al., 2009; Marques et al., 2010), leads to the speculation that IRBD patients might present changes in regions involved in language capacity. In this sense, semantic fluency in PD was associated with frontal, temporal, and cerebellar areas, whereas no correlation was found between brain structural changes and phonemic fluency (Pereira et al., 2009b).

Impairment in Trail making test (TMT) part A (TMTA) (Youn et al., 2016) and part B (TMTB) (Gagnon et al., 2009; Lerche et al., 2018; Massicotte-Marquez et al., 2008; Shin et al., 2019) has been systematically reported in previous works and might be manifesting complex visual scanning and mental flexibility difficulties in IRBD. The main limitation of TMT is that it has a motor component such that speed and agility make a strong contribution to success. Hence, the fact that IRBD shows greater impairment in TMT might be due not only to complex visual scanning and mental flexibility problems but also to subtle motor signs in terms of motor speed. Indeed, lower scores in the Purdue Pegboard (Postuma et al., 2009) support the presence of motor speed slowness in IRBD. To avoid confusion, TMTB/A ratio allows studying mental flexibility subtracting the motor effect, and interestingly it is also impaired in these patients (Lerche et al., 2018; Massicotte-Marquez et al., 2008).

There is evidence highlighting dysfunction in attention and working memory. Previous reports showed significant low performance in digit span (Gagnon et al., 2009; Terzaghi et al., 2013, 2008), corsi supraspan (Ferini-Strambi et al., 2004), and attentive matrices (Terzaghi et al., 2013). Other researchers have found that in comparison to healthy individuals, IRBD had impaired decision-making measured through the Iowa Gambling task (Delazer et al., 2012; Sasai et al., 2012), interference control (Bezdicsek et al., 2018), and abstract capacity deficit assessed with the Raven colored matrices (Terzaghi et al., 2013), and the Similarities test (Massicotte-Marquez et al., 2008).

Overall, these works suggest that attention and executive functions may be affected in IRBD patients relative to healthy individuals.

Language

Language is by far the less studied function in IRBD, which is surely endorsed on the assumption that IRBD is a prodromal stage of parkinsonism disorders. Within these few studies, works using the Boston Naming Test did not report denomination abnormalities in IRBD (Her et al., 2019; Li et al., 2018). Recently, Galbiati and co-workers, showed lower performance in IRBD with MCI in the Token Test (Galbiati et al., 2019a), a task that is commonly used to assess verbal comprehension. It is worth noting that impaired performance in the Token test has been reported in PD patients with hallucinations (Ramírez-Ruiz et al., 2006). A possible explanation other than verbal comprehension deficit might be a failure in some items with greater working memory demand, a function that has been proven to be affected in IRBD (Gagnon et al., 2009; Terzaghi et al., 2013, 2008).

Visuoperceptual functions

Visuoperceptive (VP) and visuospatial (VS) abilities in IRBD have been gaining much attention in the scientific community, mainly because VS/VP dysfunction is commonly present in DLB (Levy and Chelune, 2007; Tiraboschi et al., 2006) and PD (Garcia-Diaz et al., 2018a; Pereira et al., 2009a; Ramírez-Ruiz et al., 2006). More interestingly, since VS/VP impairment enables to differentiate between DLB and other dementias (Levy and Chelune, 2007; Tiraboschi et al., 2006), it has been proposed as a hallmark in DLB cognitive differential diagnosis (Tiraboschi et al., 2006). Nevertheless, this intriguing topic has been a little overlooked in the literature with very few works until now.

Object identification

Since most clinical neuropsychological tests assessing VP require qualitative correction, some works addressing this topic have used experimental paradigms. Back in 2010, Marques and colleagues (2010), hypothesized that VP impairment was commonly present in RBD, both in its idiopathic form and when associated with PD. In their experiment, they studied sensory, perceptive, and attentional aspects of visual information processing (Marques et al., 2010). Their results manifested a higher object identification threshold in

IRBD and PD with RBD (PD-RBD). They came to the conclusion that since object identification is mainly performed by the ventral visual pathway, their results were pointing out that RBD might affect somehow this specific pathway. Moreover, the fact that they did not observe any difference in visual perception between PD-RBD and IRBD, or between PD without RBD and HC, lead them to hypothesize that RBD condition might probably be related to a non-dopaminergic origin (Marques et al., 2010).

In a subsequent work, Plomhause and co-workers (Plomhause et al., 2014) assessed VP processing and visual attention with the fragmented picture identification task developed by Biederman (Biederman and Cooper, 1991) and the Posner Paradigm (Posner, 1980) respectively. In comparison to healthy controls, IRBD had perceptive impairment but not attentional impairment. Particularly, their results highlighted impaired perceptual priming in IRBD, classically measured by the Biederman's task. The priming effect in IRBD was only seen when the object previously presented was exactly the same, and not if it was "complementary" (i.e. the same object but different shape) (Plomhause et al., 2014). According to the author's interpretation, the response pattern observed in IRBD was consistent with impaired intermediate perceptive processing of visual information, since the initial presentation of the image did not appear to activate an overall structural representation. They hypothesized that the neural correlate of this impairment might correspond to the lateral occipital complex, which has been shown to be involved in object recognition (Plomhause et al., 2014).

Facial and emotion recognition

There is fairly evidence of facial and emotion recognition deficits in PD (Baggio et al., 2012; Garcia-Diaz et al., 2018a; Pereira et al., 2009a), nevertheless, no one to the best of our knowledge has studied this function in IRBD.

Color discrimination in IRBD

Impaired color perception is a common non-motor symptom of PD (Postuma et al., 2009; Price et al., 1992), that is present even in the early stages of the disease (Büttner et al., 1995). Whereas former works attribute the deficit to retinal anomalies (Price et al., 1992), it has been reported that color discrimination in PD is associated with posterior structural alterations, with MCI (Bertrand et al., 2012) and with RBD (Postuma et al., 2009). Until now, nearly all evidence in IRBD's color perception comes from Montreal, Québec, Canada.

They identified poorer color discrimination in IRBD compared to healthy individuals (Postuma et al., 2011, 2009, 2006).

Minor hallucinations in IRBD

Well-structured visual hallucinations (VH) are frequently present in PD and DLB. In this sense, in a large retrospective study, a lifetime prevalence of 50% in PD and 73% in DLB was identified (Williams and Lees, 2005). Research on the field, suggests that VH onset might be preceded by minor hallucinations (MH). A preliminary work reported that the prevalence of MH in isolation was 14.3% in PD (**Panel 4** includes a brief description of MH subtypes and prevalence in PD) (Fenelon, 2000). In keeping with these findings, a longitudinal study found out that after 4.4 years of follow-up, 28.5% of the PD drug-naïve patients with MH developed well-formed VH (Pagonabarraga et al., 2016). Additionally, MH are closely associated with other nonmotor symptoms such as depression (Fenelon, 2000) and RBD (Lenka et al., 2017; Pagonabarraga et al., 2016). Hence, in-depth MH investigation sounds of particular interest in IRBD (for a systematic review on MH in PD refer to Lenka, et al., 2019).

Despite its relevance, only one previous study addressed this topic in IRBD (Sasai-Sakuma et al., 2017). In a novel attempt to study complex visual illusions, they found out that IRBD patients experienced more frequently this phenomenon. In addition, their results demonstrated that patients presenting pareidolias had a greater amount of REM sleep without atonia, lower sleep efficiency, and worse cognitive impairment. They put forward the presence of pareidolias in IRBD as a possible predictive marker of Lewy body disease. The role of the dorsal visual stream in the genesis of MH in PD (Pagonabarraga et al., 2014), could lead to hypothesize that the presence of MH in IRBD underlay abnormalities in these regions, even before the motor onset.

Panel 4 Minor Hallucinations in PD

As described in Fénelon et al. (2000), minor forms of hallucinations encompass different types of hallucinatory experiences, namely feeling of presence, passage hallucinations, and visual illusions. In their work, MH were present in 25.5% of PD patients (14.3% in isolation).

- ◆ *Visual illusions*: are brief misperceptions of objects or living beings that differ from objective reality. Visual illusions include the presence of pareidolias, that is the illusory perception of faces or animals from irrelevant visual stimuli. Based on Fénelon et al., (2000), illusions occurred in 16% of PD with MH (PD-MH).
- ◆ *Feeling of presence*: The patient has the vivid sensation of the presence of somebody (more often a person than an animal) either somewhere in the room or, less often, behind him or her. Commonly occur while patients are alone. Presence hallucinations was the most frequent subtype of MH in their work, and it was present in 64% of PD-MH.
- ◆ *Passage hallucinations*: consisted of a brief appearance of a person or an animal passing through the periphery of the visual field, moving sideways. Patients usually have an irrepressible urge to look towards the illusory moving perception. This subtype was present in 33% of PD-MH.

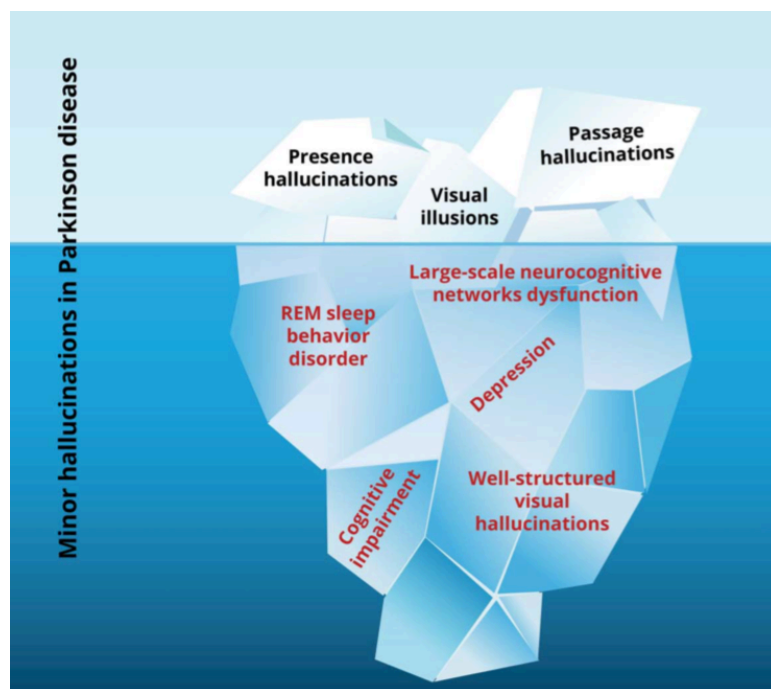


Figure 2. Schematic representation of the iceberg phenomenon in patients with Parkinson's disease and minor hallucinations extracted from Lenka, et al., 2019.

Altogether, this previous works on visuoperception identified difficulties in object identification and color discrimination, as well as increased frequency of complex visual illusions in IRBD patients. These cognitive deficits may be manifesting posterior and ventral visual pathway abnormalities.

Visuospatial and Visuoconstructive functions

Visuoconstructive (VC) dysfunction has been reported in PD and DLB (Garcia-Diaz et al., 2014; Gnanalingham et al., 1996), and is commonly assessed using copy drawing and building tasks. Neuropsychological investigations in IRBD demonstrated lower performance in copy drawing tests. For instance, two previous works identified lower scores in drawing Clock (Iranzo et al., 2006; Youn et al., 2016), other authors in ROCF copy (Fantini et al., 2011; Li et al., 2018; Zhang et al., 2019), and Pentagon's test (Galbiati et al., 2019a). Even so, impairment in copy drawing tests remains unclear (Gagnon et al., 2009; Her et al., 2019; Li et al., 2018; Massicotte-Marquez et al., 2008; Shin et al., 2019; Terzaghi et al., 2013, 2008). On the other hand, no differences with healthy individuals have been reported in block design tests (Gagnon et al., 2009; Li et al., 2018; Massicotte-Marquez et al., 2008). The main limitation of using copy drawing and building tasks is that they have a significant motor component, so speed slowness and subtle motor signs may affect the individual's performance. Therefore, the conclusion of the aforementioned studies is not straightforward. At the same time, some works assume that poor performance in constructive tasks is showing VS deficits in IRBD. However, as stated above, it is difficult to clarify if these findings are due to VS/VC deficits or motor subtle signs. So, it would seem reasonable including tests assessing this function in isolation, instead. Nevertheless, the specific and independent assessment of VS functions has been neglected in the literature, with only two previous works attempting to assess this function (Iranzo et al., 2006; Pereira et al., 2019). Unfortunately, the first work lacked a control group, still, they reported VS deficit in comparison to normalized scores (Iranzo et al., 2006). The other study showed a clear tendency to significance in VS impairment in IRBD relative to HC (Pereira et al., 2019). Further research including VS tests is needed to elucidate whether IRBD presents impairment in this domain.

IRBD cognitive profile relative to PD

Few researchers have addressed the question of how the IRBD's cognitive profile is relative to PD's. Although some results are available, the evidence is not conclusive. Broadly speaking, preliminary studies have found that IRBD and PD-RBD have similar phonetic fluency (Marques et al., 2010), VS (Pereira et al., 2019), VC (Zhang et al., 2019) and VP (Marques et al., 2010) impairment. Contrary, others reported that in comparison

to IRBD, PD-RBD had lower performance in attention (Gagnon et al., 2009; Zhang et al., 2019), immediate recall, and VC tasks (Gagnon et al., 2009). In comparison to PD without RBD, IRBD patients showed worse scores in executive functions, attention (Gagnon et al., 2009), semantic fluency (Gagnon et al., 2009; Pereira et al., 2019) and verbal memory (Gagnon et al., 2009). Additionally, color impairment has been also identified in PD-RBD, relative to controls (**Figure 6**). The authors conclude that color perception is more linked to the presence of RBD than PD since statistically significant differences were also found between PD-RBD and PD without RBD (Postuma et al., 2009).

It could be speculated that differences between IRBD and PD patients are more evident when PD patients are distinguished according to RBD presence. IRBD and PD-RBD seem to share impairment in VP functions, with greater difficulties in visual scanning and VC in PD-RBD. Hopefully, the next few years will witness a considerable growing interest in this challenging topic.

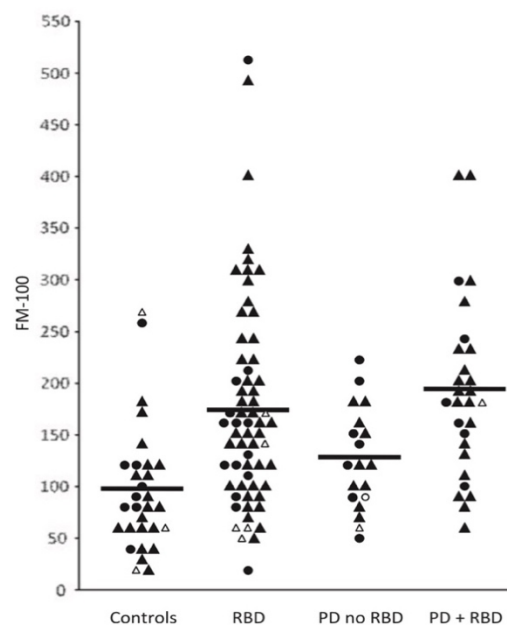


Figure 6. Farnsworth–Munsell 100-hue test measures in normal controls, in patients with Idiopathic REM sleep behavior disorder (IRBD), PD patients with or without REM sleep behavior disorder (PD no RBD and PD + RBD). Horizontal bar indicates mean value for group. Black triangle= males ≥ 50 years; open triangle= males < 50 years; filled circle= females ≥ 50 years; open circle= females < 50 years (Postuma et al., 2009).

Longitudinal studies

Longitudinal research attempting to find markers of phenoconversion in IRBD is gaining much attention, with a particular interest in understanding what differentiates those patients evolving to PD from those evolving to DLB. Under these circumstances, the study of neuropsychological changes in IRBD over time has become an intriguing area. Some works identified decrease performance in executive functions and attention in IRBD across time (Terzaghi et al., 2013; Youn et al., 2016). Although they also included a group of healthy individuals, they only found time changes in IRBD. On the other hand, one work showed differences in cognition in IRBD relative to normal aging after 2 years. They found out that IRBD patients had lower performance in working memory, visuoconstruction, and delayed verbal memory in comparison to controls (Fantini et al., 2011). Nevertheless, none of these former works reported an interaction between group and time, so the aging effect cannot be ruled out.

Converters vs non-converters

An alternative approach is addressing the question of how different cognitive profile is at baseline in those patients that eventually phenoconvert to a Synucleinopathy. Within this framework, the proportion of MCI at baseline was found in 22-50% of the converters, and in 12-19% of those that remained disease free (Génier Marchand et al., 2017; Terzaghi et al., 2019). When studying retrospectively those patients that developed dementia, Postuma and co-workers observed that all patients presented MCI at baseline (Postuma et al., 2012c). In this context, previous research manifested greater impairment in executive functions, attention (Génier Marchand et al., 2017; Terzaghi et al., 2019; Youn et al., 2016), semantic fluency (Génier Marchand et al., 2017), color discrimination (**Figure 7**) (Postuma et al., 2011) and verbal memory (Terzaghi et al., 2019) in IRBD converters in comparison to non-converters. Worth mentioning is the fact that almost all works showing decline in executive functions used TMT test. As stated above, this test demands motor functioning; for this reason, another plausible explanation is that these findings may also be reflecting subtle motor signs in IRBD that eventually develop parkinsonism. Contrary to previous papers, Pereira and colleagues (2019) did not found differences in any neuropsychological test between converters and non-converters (Pereira et al., 2019).

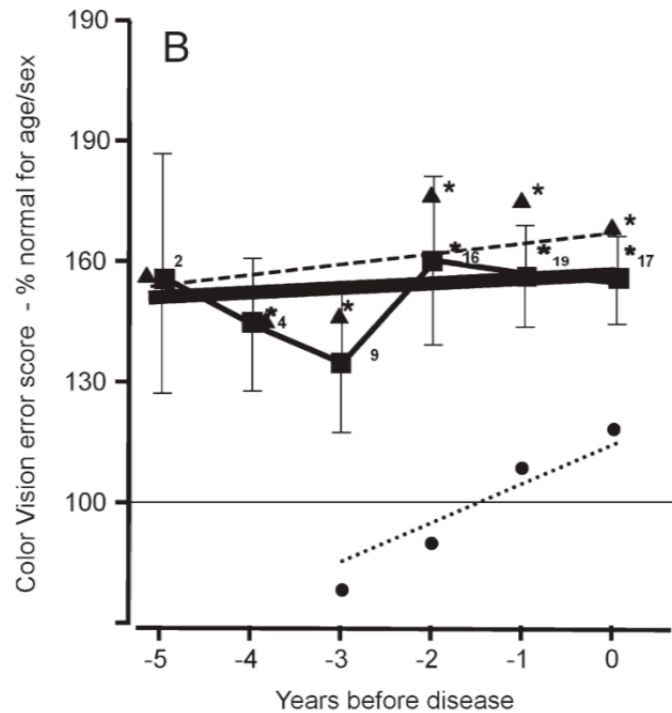


Figure 7. Color vision testing in preclinical intervals of disease. Year 0 refers to examination at time of disease diagnosis, and years -1 to -5 to examinations before disease diagnosis. Square= entire disease group; triangle= dementia/ PD-dementia; circle= tremor-onset PD. Error bars are for the disease-group as a whole (standard error). The number beside each data point represents the number of assessments at that time point for the disease group as a whole. * significantly different than age/sex-adjusted expected values (1-sample t test). The solid line represents the regression line for the entire disease group, and dotted lines represent regression lines for each disease subgroup (dementia/PD-dementia and tremor-onset PD). (Postuma et al., 2011)

As Génier Marchand et al. (2018) put forward in their paper, the heterogeneity seen among IRBD patients, is probably due to the fact that some IRBD patients will evolve to PD, others to DLB, and others will remain idiopathic. That could be the reason why their presentation, clinical phenotype, and progression may vary across patients (Génier Marchand et al., 2018). With this idea in mind, the next subsection does an in-depth analysis of their interesting work, presenting the neuropsychological profile of IRBD patients that finally phenoconvert to an overt DLB. This intriguing topic will surely help both clinical and research practice.

Cognitive profile of IRBD patients that will eventually evolve to DLB

In their preliminary work, Marchand et al. observed that cognitive impairment clearly distinguishes those IRBD patients that will finally evolve to a DLB than those that will develop a PD. Their results showed that when comparing those converting to dementia

with those converting to a parkinsonism, the firsts performed worse in verbal learning, immediate and delayed visual memory, as well as in a task involving visuospatial and visuoconstructive functions. Logistic regression analysis showed that in IRBD, higher age, poorer performance on mental flexibility, visuospatial and visuoconstructive functions were independent trending predictors of developing an overt dementia (Génier Marchand et al., 2017).

In a major advance, the same research group (2018) examined throughout 6 years the cognitive profile of 109 IRBD patients (**Figure 8**). Afterward, they assessed the progression of cognitive decline before disorder diagnosis in three groups of IRBD patients classified as follows: those IRBD that will remain idiopathic (still-idiopathic), those that will eventually develop an overt DLB (DLB-converters) and those that will convert to PD (PD-converters). Their results showed that after 6 years of systematic follow-up, DLB-converters performed worse than still-idiopathic patients in TMTB almost at all time points. Interestingly, verbal fluency, the ROCF copy, and block design performance were worse in DLB-converters in comparison to still-idiopathic and PD-converters, only soon before conversion (Génier Marchand et al., 2018).

As for verbal episodic memory, DLB-converters showed a progressive decline in the Rey Auditory-Verbal Learning Test (RAVLT), particularly in learning (sum of trials 1 to 5), immediate and delayed recall, but also in recognition. On the contrary, no decline in the RAVLT test was detected in PD-converters or still-idiopathic patients. Just few years before DLB diagnosis, DLB-converters performed significantly worse than PD-converters and still-idiopathic patients in immediate and delayed recall. Recognition in DLB-converters worsened in the year when the diagnosis was done in comparison to the two other groups (Génier Marchand et al., 2018). These results outline that DLB-converters present progressive memory decline that will eventually involve not only evocation but also recognition deficits.

Overall, this recent evidence reveals that IRBD patients that will evolve to DLB present executive and memory decline throughout years of the disease, with later semantic fluency and visuoconstructive impairment.

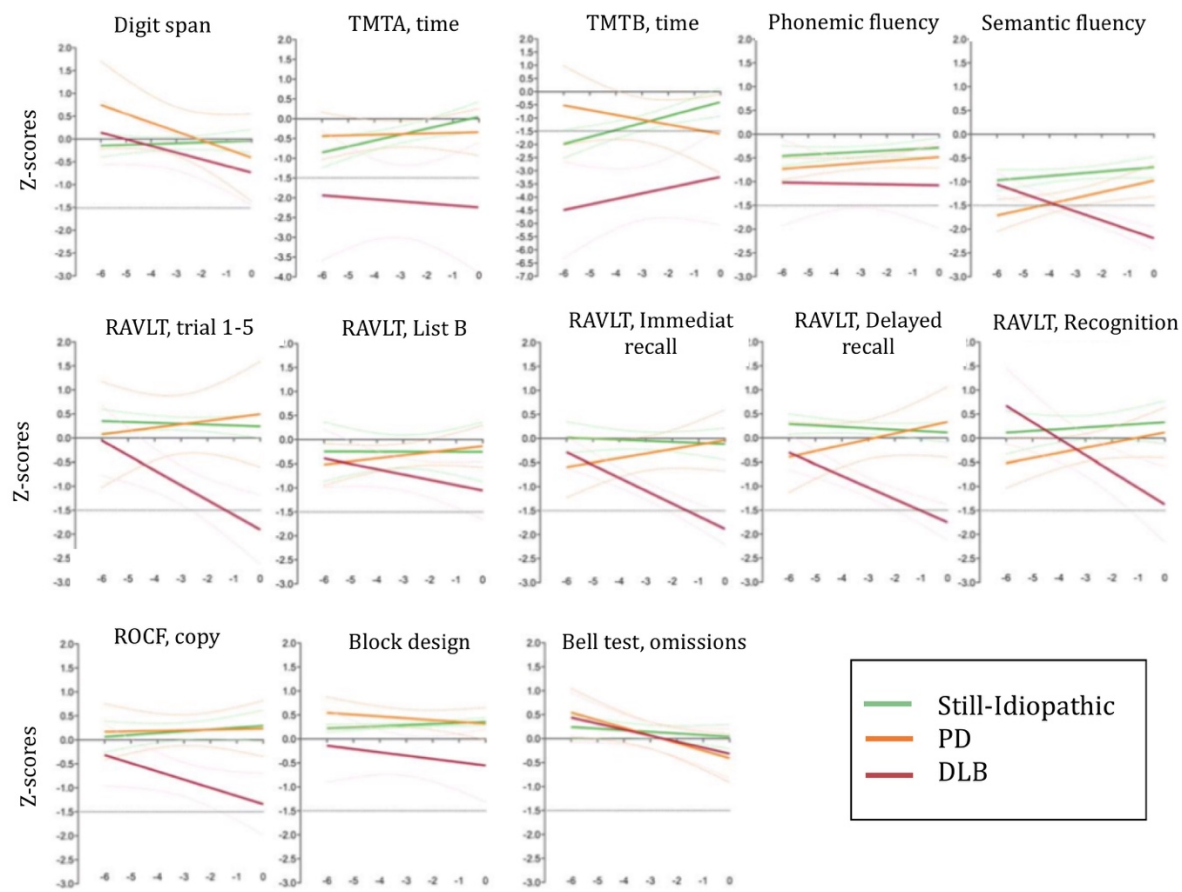


Figure 8. Performance changes on neuropsychological tests (z scores) over time shown by regression lines. Dotted colored lines represent confidence intervals (95%) of the slope. 0= last follow-up for patients who remained idiopathic and year of conversion for PD and DLB patients; -1, -2, -3, -4, -5, -6= 6 previous follow-up years. Black dotted line represents -1.5 thresholds for z scores, which is considered to be clinically impaired performance. PD, Parkinson's disease; DLB, dementia with Lewy bodies; TMT, Trail Making Test; RAVLT, Rey Auditory-Verbal Learning Test; ROCF, Rey-Osterrieth Complex Figure (Génier Marchand et al., 2018).

Cognitive tests that may predict clinical progression in IRBD

This is another intriguing question that may help to select neuropsychological tests in clinical practice, but also in clinical trials that attempt to study those patients at higher risk to convert. Researches that have addressed this question found out executive functions and semantic fluency to be cognitive markers of progression in IRBD (Génier Marchand et al., 2018; Génier Marchand et al., 2017; Terzaghi et al., 2019). In particular, in Terzaghi et al. work, the executive domain, measured using Raven's colored matrices, Weigl's sorting Test, Frontal assessment battery, and verbal fluency was associated with phenoconversion (Terzaghi et al., 2019). In the same line, in Génier Marchand and colleagues' research, Stroop Color Word Test, TMTB, and ROCF copy performance were

independent predictors of dementia in IRBD (Génier Marchand et al., 2017). In a subsequent study of the same group, they highlighted that the TMTB was the test that better detects early prodromal dementia stages, whereas semantic fluency and verbal episodic learning tests such as the RAVLT test, were the best for monitoring changes over time (Génier Marchand et al., 2018). Finally, in a 5-year follow-up study with IRBD, abnormal color vision was associated with an increased risk of conversion to an overt alpha-Synucleinopathy, mostly to DLB (Postuma et al., 2015b).

Taken as a whole, TMTB, semantic fluency, color perception, and verbal episodic learning tests may be helpful to detect those IRBD patients that will eventually progress to an overt dementia.

Summary

Altogether, the evidence outlines that in comparison to healthy individuals, IRBD patients present impairment in verbal memory, semantic fluency, object identification, color discrimination, mental flexibility, and visuoconstruction. It should be pointed out, that there are slightly different cognitive profiles among IRBD patients, depending on their final onset. Those IRBD that will evolve to DLB present greater progressive executive and memory decline throughout years of the disease, with later semantic fluency, visuoconstructive difficulties, and even greater memory impairment. Finally, when compared to PD patients, IRBD and PD-RBD seem to share impairment in visuo-perceptual abilities, with higher dysfunction in attention and visuoconstruction in PD-RBD.

Table 4. Studies of cognitive impairment in IRBD

Reference	Sample	FFEE/ Attention	VS/VP/VC	Language	Memory
Ferini-Strambi et al., 2004	17 IRBD 17 HC	IRBD<HC	Corsi Supraspan ROCF copy		
Iranzo et al., 2006	44 IRBD (compared to norm)		VOSP and drawing Clock		
Postuma et al., 2006	25 IRBD 25 HC	IRBD<HC	FM-100 Hue Test		
Massicotte-Marquez et al., 2008	14 IRBD 14 HC	IRBD<HC	TMTB, ratio TMTB/A, Similarities (WAIS-III), Stroop flexibility condition, Semantic fluency		RAVLT (immediate, sum trials 1-5, list B, retention, recognition).
Terzaghi et al., 2008	23 IRBD 23 HC	IRBD<HC	Word span, Digit span forward		ROCF delayed recall, Logic memory
Postuma et al., 2009	68 IRBD 34 PDRBD 21 PDNRBD 36 HC	IRBD<HC	FM-100 Hue Test		
Gagnon et al., 2009	32 IRBD 18 PDNRBD 22 PDRBD 40 HC	IRBD<HC	Digit span, TMTB, phonemic and semantic fluency		RAVLT (sum trials 1-5, list B, immediate, delayed recall)
		IRBD<PDNRBD	Digit span, TMTB, phonemic and semantic fluency		RAVLT (sum trials 1-5, list B, delayed recall)
		IRBD>PDRBD	Bells Test	ROCF copy; Block Design	RAVLT immediate recall
Marques et al., 2010	10 IRBD 10 PDNRBD 10 PDRBD 8 HC	IRBD<all groups			Buschke list free recall
		IRBD/PDRPBD <HC	Phonemic fluency	Object identification	
Fantini et al., 2011*	24 IRBD 12 HC	IRBD<HC		ROCF copy	Logic memory delayed recall
		IRBD _{F-up} <HC _{F-up}	Corsi Supraspan	ROCF copy	Logic memory recall
Sasai et al., 2012	38 IRBD 34 HC	IRBD<HC	Iowa gambling task		
Delazer et al., 2012	16 IRBD 45 HC	IRBD<HC	Iowa gambling task		

Reference	Sample	FFEE/ Attention	VS/VP/VC	Language	Memory
Terzaghi et al., 2013*	20 IRBD	IRBD<HC	Word and Digit span, Raven colored matrices, Attentive matrices, Wisconsin Test		RAVLT delayed recall, Logical memory, ROCF delayed
	20 HC	IRBD _b >IRBD _{f-up} * matrices*			
Plomhause et al., 2013	15 IRBD	IRBD<HC	Impaired perceptual priming (Biederman task)		
	15 PDRBD 15 PDNRBD 20 HC				
Youn et al., 2016*	96 IRBD	IRBD<HC	Constructional praxis, drawing Clock		
	57 HC	IRBD _b >IRBD _{f-up} * Digit span*			
Rolinski et al., 2016b	21 IRBD	IRBD<HC PD<HC			Visual short-term memory
	26 PD 26 HC				
Sasai-Sakuma et al., 2017	202 IRBD 46 HC	IRBD> HC	Presence of pareidolias		
Génier Marchand et al., 2017*	34 IRBDc	IRBDc< IRBDnc	TMTA, TMTB, ratio TMTB/A, semantic fluency		
	42 IRBDnc				
Bezdicek et al., 2018	60 IRBD	IRBD<HC	Stroop interference		RAVLT, immediate, delayed recalls, and recognition; Prospective memory
	30 HC				
Lerche et al., 2018*	962 HC	pRBD<HC	TMTB, ratio TMTB/A, semantic fluency		
	183 pRBD	pRBD time effect*	TMTB, ratio TMTB/A*		
Li et al., 2018	28 IRBD	IRBD/PD<HC	ROCF copy		RAVLT delayed recall; Prospective memory (CAMPROMPT)
	25 PD 21 HC				
Génier Marchand et al., 2018*	20 IRBD _{PD}	Between groups	TMTA, semantic fluency	ROCF copy	RAVLT recognition
	28 IRBD _{DLB} 71 IRBD _{still}				
Rahayel et al., 2018b	17 IRBD-MCI 35 IRBD-NMCI 41 HC	IRBD-MCI<IRBD-NMCI	FM-100 Hue Test		

Reference	Sample	FFEE/ Attention	VS/VP/VC	Language	Memory
Marcone et al., 2019	38 IRBD 20 HC	IRBD<HC			Prospective memory
Terzaghi et al., 2019*	30 IRBDc 33 IRBDnc	IRBDc< IRBDnc*	Attentive matrices and FAB*		RAVLT immediate and delayed recall*
Galbiati et al., 2019a	80 IRBD 40 HC	IRBD<HC	Pentagon test (closing-in and total)		
		IRBD-MCI<IRBD-NMCI	Pentagon test (rotation and closing-in), ROCF copy	Token Test	ROCF delayed recall
		IRBD<HC	SDMT		
Pereira et al., 2019*	56 PDpRBD 94 PDNRBD 31 HC	IRBD<PDNRBD	JLO		HVLT-R recognition
		IRBD<PDRBD	Semantic fluency		
Shin et al., 2019	25 IRBD 13 HC	IRBD<HC	TMTB		Immediate, delayed and recognition verbal learning test
Her et al., 2019	15 IRBD 19 HC	IRBD<HC	Verbal fluency		Verbal learning test
Zhang et al., 2019	15 IRBD 12 PDRBD 23 HC	IRBD<HC	ROCF copy (time)		
		IRBD>PDRBD	TMTA		RAVLT delayed recall

Only those contrasts involving IRBD group are listed in the table. Abbreviations: CAMPROMPPT, The Cambridge Prospective Memory Test; FAB, Frontal assessment battery; FFEE, executive functions; FM-100 Hue Test, Farnsworth-Munsell 100 Hue Test; HC, healthy controls; HVLT-R, Hopkins Verbal Learning Test-revised; IRBD, Idiopathic rem sleep behavior disorder; IRBD_b, IRBD at baseline; IRBD_{DLB}, IRBD that convert to DLB; IRBDc, IRBD converters; IRBD_{↑up}, IRBD at follow-up; IRBD-MCI, IRBD with mild cognitive impairment; IRBDnc, IRBD non-converters; IRBD-NMCI, IRBD patients without mild cognitive impairment; IRBD_{PD}, IRBD patients that convert to PD; IRBD_{still}, IRBD that remained disease free after follow-up; JLO, Benton Judgment of Line Orientation test; PD, Parkinson's disease patients; PDpRBD, PD patients with probable RBD (RBD was determined by the RBD Questionnaire ≥5); PDNRBD, PD patients without RBD; PDRBD, PD patients with RBD; RBD, rem sleep behavior disorder; pRBD, subjects with probable RBD (RBD was determined by the RBD Questionnaire ≥5); RAVLT, Rey Auditory Verbal Learning test; ROCF, Rey-Osterrieth's complex figure; TMTA, Trail making test part A; TMTB, Trail making test part B; SDMT, Symbol digits modalities test; VC, visuoconstruction; VOPS, The Visual Object and Space Perception Battery; VP, visuoception; VS, visuospatial. * Those studies showing longitudinal results.

1.1.4. Brain imaging in IRBD

Magnetic Resonance Imaging (MRI) is one of the most commonly used techniques in the field of neuroscience. Particularly since MRI allows detecting regional and signal abnormalities, it is widely used in the study of brain changes associated with neurodegeneration. In the past decade, the characterization of brain structure and function in IRBD as a prodromal stage of Synucleinopathies has become an emergent field of study. This subsection exposes an overview of previous literature on neuroimaging in IRBD. First, I will present a systematic review of literature reporting structural, functional, and metabolic abnormalities underlying IRBD. Second, I will expose previous works regarding neuroanatomical and neurofunctional bases of clinical and cognitive manifestations in IRBD. **Table 5, Table 6, Table 7, and Table 8** at the end of the current section, present the main structural, functional, and metabolic findings in IRBD.

Gray matter changes

There is a considerable amount of procedures that enable us to understand brain structure and its relationship with neurodegeneration. The most common methodologies to explore gray matter (GM) integrity include voxel-based morphometry (VBM), Cortical thickness (CTh), and volumetric analysis.

A growing body of structural MRI research supports the view that GM changes are common in IRBD patients. Based on the implication of the striatum in parkinsonism disorders, preliminary work in this field focused on the study of striatal volumes. They found reduced bilateral putamen in IRBD patients in comparison to healthy individuals (Ellmore et al., 2010a). Later, in 2012, using a 1.5T MRI, a VBM approach showed IRBD had GM volume reduction in the cerebellum, the pons, and in the left parahippocampal gyrus (**Figure 9A**) (Hanyu et al., 2012). The authors confirmed the expected hypothesis that brainstem abnormalities may be responsible for the occurrence of IRBD and may stand out striking similarities with Lewy body-like diseases.

In 2014, Rahayel and colleagues published structural data obtained with a 3T MRI that can be more sensitive to subtle cortical changes than 1.5T scanners. Their research using VBM showed GM volume loss in the right superior frontal sulcus (**Figure 9E**) (Rahayel et al., 2014). Shortly thereafter, with a larger sample of IRBD, the same group reported lower

GM volume in the frontal cortices, anterior cingulate gyri, and caudate nucleus, as well as reduced global volume in the right putamen in comparison to HC. Additionally, using the shape analysis technique, they revealed atrophy in external and internal left pallidum, whereas caudate, putamen and thalamus did not manifest any shape variation in the IRBD patients (**Figure 9C-D**) (Rahayel et al., 2018a).

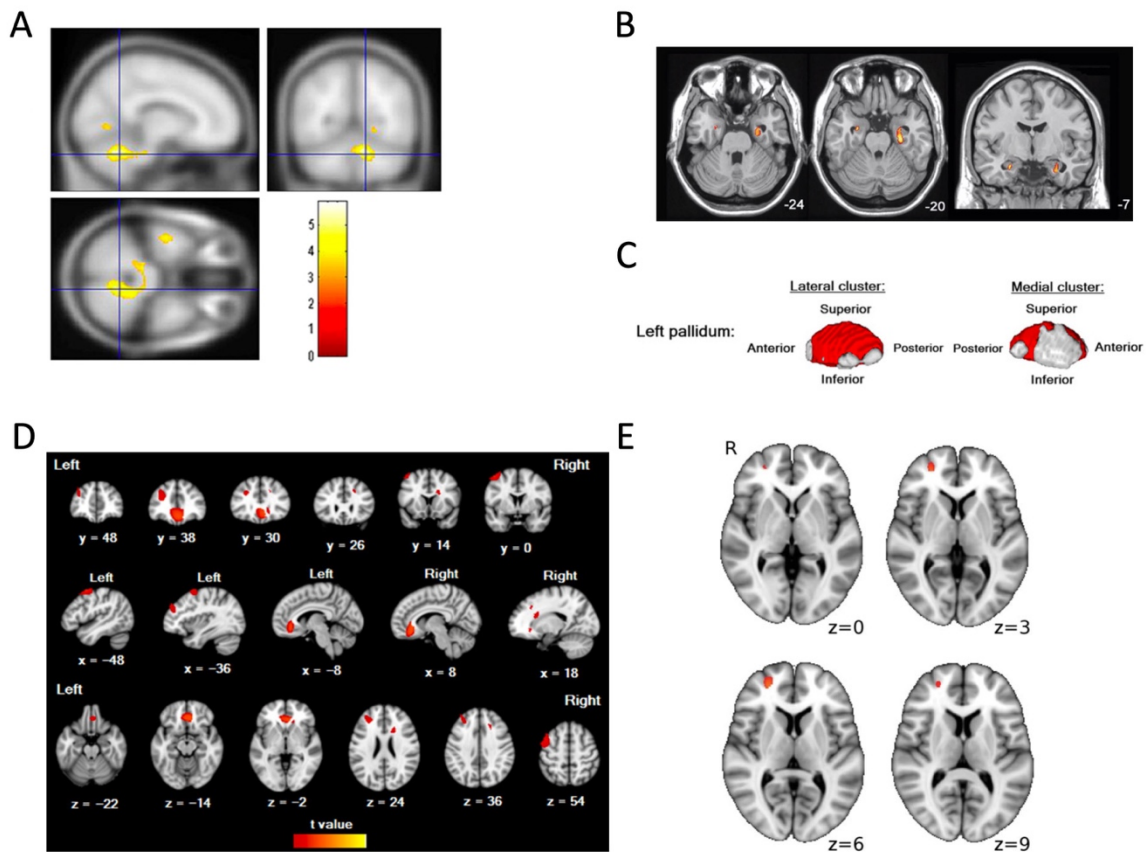


Figure 9. Gray matter (GM) volume changes in IRBD in comparison to healthy individuals. **A.** Reduced GM volume in IRBD (Hanyu et al., 2012); **B.** In red-yellow areas showing increase GM density values in IRBD (Scherfle et al., 2011); **C.** Shape abnormalities in IRBD patients, red cluster is showing atrophy in left pallidum (Rahayel et al., 2018a); **D.** Reduced GM volume in IRBD (Rahayel et al., 2018a); **E.** Reduced GM in IRBD (Rahayel et al., 2014).

There are some slight discrepancies in the topographic cortical thickness pattern underlying IRBD degeneration. In the first attempt to define this pattern, IRBD patients exhibited cortical thinning encompassing only the right hemisphere. Particularly, IRBD had atrophy in the right medial frontal lobe and a region extending from the cingulate and paracingulate gyri to the gyrus rectus, the dorsolateral part of the superior frontal gyrus, and in the right lingual and fusiform gyri (**Figure 10A**) (Rahayel et al., 2014). A subsequent study of the same group identified bilateral cortical thinning in IRBD, in regions such as the medial superior frontal, orbitofrontal and anterior cingulate cortices,

and the right dorsolateral primary motor cortex (**Figure 10B**) (Rahayel et al., 2018a). Differences among their studies might be explained by two factors, first, their second study included a larger sample, and second, they did not use the same technique to analyze cortical thickness. Recently, in Pereira and colleagues' work (2019), IRBD patients manifested cortical atrophy in left lateral occipital and postcentral gyri, which extended to left inferior parietal and supramarginal areas (**Figure 10C**). On combining these previous studies, cortical thinning in IRBD seems to be present in the cingulate cortex, the lateral prefrontal and somatosensorial and motor areas, regions that are affected in both PD and DLB.

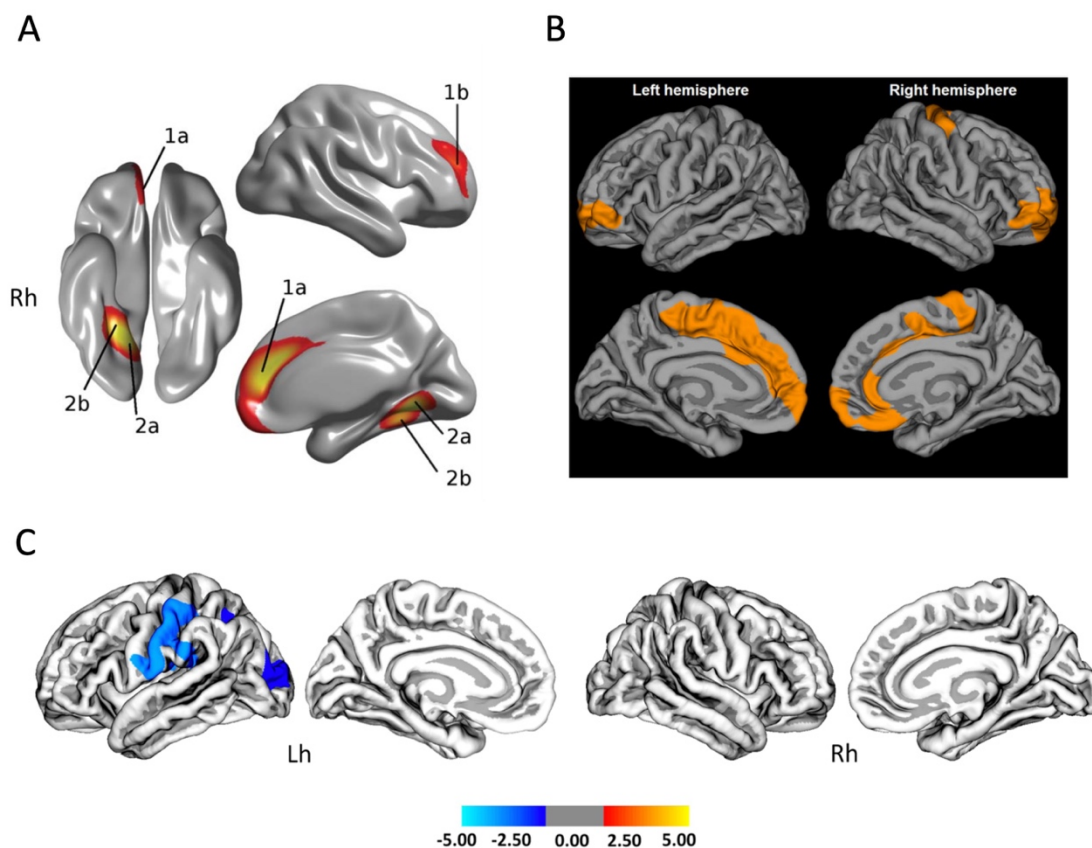


Figure 10. Cortical thickness abnormalities in IRBD in comparison to healthy individuals. **A.** (Rahayel et al. 2014); **B.** (Rahayel et al., 2018a); **C.** (Pereira et al., 2019).

Nevertheless, there are discrepancies regarding the presence of atrophy in structures such as the brainstem and the hippocampus. Whereas one study reported GM reductions in the tegmental portion of the pons (Hanyu et al., 2012), when using 3T MRI scanners, differences in this region were not detected (Rahayel et al., 2018a). In the same line, proton magnetic resonance spectroscopy was not able to detect brainstem abnormalities in patients with IRBD (Iranzo et al., 2002). As regards the hippocampus, only one work

using VBM has focused on this structure, showing increases in GM densities in both hippocampi (**Figure 9B**) (Scherfler et al., 2011). But bearing in mind that hypermetabolism in the hippocampus is also identified (see **Metabolic studies**), further research is needed to elucidate the hippocampal involvement in IRBD patients, and the sensibility of MRI approaches.

Given the fact that most IRBD patients will eventually develop an overt Synucleinopathy, there is growing interest in knowing the similarities among IRBD, PD, and DLB patients. Nonetheless, still, only a few works focused on such objectives. In 2010, Ellmore et al. found significantly smaller left putamen in IRBD in comparison to age-matched early PD patients. The authors interpreted such unexpected results, as a compensatory structural increase in striatal volume due to a deficit in dopaminergic function (Ellmore et al., 2010b). A recent work using structural MRI of the Parkinson's Progression Markers Initiative (PPMI) database found no differences in cortical thickness and subcortical measures between PD de novo and patients with IRBD. The authors suggested that the absence of significant results could be due to the fact that the prevalence of future PD converters in their sample was probably high, and thus subtle PD-like GM changes in IRBD patients were already ongoing (Pereira et al., 2019).

Although there are still some discrepancies, these previous investigations highlight the presence of GM changes in the anterior cingulate and in somatosensorial and motor areas in IRBD, with still unclear results in subcortical and hippocampal structures. Further data collection is required to better characterize gray matter abnormalities in IRBD, with a particular focus on the hippocampal formation.

White matter changes

Diffusion Tensor Imaging (DTI) approach provides information about microscopic changes in white matter (WM) tissue. Very little is known about how does IRBD affects WM integrity. The first investigation to explore this topic demonstrated with a whole-brain analysis, that relative to healthy individuals, IRBD exhibit decreased axial diffusivity in the pons, in bilateral corona radiata, and the SN and increased fractional anisotropy (FA) bilaterally in the internal capsule and olfactory regions. Whereas the fornix, the right

visual stream, and the left superior temporal lobule had both reduced FA and increased radial diffusivity (RD) (**Figure 11**) (Unger et al., 2010).

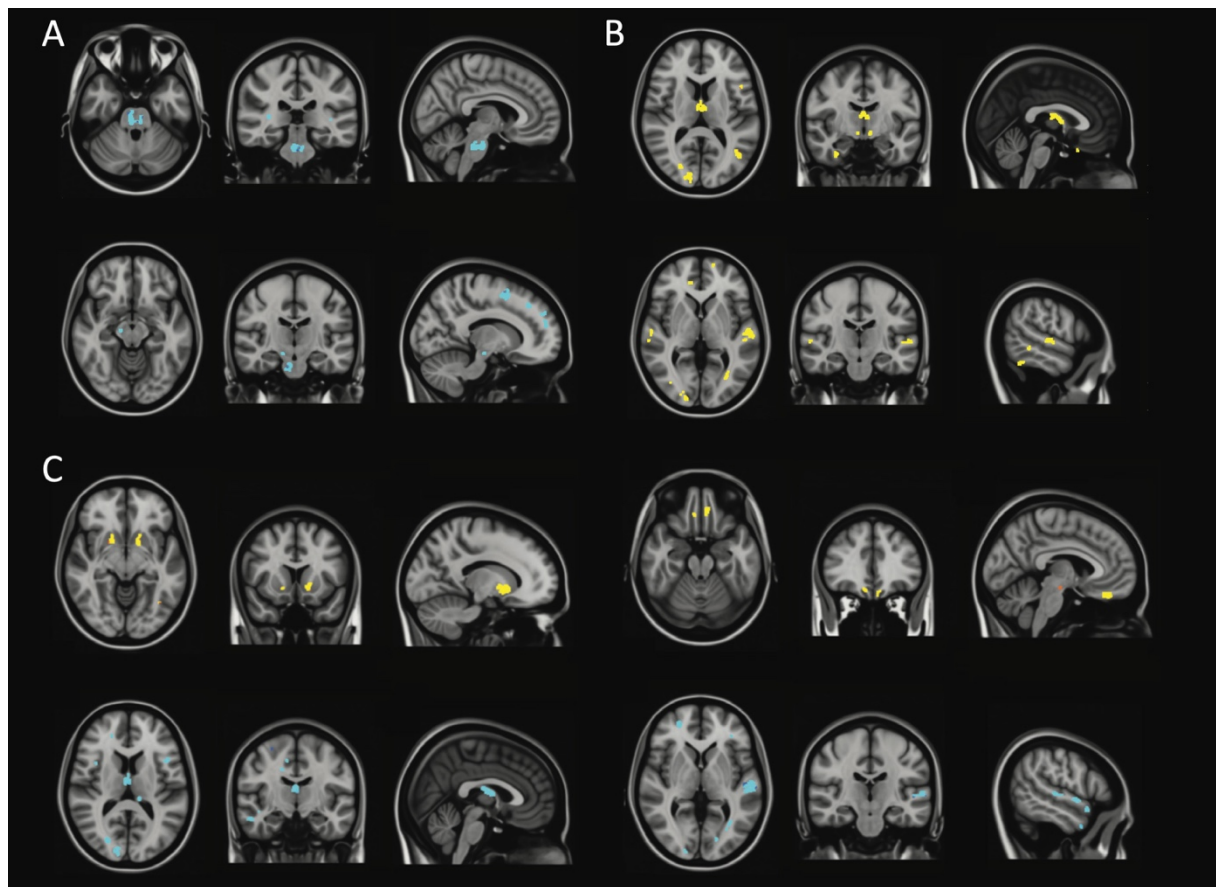


Figure 11. White matter abnormalities in IRBD patients (Unger et al., 2010). **A.** In blue axial diffusivity changes; **B.** In yellow changes in radial diffusivity; **C.** Changes in fractional anisotropy, increases are shown in red-yellow, and decreases in light-blue-blue.

In the same line, subsequent investigations identified reduced FA in the mesencephalic tegmentum, the rostral pons (**Figure 12A**) (Scherfler et al., 2011) and the SN (Pyatigorskaya et al., 2017), as well as increased mean diffusivity (MD) in the pontine tegmentum and reticular formation (**Figure 12B**) (Scherfler et al., 2011). Of particular interest are the WM abnormalities recently identified in IRBD relative to clinical PD patients. With increase MD in IRBD mainly found in the right hemisphere, namely in the corpus callosum, the superior and inferior longitudinal fasciculus, the corticospinal tract, the forceps major, and the corona radiata (Ohlhauser et al., 2019) (see **Figure 12C**). The authors interpret this right predominance as early asymmetric neurodegeneration in prodromal stages.

Altogether, these previous works demonstrate IRBD may present microstructural WM abnormalities in regions that regulate REM sleep, and that are known to have neurodegenerative pathology in early PD (**Table 5** summarizes structural findings in IRBD).

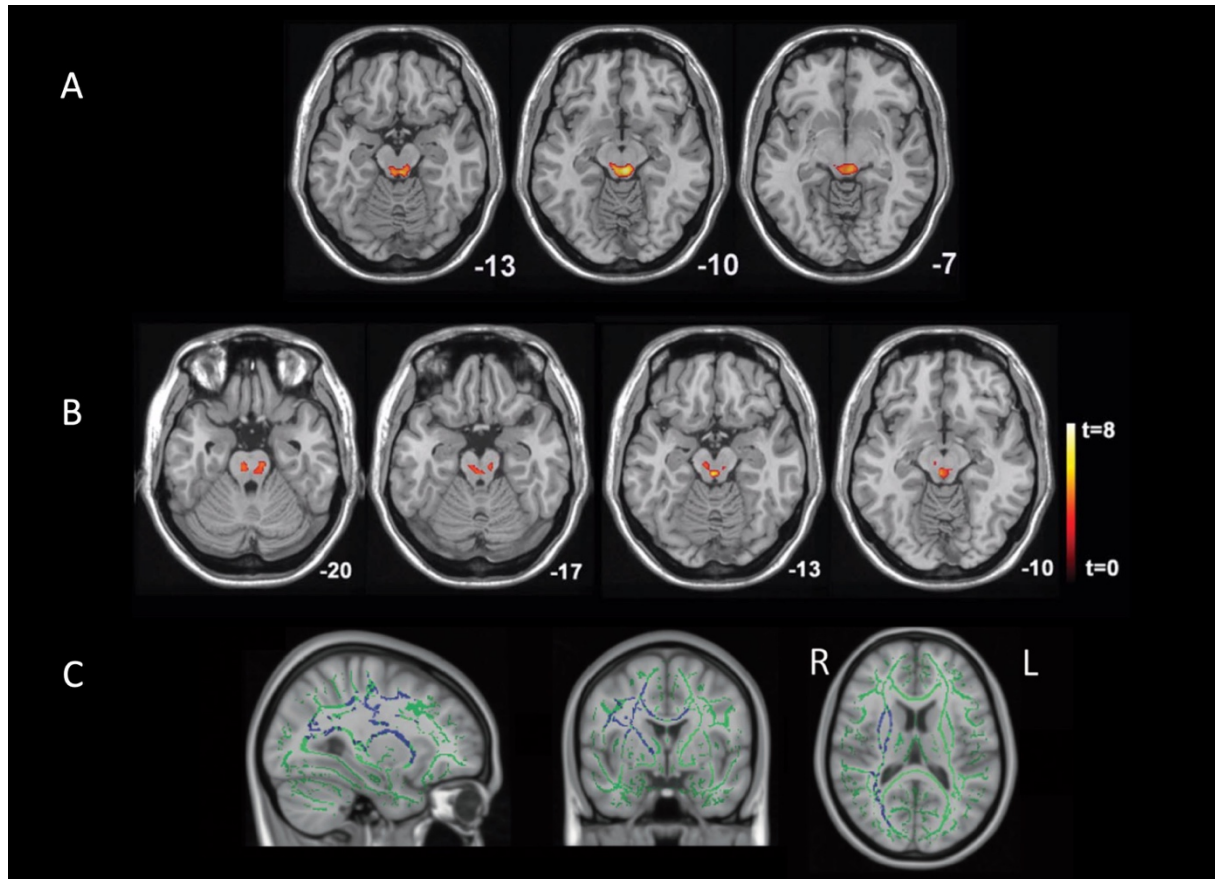


Figure 12. White matter abnormalities in IRBD (continuation). **A.** In red-yellow areas of decreased fractional anisotropy in comparison to healthy controls (HC) (Scherfler et al., 2011); **B.** In red-yellow areas with significantly increased mean diffusivity (MD) in comparison to HC (Scherfler, et al. 2011); **C.** In blue Increased MD in IRBD patients in comparison to clinical Parkinson' disease patients (Ohlhauser et al., 2019).

Structural connectivity

In broad terms, connectivity is how brain regions communicate with one another and how information is passed from one brain area to the next (Bijsterbosch et al., 2017). Structural connectivity is commonly referred to the existence of WM tracts connecting different brain regions, which is usually derived from diffusion-weighted imaging (DWI) through tractography. Moreover, T1-weighted MRI is also used to build connectivity matrices based on the correlation coefficient between all pairs of regions.

The characterization of human structural connectome in healthy, but also in pathological brains, is increasingly becoming a focus of interest in neuroimaging research. Unfortunately, very little is known about structural connectivity in IRBD, with only two previous works using this approach (K. M. Park et al., 2018; Wen et al., 2017), and only one of them including exclusively IRBD patients (K. M. Park et al., 2018). The first investigation (Wen et al., 2017) used deterministic tractography to explore structural connectivity in prodromal PD (IRBD or/and hyposmic patients) and de novo PD patients. They also applied graph analysis that showed higher small worldness and cluster coefficient in prodromal PD patients relative to HC and PD de novo. Besides, to further identify specific connections where WM structural connectivity was altered, they used Network-based Statistics (NBS). This technique revealed that prodromal PD patients had greater connectivity between regions involved in motor, olfactory, and sleep functions compared to HC and de novo PD (see **Table 6** and **Figure 13** for further details). The authors considered that finding increased network efficiency and connectivity in prodromal PD might suggest neural compensation in premotor PD phases.

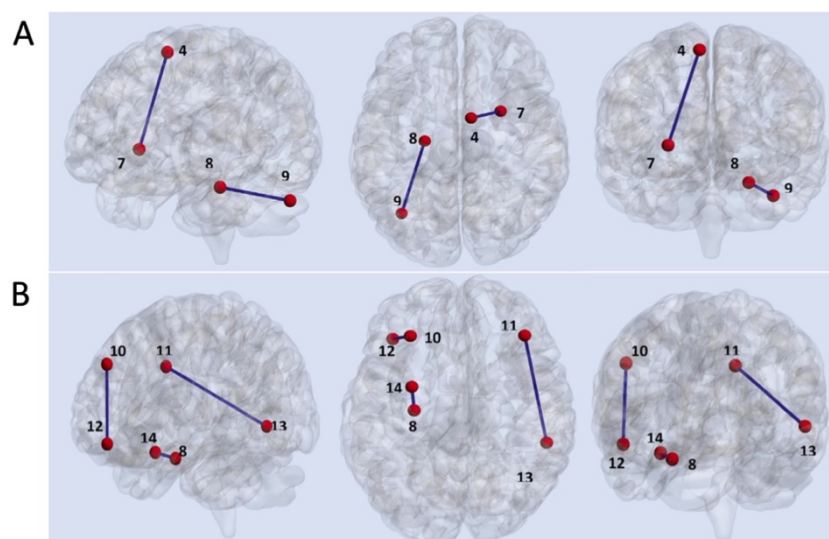


Figure 13. Structural connectivity characterization of prodromal PD (hyposmic and/or IRBD patients). **A.** Prodromal PD have greater connectivity than HC; **B.** Prodromal PD have greater connectivity than de novo PD (Wen et al., 2017).

More recently, Park and colleagues (2018) used T1-weighted images to define 73 regions of interest and obtained correlation matrices between every pair of nodes. They finally computed graph analysis that showed decreased global network, average degree, global efficiency, and local efficiency, and increased characteristic path length in IRBD patients in comparison to HC. The authors concluded that IRBD manifests topological

disorganization of the global brain network, which may be implicated in the pathogenesis of IRBD (K. M. Park et al., 2018). The main limitation of this work is its small sample size of 10 IRBD patients. Within this in mind, further research is required to characterize how IRBD affects structural connectivity.

Functional MRI changes

If structural connectivity offers the possibility to study the structural integrity of tracts connecting different brain regions, functional connectivity explores the dependence and coactivation of the signal from these areas. That is to say, to study functional connectivity, the similarity of the signals from different brain regions is measured, if the signals are similar, this is likely to mean that they are connected (Bijsterbosch et al., 2017). This approach enables us to explore how well the brain is connected under certain conditions. Within this framework, the characterization of brain functional connectivity in neurodegeneration is attracting considerable attention and may explain subtle abnormalities even in the early stages of the disease. The last two decades have witnessed a huge growth of publications showing functional connectivity abnormalities in neurodegenerative disorders (Hohenfeld et al., 2018).

Nevertheless, functional connectivity constitutes a relatively new topic of research on IRBD literature, with only a few studies published to date (**Table 6** summarizes functional findings in IRBD). Preliminary work in this field was carried out less than a decade ago. In 2013 Ellmore, et al. focused its work on SN functional connectivity in IRBD. Their results highlighted that IRBD patients had reduced connectivity between SN and the left putamen in comparison to HC, but increased connectivity in comparison to PD patients. Moreover, IRBD showed higher connectivity than HC between the SN and the right superior occipital gyrus, but lower than PD patients (Ellmore et al., 2013). Taking into account these findings, they concluded that altered nigrostriatal and nigrocortical connectivity characterizes IRBD before motor impairment.

More recently, Rolinski et al. (Rolinski et al., 2016a) proposed a new approach to detect functional connectivity changes in an ad hoc defined basal ganglia (BG) network (BGN). They demonstrated that both IRBD and PD patients had reduced coactivation within the BGN when compared to HC, but also between the BG and frontal regions, namely the cingulate and paracingulate gyri, the orbital cortices and the inferior and middle frontal

gyri (**Figure 14**). Interestingly, this abnormal BGN connectivity enables to differentiate both IRBD and PD from controls with high sensitivity (95.8%) and specificity (73.9%), drawing our attention to the potential role of functional connectivity to detect early dysfunction in BG. In addition to that, they also studied the relationship between the BG connectivity and the metabolic changes assessed through single-photon emission computed tomography (SPECT), finding no correlation between these measures. In keeping with a previous work (Ellmore et al., 2013), they reached the conclusion that the PD-like functional connectivity signature exists even before the motor onset in IRBD. In particular, they underline the idea of using BG functional connectivity as a biomarker for detecting early BG malfunction, thus helping to identify at-risk patients (Rolinski et al., 2016a).

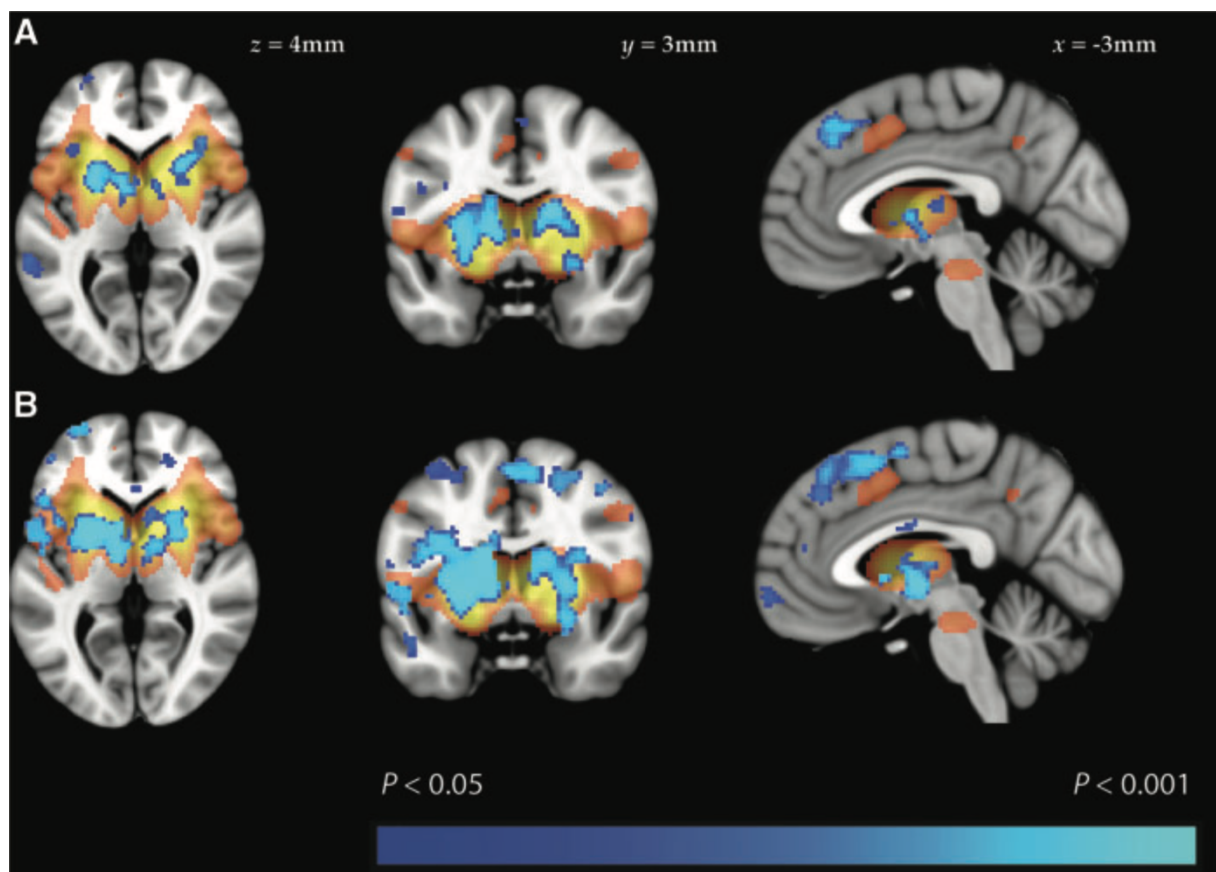


Figure 14. Results of resting state functional MRI analysis (Rolinski et al., 2016a). Group difference maps illustrate clusters of significantly reduced connectivity (blue) in patients with **A.** Parkinson's disease and **B.** Idiopathic rapid eye movement sleep behavior disorder, when compared to healthy individuals. A map of an ad hoc Basal Ganglia network is shown in orange.

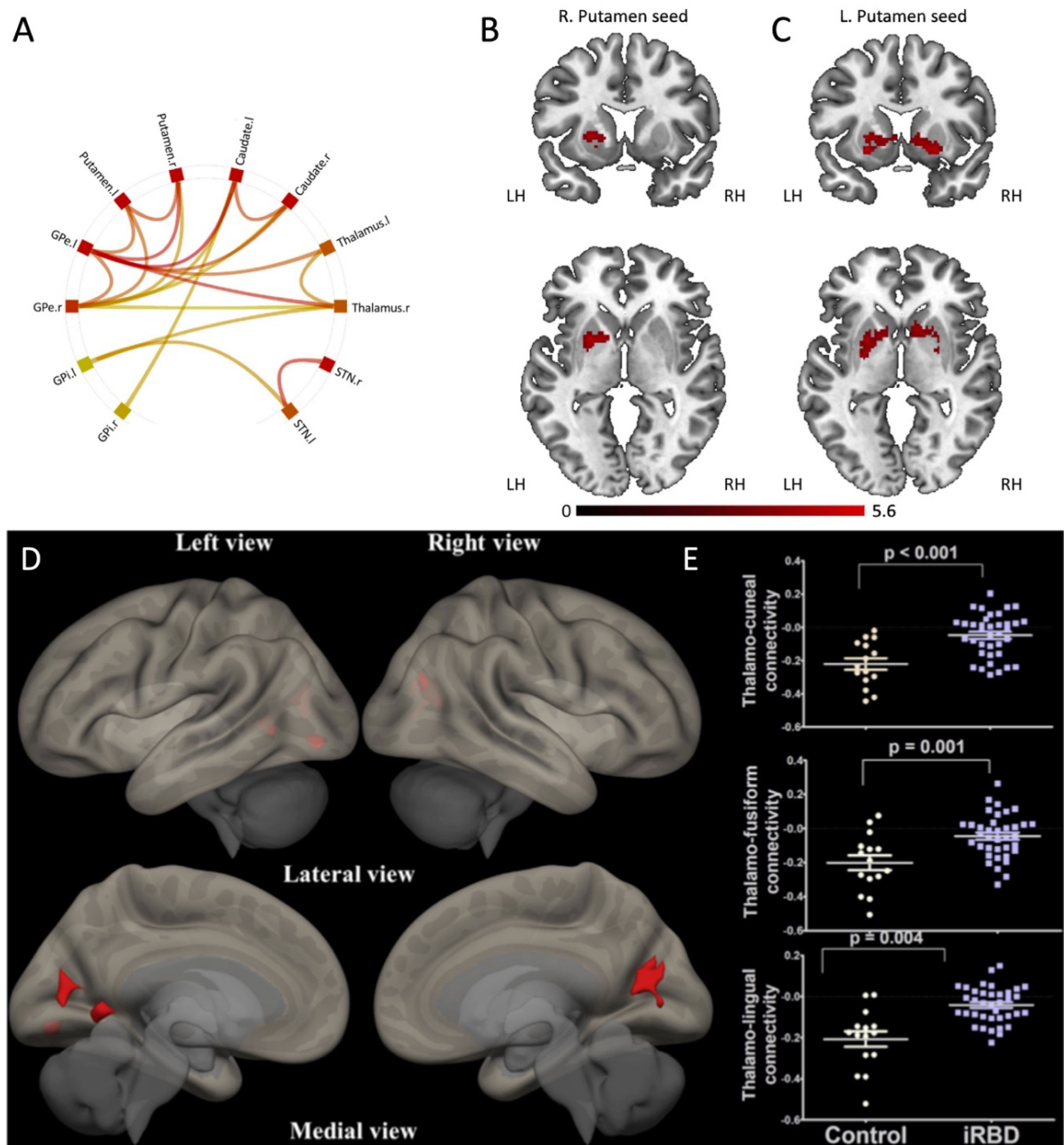


Figure 15. Thalamo functional connectivity in IRBD. **A-C.** Alterations in striato-thalamo-pallidal functional connectivity (FC) in prodromal PD (IRBD and/or hyposmia) (Dayan and Browner, 2017). **A.** Region-to-region FC in the basal ganglia using non-parametric network-based statistic analysis. In red those connections where the prodromal PD showed reduced FC relative to the control group. **B.** Seed-to-voxel FC analysis were the prodromal PD group showed reduced FC relative to HC between the right putamen and a cluster encompassing the left putamen and pallidum; **C.** Seed to voxel analysis were the prodromal PD relative to HC had reduced FC between the left putamen and clusters in the left pallidum, and right putamen and pallidum. **D-E.** Altered thalamo-cortical FC between IRBD and controls (Byun et al., 2020). **D.** Statistical maps of the regions where FC with the left thalamus was significantly increased in IRBD compared to the controls; **E.** The average FC value of left thalamus to right cuneus, left fusiform gyrus, and left lingual gyrus across controls and IRBD patients. Abbreviation: IRBD, idiopathic rapid eye movement sleep behavior disorder.

In 2017 Dayan and Browner took a new look at BG dysfunction in prodromal PD purposing a different approach that allowed them to study the BG as a network itself (Dayan and Browner, 2017). This work included patients suffering IRBD and/or hyposmia because it was focused on prodromal PD stages, therefore some caution should be taken in the generalization of these results to the IRBD population. This research revealed that relative to controls, the prodromal PD group presented reduced striato-thalamo-pallidal functional connectivity (**Figure 15A-C** and **Table 6**). Nonetheless, their technique was unable to reveal disrupted connectivity of the SN. More recently, using a seed to whole-brain approach Byun, et al. reported increased connectivity relative to HC between the left thalamus and posterior regions in IRBD patients (**Figure 15D-E**) (Byun et al., 2020). They interpreted such a result as a compensatory mechanism for cognitive impairment in IRBD.

In light of this evidence, Postuma (2016) put forward resting-state functional MRI (fMRI) as a new biomarker of prodromal neurodegeneration in PD (Postuma, 2016), which will probably enhance the number and quality of works that seek to attempt this topic.

One of the major drawbacks of these previous works is that they focused their analyses on previously selected regions including mainly the basal ganglia (Dayan and Browner, 2017; Ellmore et al., 2013; Rolinski et al., 2016a). Although their approaches are fully justified on the assumption that IRBD is a prodromal stage of PD, this raises many questions about functional whole-brain connectivity in IRBD, which until now have not been dealt with in-depth and remains an intriguing challenge.

Metabolic studies

Molecular imaging is a powerful instrument that helps to understand the underlying metabolic mechanisms of certain diseases. Positron emission tomography (PET) and SPECT are among the most widely used functional nuclear imaging techniques in neuroscience. Particularly, PET and SPECT have a longer tradition than fMRI research on the characterization of brain function in neurodegeneration. Currently, there is a considerable amount of literature using nuclear imaging in IRBD. In this subsection, a brief overview of those works that are somehow related to the framework of this Doctoral Thesis will be given (**Table 7** list the most important metabolic findings in IRBD). For a

recent systematic review and meta-analysis refer to Bauckneht and colleagues (**Figure 16**) (Bauckneht et al., 2018).

One of the major topics to be investigated in this field was the identification of dopaminergic abnormalities in IRBD, just as it has been widely identified in PD (Kaasinen and Vahlberg, 2017). In this sense, initial studies using dopaminergic imaging, reported dopamine deficit in IRBD (**Figure 17A**) (Albin et al., 2000; Eisensehr et al., 2000), with 20-40% of the IRBD patients showing abnormal DAT imaging relative to healthy individuals (Iranzo et al., 2010b; Kim et al., 2010). Of particular interest is the fact that compared to PD, IRBD patients had less prominent DAT deficit in the striatum (Arnaldi et al., 2015; Eisensehr et al., 2003; Zoetmulder et al., 2016), supporting the ongoing idea that IRBD may be a prodromal stage of PD.

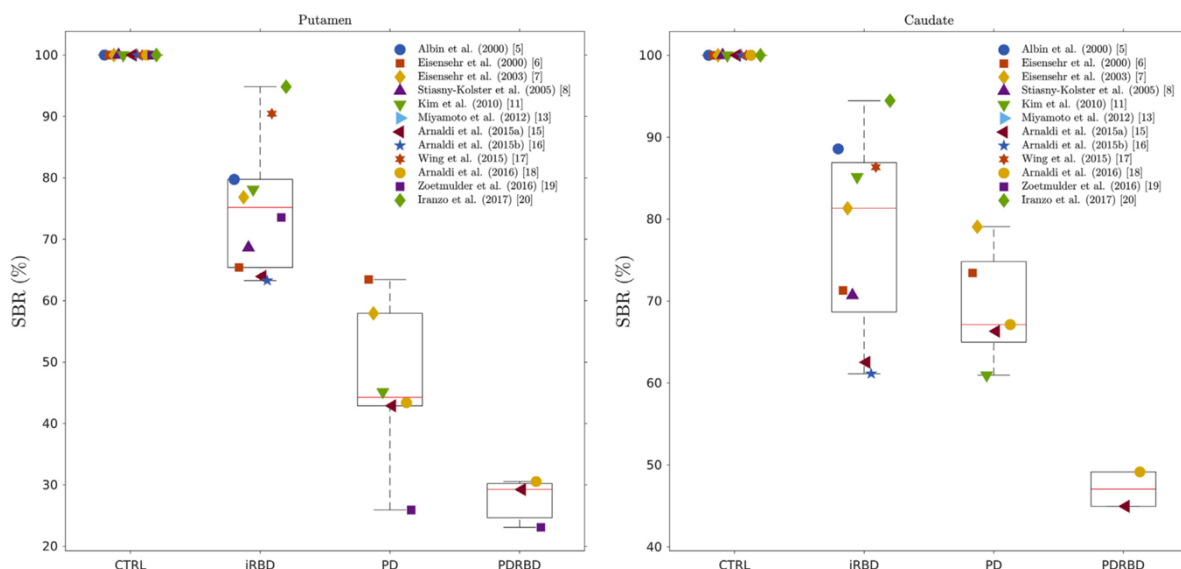


Figure 16. Box plots of PET/SPECT presynaptic dopaminergic standardized binding ratio of some selected studies, expressed as percentages with respect to each study's own healthy cohort (Bauckneht et al., 2018).

Much work on the potential of nuclear MRI has been carried out to reveal brain perfusion changes in IRBD compared to healthy individuals. Broadly, previous literature showed that relative to healthy controls, IRBD patients had increased blood flow in subcortical regions, namely the pons and putamen (Holtbernd et al., 2014; Mazza et al., 2006; Vendette et al., 2011), the cerebellum (Holtbernd et al., 2014), and hippocampus (Ge et al., 2015; Mazza et al., 2006; Vendette et al., 2012, 2011); whereas decreased regional flow was detected in frontal (Ge et al., 2015; Hanyu et al., 2011; Mazza et al., 2006; Vendette et al., 2012, 2011), temporal, parietal (Ge et al., 2015; Hanyu et al., 2011; Mazza et al., 2006;

Sakurai et al., 2014; Vendette et al., 2012, 2011) and occipital (Sakurai et al., 2014) cortices (**Figure 17B-D**). Overall these studies suggested that region-specific metabolic abnormalities exist in IRBD patients, affecting both subcortical and cortical structures.

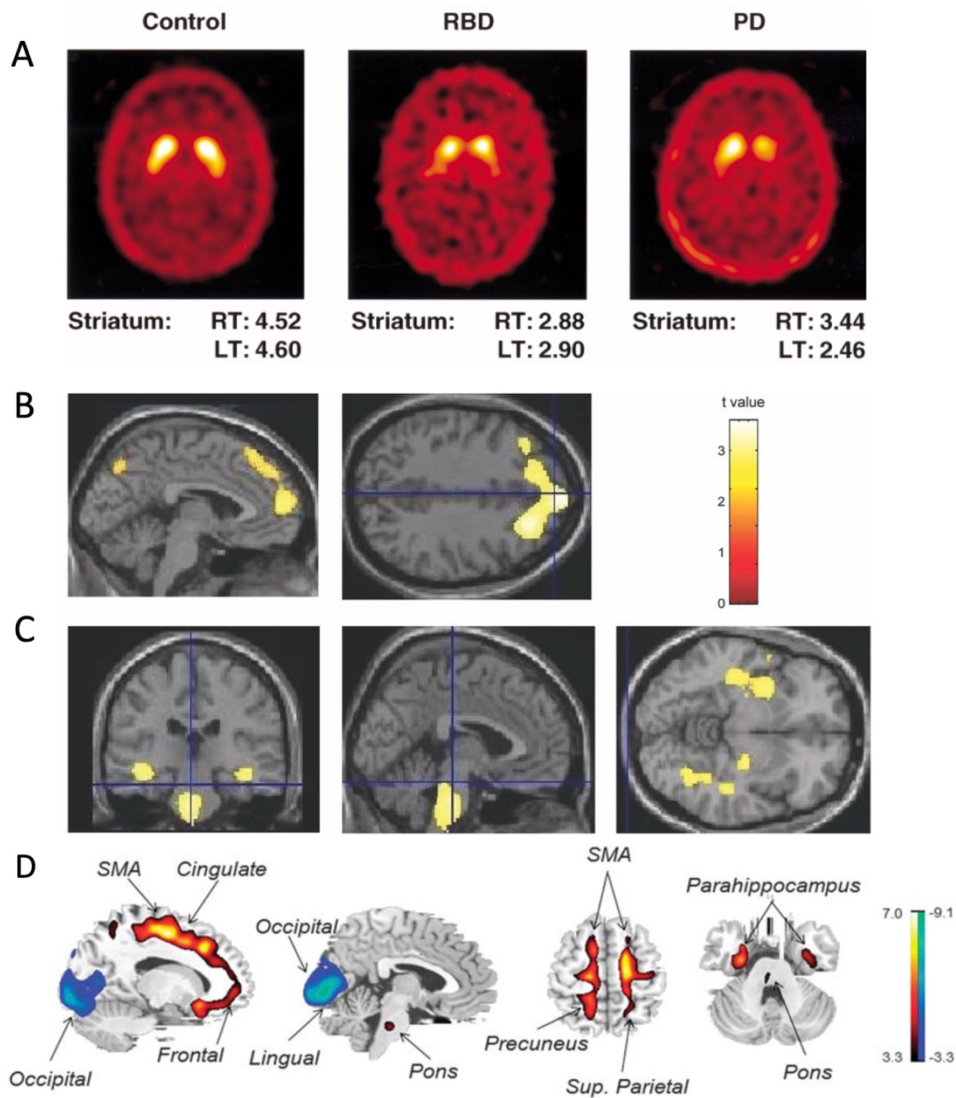


Figure 17. Metabolic changes in IRBD. **A.** Preliminary study showing dopamine deficit in Idiopathic rapid eye movement sleep behavior disorder (IRBD). The image shows the [^{123}I]IPT-SPECT of one patient with IRBD, one with PD and one healthy control (HC). Note the bilaterally reduced IPT binding ratio in the IRBD patient, whereas in the PD patient the reduction is asymmetrical, being more pronounced contralateral to the symptomatic body side of the patient (Eisensehr et al., 2000). RT, right; LT, left; **B.** IRBD patients had hypoperfusion in comparison to HC (Vendette et al., 2011); **C.** hyperperfusion in IRBD relative to HC group (Vendette et al., 2011); **D.** Normalized glucose metabolism in the IRBD patients increased (red) and decreased (blue) relative to HC (Ge et al., 2015).

Most neurodegenerative disorders are characterized by specific glucose metabolic patterns, commonly detected with ^{18}F -FDG-PET brain imaging (Brown et al., 2014). In keeping with the specific pattern of altered glucose metabolism found in PD (Eidelberg,

2009; Eidelberg et al., 1994; Teune et al., 2014, 2013); it has been identified a metabolic pattern underlying IRBD (Meles et al., 2018; Wu et al., 2014). This so called IRBD-related pattern (IRBDRP) (**Figure 18A-B**) has been also recognized using ^{18}F -FDG-PET (Meles et al., 2018; Wu et al., 2014). The IRBDRP is characterized by hypermetabolism in the cerebellum, brainstem, thalamus, sensorimotor cortex and hippocampus (Meles et al., 2018; Wu et al., 2014), and by hypometabolism in the middle cingulate, parietal (Meles et al., 2018), temporal and occipital (Meles et al., 2018; Wu et al., 2014) cortices. Interestingly, this IRBDRP partially overlaps with the PD-related pattern (**Figure 18C-D**) (Meles et al., 2018). Suggesting that IRBDRP may be an early manifestation of the PD-related pattern (PDRP) (Meles et al., 2018) and thus, may be useful to identify those at higher risk to develop a parkinsonism disorder (Wu et al., 2014). Further longitudinal research on this topic is needed to determine how the IRBDRP change throughout the disease.

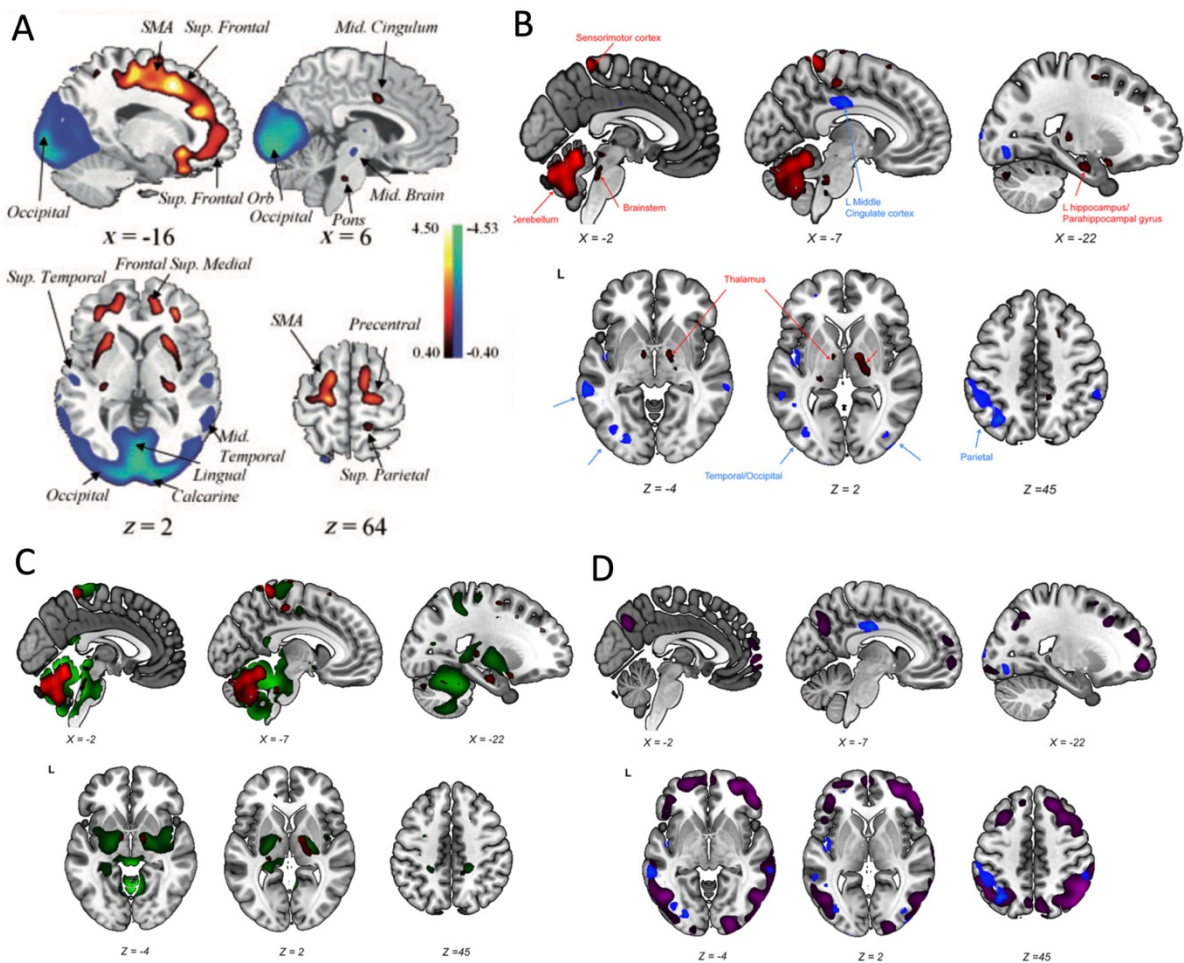


Figure 18. A-B show IRBD-related pattern (IRBDRP) identified in ^{18}F -FDG PET scans from IRBD and healthy individuals using spatial covariance analysis. Voxel with positive regions weights (hypermetabolism) are color-coded red; those with negative region weights (hypometabolism)

are color-coded blue. **A.** IRBDRP identified in Wu and colleagues (2014); **B.** IRBDRP identified in Meles and colleagues (2018); **C.** IRBDRP and PDRP overlap, relatively hypermetabolic regions of PDRP (green) and IRBDRP (red) (Meles et al., 2018); **D.** IRBDRP and PDRP overlap, relatively hypometabolic regions of PDRP (purple) and IRBDRP (blue) (Meles, et al., 2018).

The evidence of molecular imaging research in IRBD points out the striatal dopaminergic deficit and cortical and subcortical functional abnormalities that are consistent with the metabolic PD-like pattern.

Iron deposition

The increased iron deposition has been consistently reported to occur in parkinsonism disorders, and it is thought to contribute to the damage of dopaminergic neurons in this disease (Berg and Hochstrasser, 2006). In light of this evidence, the scientific community has hypothesized that this increment in brain iron deposition will probably be present in PD prodromal stages. There is still considerable uncertainty with regards to this topic since while some preliminary research demonstrated increase iron deposition in IRBD relative to controls (Sun et al., 2019), others did not identify any significant result (J. H. Lee et al., 2014). Interestingly, one of these previous works found that PD patients had elevated iron levels in the SN in comparison to IRBD (Sun et al., 2019).

Longitudinal studies

Owing to a recent multicentric study, there is robust evidence on the phenoconversion rate in IRBD. They determined that the estimated 12-year risk of converting to an overt neurodegenerative disease is 73.5% (Postuma et al., 2019). For this reason, longitudinal data in individuals suffering from IRBD is generating considerable interest, mainly in terms of detecting those at-risk patients (for a systematic review and meta-analysis of longitudinal data refer to (Galbiati et al., 2019b)). Despite decades of research, few works have examined brain markers that enable to predict those patients that will eventually convert to a defined parkinsonism disorder.

In 2010, Iranzo et al. carried out the first longitudinal work using neuroimaging. After 2.5 years follow-up, those IRBD patients that had pathological dopamine transporter (DAT) binding in the striatum at baseline, showed an increased risk for the development of Synucleinopathies (Iranzo et al., 2010b). The impact of this preliminary contribution lied in the possibility of detecting subclinical changes in IRBD that might be useful markers to

identify individuals at increased risk of phenoconversion. Shortly thereafter, additional works found out other prognostic biomarkers. For instance, larger increased hippocampal perfusion (Dang-Vu et al., 2012) latent abnormalities in the PDRP expression (Holtbernd et al., 2014; Yoon et al., 2019), and bilateral putaminal activity (Iranzo et al., 2017a; Miyamoto et al., 2020), were all able to predict a subsequent phenoconversion to a defined parkinsonism in IRBD.

More recently, Pereira and co-workers (Pereira et al., 2019) reported that IRBD patients that eventually convert to a Lewy body disease had widespread thinning in the left superior frontal, right precentral and right lateral occipital gyri compared to non-converters, without differences in subcortical regions (**Figure 19**). One of the main issues of this study is that they did not include MRI follow-up for the control group, which does not allow answering the question of how different brain changes are in IRBD over time in comparison to normal aging.

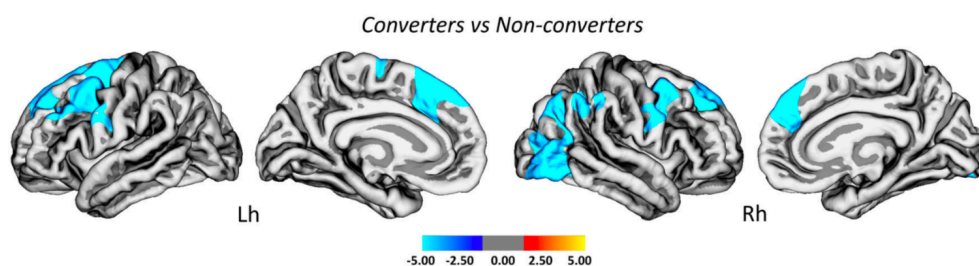


Figure 19. Cortical thinning in IRBD patients that converted to a Lewy body disorder compared to non-converters. Vertex-wise comparisons of cortical thickness between patients with idiopathic REM sleep behavior disorder (IRBD) that progressed to a Lewy body disorder at follow-up (converters) compared to IRBD patients that remained disease free (nonconverters). The color scale bar shows the logarithmic scale of p values ($-\log_{10}$). Lh, left hemisphere, Rh right hemisphere (Pereira et al., 2019).

Overall, previous longitudinal data highlight that metabolic abnormalities and thinning in frontal and occipital cortices in IRBD are associated with a greater likelihood of developing a presumed Synucleinopathy. Although this research has illuminated promising results, there is still a need for extensive longitudinal studies using neuroimaging techniques.

Neural correlates

In this subsection, an overview of the neural substrates underlying cognitive impairment in IRBD will be given. A summary of cognitive and clinical neural correlates is presented

in **Table 8**. Neuroanatomical correlates of olfactory dysfunction will be further addressed in the next section (**1.2.3. Neural correlates of olfactory dysfunction**), where olfactory function in IRBD will be thoroughly reviewed.

Brain correlates of clinical features

Previous research has aimed at seeking neuroanatomical and neurofunctional bases of clinical manifestations in IRBD. In this context, RBD duration has been associated mainly with frontal (Ge et al., 2015; Rahayel et al., 2018a), posterior (Ge et al., 2015), and cerebellar (Ge et al., 2015) changes. Regarding muscle activity during REM sleep (Bedard et al., 2019; Dušek et al., 2019; Rahayel et al., 2018a; Zoetmulder et al., 2016) and % of REM sleep without atonia (Ehrminger et al., 2016), it has been shown an association with cholinergic alterations in the mesopontine and paracentral areas (Bedard et al., 2019), DAT binding in the putamen (Dušek et al., 2019; Zoetmulder et al., 2016), caudate and fusiform atrophy (Rahayel et al., 2018a) and lower neuromelanin signal of the locus coeruleus/subcoeruleus (Ehrminger et al., 2016). As for neuropsychiatric symptoms, a recent study proposes caudate, left calcarine, and right cuneus atrophy to underly depression in IRBD patients (Bourgouin et al., 2018).

Motor status in IRBD has been related to hippocampal perfusion (Dang-Vu et al., 2012), caudate and cerebellum volumes (Rahayel et al., 2018a), and frontal and fusiform thinning (Pereira et al., 2019). Interestingly, when motor output was assessed more thoroughly, slower finger tapping of the right hand was associated with cortical thinning in the right paracentral and superior parietal lobule cortices, and bilaterally with the pallidum. Whereas in the left hand correlated with cortical thinning in the right postcentral and superior parietal lobule cortices (Rahayel et al., 2018b). In their recent work, Yamada, et al. (2019) examined functional connectivity abnormalities in sensorimotor regions underlying mild motor impairment in IRBD. They evidenced that in comparison to IRBD with normal motor function, patients with mild motor impairment showed increased connectivity between the cerebellum and primary somatosensory and premotor cortices. They hypothesize that increased cerebellar-cortical functional connectivity may represent compensation for mild nigrostriatal dopaminergic dysfunction (Yamada et al., 2019).

Taken as a whole, these previous findings highlight that the IRBD clinic is better understood when we look at different kinds of neuroimaging techniques, identifying the important role of regions such as the premotor, somatosensory, and posterior cortices, the hippocampi, cerebellum, and striatal regions.

Brain correlates of cognitive impairment in IRBD

Because there is extensive evidence of cognitive impairment in RBD (see subsection **1.1.3 Neuropsychological impairment in IRBD**), there is a growing interest in investigating the substrates underlying cognitive impairment in these patients. However, few researchers have addressed this question.

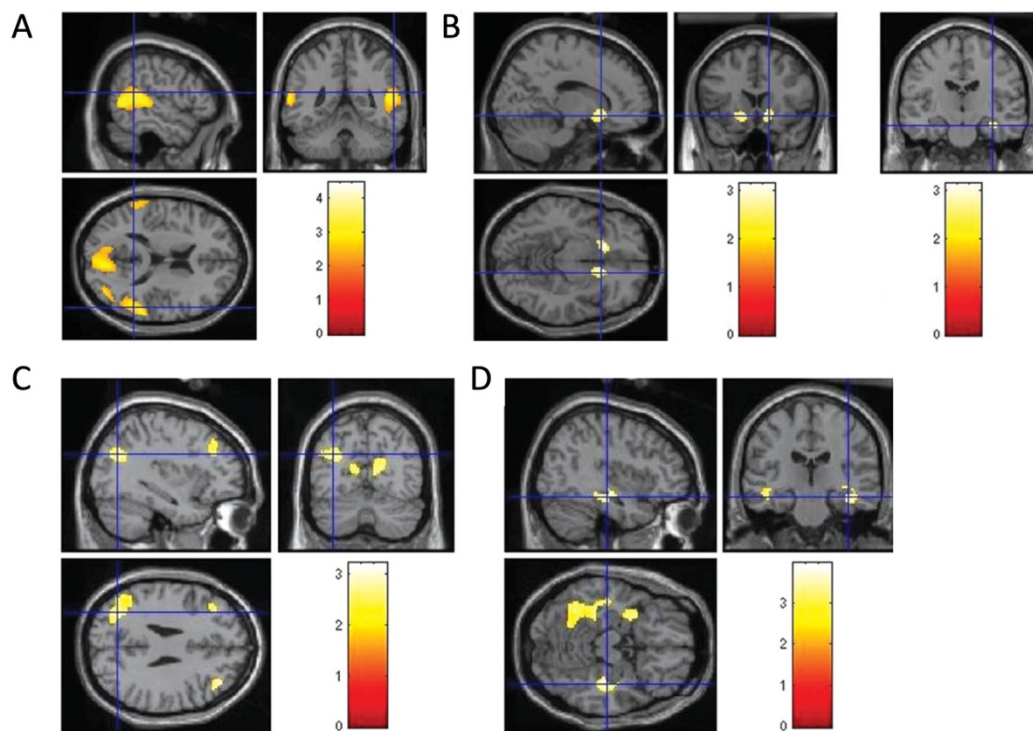


Figure 20. Regional cerebral blood flow (rCBF) characterization in IRBD patients with mild cognitive impairment (IRBD-MCI) and IRBD patients without MCI (IRBD-NoMCI). **A.** Brain areas in which rCBF is lower in IRBD-MCI than in IRBD-NoMCI patients; **B.** Brain areas in which rCBF is higher in IRBD-MCI than in IRBD-NoMCI patient; **C.** Brain areas in which rCBF is lower in IRBD-MCI patients than in controls; **D.** Brain areas in which rCBF is higher in IRBD-MCI patients than in controls (Vendette et al., 2012).

What we know about brain abnormalities in IRBD patients with MCI (IRBD-MCI) is largely based on two previous studies. Back in 2012, Vendette and colleagues (2012), reported cortical hypoperfusion in the occipital, temporal, and parietal regions in IRBD-MCI compared with controls, and IRBD without cognitive impairment. Moreover, IRBD-MCI

showed increased hyperperfusion in the right hippocampus, the putamen, and the left paracentral gyrus (**Figure 20**) (Vendette et al., 2012). They concluded that this specific posterior cortical pattern in IRBD-MCI was similar to that found in DLB and PD with dementia. Subsequent work focused on structural data, found out that IRBD-MCI had cortical thinning in the frontal, cingulate, temporal, the insula and occipital cortices, and abnormal surface contraction in the putamen and thalamus. On the other hand, patients without MCI had cortical thinning restricted to the frontal cortex. It is worth mentioning that thinning in the right middle temporal cortex was able to discriminate (AUROC= 0.79) those patients having MCI from those with normal cognition (**Figure 21**) (Rahayel et al., 2018b).

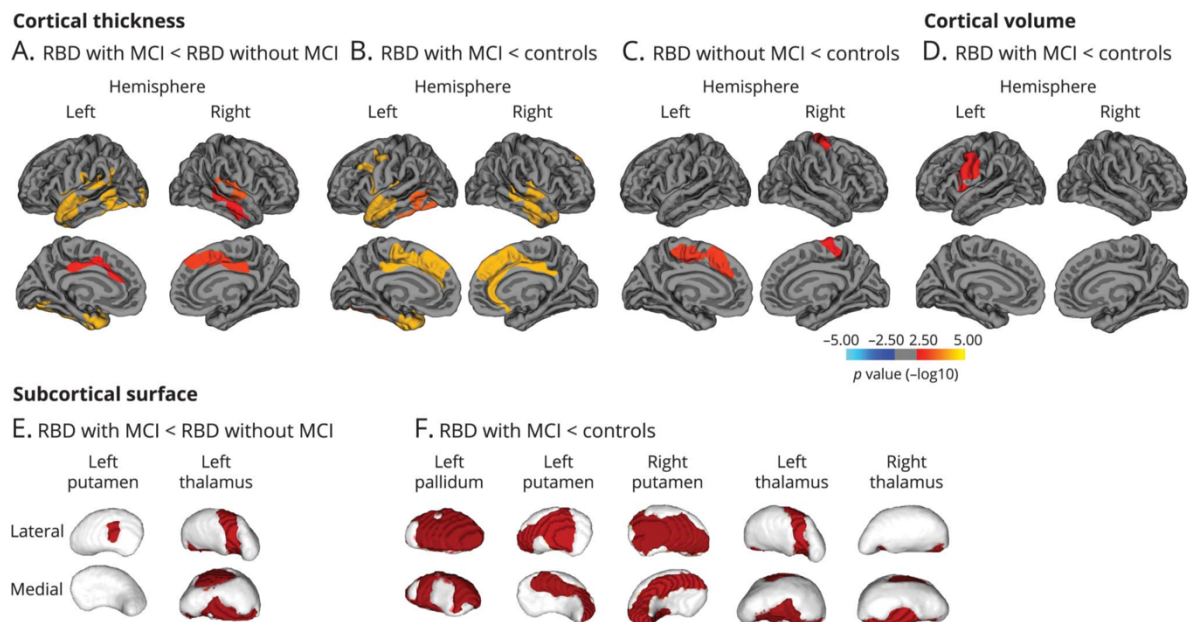


Figure 21. Cortical and subcortical abnormalities in IRBD patients with MCI, IRBD patients without MCI, and controls. **A-C** show differences in cortical thickness (see specific contrasts in the figure). **D.** Decreased cortical volume in IRBD patients with MCI compared to controls. The color bar indicates the logarithmic scale of p values ($-\log_{10}$) for between-group differences, with red-yellow areas representing reductions in the first compared to the last group in the contrast **E.** Compared to IRBD patients without MCI, IRBD patients with MCI showed abnormal contraction in the left putamen and thalamus **F.** IRBD patients with MCI also had abnormal contraction compared to controls in the bilateral putamen and thalamus and the left pallidum (Rahayel et al., 2018b).

As for specific cognitive domains, works addressing color perception correlates in IRBD (**Figure 22**), found that it was associated with the hippocampal region (Dang-Vu et al., 2012), frontal and occipital perfusion (Vendette et al., 2011), and with GM changes in the right cuneus and hippocampus (Rahayel et al., 2018b). Additionally, lower performance

in visuospatial functions (**Figure 23A-B**) was associated with thinning in the frontal, insular (Rahayel et al., 2018b), temporal, parietal and occipital cortices (Pereira et al., 2019; Rahayel et al., 2018b), and with the right hippocampus (Rahayel et al., 2018b).

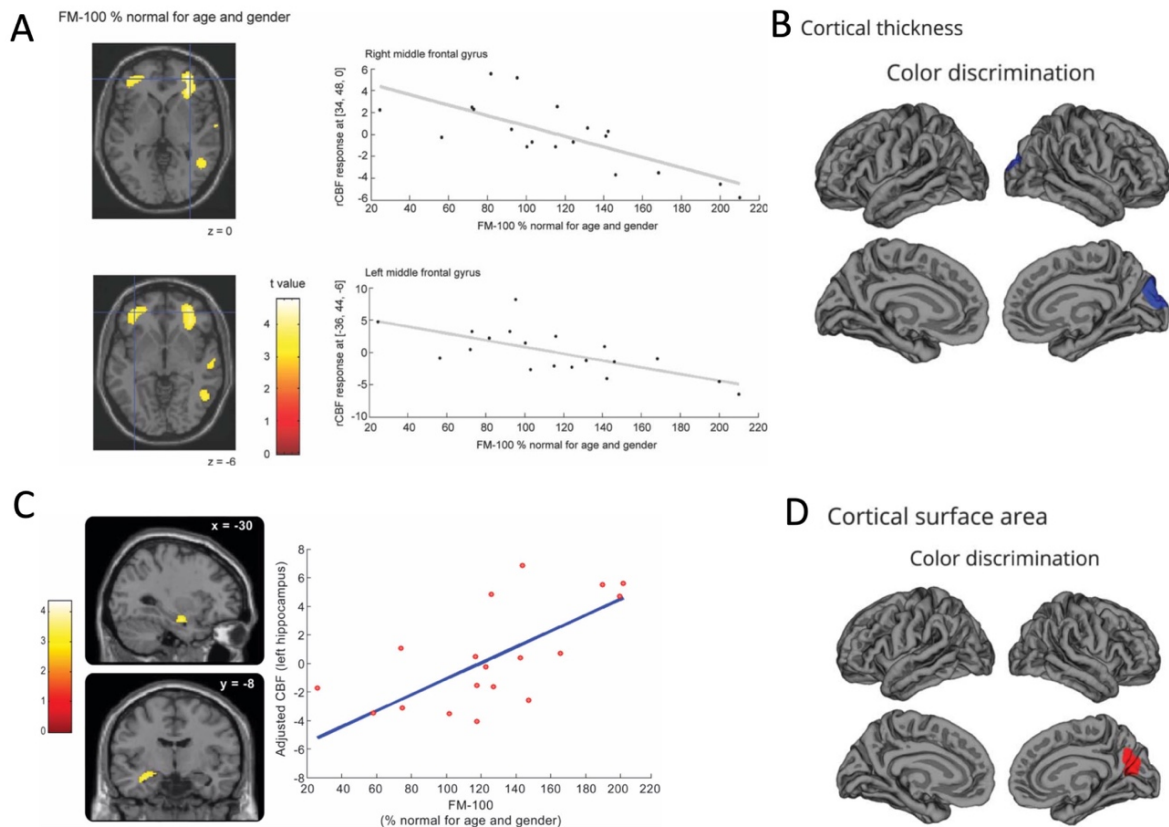


Figure 22. Color perception measured by Farnsworth-Munsell 100 Hue Test scores (FM-100) neuronal correlates. **A.** Correlation between brain perfusion in the middle frontal gyrus and FM-100 score in IRBD (Vendette et al. 2011); **B.** Correlation between cortical thickness and FM-100 scores (Rahayel et al., 2018b); **C.** Brain perfusion in the left hippocampus was correlated with FM-scores across IRBD patients (Dang-Vu et al., 2012); **D.** Increased cortical surface area was associated with lower performance in color discrimination (Rahayel et al., 2018b).

With regard to attention and executive functions, impairment in this domain correlated with thinning in the frontal, temporal and occipital cortices (Rahayel et al., 2018b) (**Figure 23A**) as well as with metabolic changes in PD-like regions (Yoon et al., 2019). On learning and memory, lower performance was associated with cortical thinning in the frontal (Pereira et al., 2019), temporal, insular, and occipital (Pereira et al., 2019; Rahayel et al., 2018b) cortices, with changes in right hippocampus (Rahayel et al., 2018b) (**Figure 23A-B**), with higher thalamic-fusiform connectivity (Byun et al., 2020) and with reduced striatal dopamine uptake (Bezdicek et al., 2018).

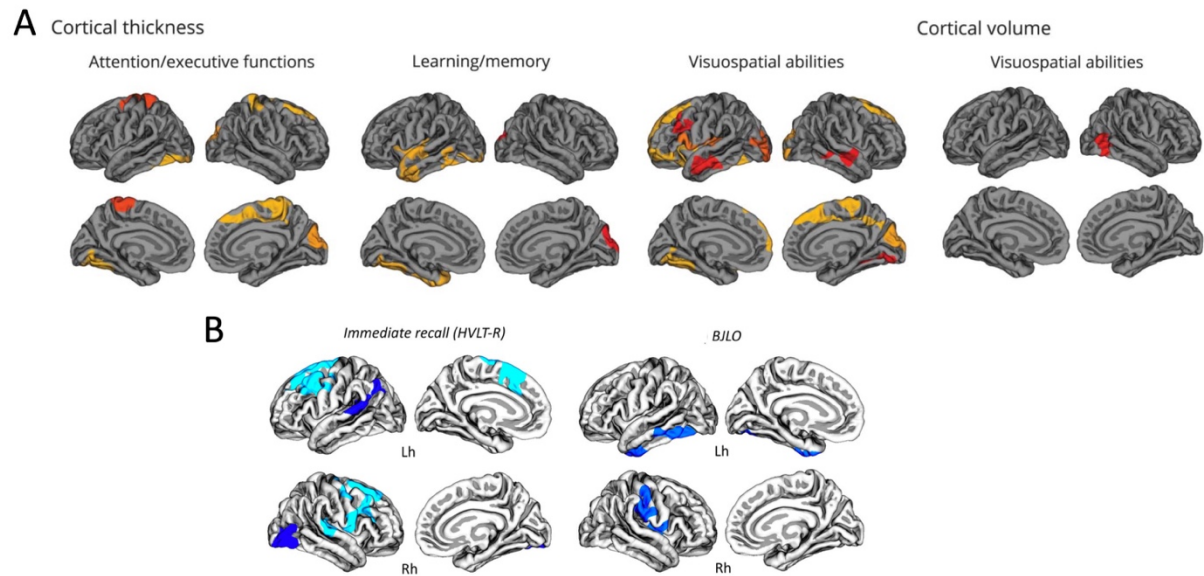


Figure 23. Cortical abnormalities are associated with cognitive scores in patients with IRBD. **A.** Cortical thinning was associated with lower performance in attention and executive functions, learning and memory and visuospatial abilities (Rahayel et al., 2018b); **B.** Cortical thickness was associated with immediate recall and visuospatial abilities (Pereira et al., 2019).

Although only some studies are available, the evidence points out that changes in posterior brain regions are reflecting cognitive dysfunction in IRBD-MCI. This conclusion arose from Rahayel et al. (Rahayel et al., 2018b) who suggested that most of the markers of neurodegeneration in IRBD are associated with occipital abnormalities stressing that posterior region may have an important role in IRBD phenoconversion.

Summary

Altogether, structural neuroimaging in IRBD identified cortical thinning in the cingulate cortex, the lateral prefrontal and somatosensorial, and motor areas, as well as subcortical GM loss mainly in putamen, brainstem, and pallidum. With only one work focused on hippocampal structure, finding increased volume. As for WM, abnormalities are shown in the pons, the SN, and in olfactory regions. Functional imaging, highlights disrupted functional connectivity within the BG and also in cortico-striatal networks in IRBD. Nevertheless, whole-brain connectivity remains unexplored. SPECT and PET imaging showed striatal dopaminergic deficit, as well as cortical and subcortical metabolic abnormalities with a PD-like pattern.

Cognitive impairment in IRBD is characterized by posterior cortical pattern and hippocampal hypermetabolism. Longitudinal research demonstrated that decrease DAT binding in the striatum, increased perfusion in the hippocampus, and thinning in the left superior frontal, right precentral, and right lateral occipital gyri are associated with clinical progression to an overt Synucleinopathy.

Table 5. Studies of structural changes in IRBD

Reference	Sample	MRI analysis	Contrast direction	Brain regions
Ellmore et al., 2010	5 IRBD 5 PDnovo 7 HC	Subcortical volumetry	HC>IRBD	Volume decrease in bilateral putamen
			PDnovo>IRBD	Volume decrease in L putamen
Unger et al., 2010	12 IRBD 12 HC	DTI	HC>IRBD	↓AD Pons, bilateral corona radiata and the R SN ↓FA Fornix, the R visual stream and the L superior temporal lobe
			HC<IRBD	↑FA bilateral internal capsule and olfactory regions ↑RD Fornix, the R visual stream, and the L superior temporal lobe
			HC>IRBD	↓FA mesencephalic tegmentum and the rostral pons
Scherfler, et al., 2011	26 IRBD 14 HC	DTI		↑MD pontine tegmentum and reticular formation
		VBM	HC<IRBD	Bilateral hippocampi and parahippocampal gyrus GM density
Hanyu et al., 2012	20 IRBD 18 HC	VBM	HC>IRBD	GM volume decrease in R and L anterior lobes of the cerebellum, tegmental portion of the pons and L parahippocampal gyrus
Rahayel et al., 2014	24 IRBD 42 HC	Gth		- Cortical thinning in the R medial frontal lobe, R lingual and fusiform gyri.
		VBM	HC>IRBD	- GM volume decrease in the R superior frontal sulcus
		DTI		- No differences in WM measures
De Marzi et al., 2016	15 IRBD 42 HC 104 PD	SWI	HC>IRBD	Loss of dorsolateral nigral hyperintensity
Ehrminger et al., 2016	21 IRBD 21 HC	Neuromelanin (ROI coeruleus-subcoeruleus)	HC>IRBD	↓ signal intensity in locus coeruleus/subcoeruleus complex
Pyatigorskaya et al., 2017	19 IRBD 18 HC	DTI Neuromelanin (ROI in the SN)	HC>IRBD	↓FA in the SN ↓ signal intensity in the SN
Rahayel et al., 2018a	41 IRBD 41 HC	Cortical thickness VBM		- Cortical thinning in the bilateral medial superior frontal, orbitofrontal, anterior cingulate cortices and the R dorsolateral primary motor cortex
		Shape analysis Subcortical Volumetry	HC>IRBD	- GM volume decrease in the frontal lobes, anterior cingulate gyri caudate nucleus - Surface contraction in the L pallidum - GM volume loss in R putamen

Reference	Sample	MRI analysis	Contrast direction	Brain regions
Rahayel et al., 2018b	52 IRBD 41 HC	Cortical thickness VBM Shape analysis	HC> IRBD-MCI	- Cortical thinning in the temporal, frontal and cingulate - GM volume decrease in precentral and postcentral cortex - Surface contraction in the L pallidum and bilateral putamen and thalamus
			HC> IRBD-NMCI	Cortical thinning in the frontal cortex (bilateral paracentral, R precentral cortex and L superior medial frontal)
			IRBD-NMCI>IRBD-MCI	- Cortical thinning in the frontal, cingulate, temporal and occipital cortices - Surface reduction in the L putamen and thalamus
Ohlhauser et al., 2019	20 proPD [‡] 17 cPD [‡] 21 HC [‡]	DTI	IRBD>cPD	↑MD R corpus callosum, internal/external capsule, superior and inferior longitudinal fasciculus, the corticospinal tract, forceps major, corona radiata and posterior thalamic radiation
			HC>IRBD	Cortical thinning in the L lateral occipital and postcentral gyri, inferior parietal and supramarginal areas
Pereira et al., 2019	27 IRBD [‡] 151 PDnovo [‡] 31 HC [‡]	Cortical thickness	HC>IRBDc	Bilateral frontal precentral and occipital areas
			IRBDnc>IRBDc	Cortical thinning in L superior frontal, R precentral and lateral occipital gyri
			HC>IRBD	L Pars opercularis, R isthmus cingulate cortex
K. M. Park et al., 2018	10 IRBD 14 HC	GM volumetry	HC<IRBD	R orbitofrontal, rostral middle frontal and caudate, and L Thalamus
			HC<IRBD	
Sun et al., 2019	25 IRBD 32 PD 50 HC	Iron deposition (subcortical ROIs)	HC<IRBD	↑iron bilateral SN
			PD>IRBD	↑iron bilateral SN

Abbreviations: AD, axial diffusivity; cPD, clinical PD; DTI, Diffusion-tensor imaging; FA, fractional anisotropy; HC, healthy control; IRBD, idiopathic REM sleep behavior disorder; IRBDc, IRBD patients that converted to a Lewy body disorder; IRBD-MCI, IRBD with mild cognitive impairment; IRBDnc, IRBD patients that did not phenoconvert; IRBD-NMCI, IRBD without mild cognitive impairment; MD, mean diffusivity; L, left; PD, Parkinson's disease; PDnovo, PD de novo patient; proPD, prodromal PD patients; R, right; RD, radial diffusivity; ROI, region of interest; SN, substantia nigra; SWI, susceptibility-weighted imaging; VBM, voxel-based morphometry. [‡] Parkinson's Progressions Markers Initiative data (<https://www.ppmi-info.org>).

Table 6. Studies of functional and structural connectivity in IRBD

Reference	Sample	MRI analysis	Contrast direction	Brain regions
Functional connectivity				
Ellmore et al., 2013	10 IRBD	Seed-to-whole brain (SN as seeds)	HC>IRBD	↕FC between L SN and L putamen
	11 PD		IRBD>PD	↕FC between L SN and L putamen
	10 HC		HC<IRBD	↕FC between R SN and R superior occipital gyrus
			IRBD<PD	↕FC between R SN and R superior occipital gyrus
Rolinski et al., 2016a	26 IRBD 48 PD 23 HC	Resting state fMRI (Basal ganglia network)	HC>IRBD HC>PD	↕coactivation within the BG and with frontal regions (cingulate and paracingulate gyri, the orbital cortices and the inferior and middle frontal gyri)
Dayan et al., 2017	17 proPD‡ 18 HC‡	ROI-to-ROI of the BG (NBS) Seed-to-whole brain (Putamen as seed)	HC> proPD	↕striato-thalamo pallidal FC ↕FC between the L putamen and bilateral pallidum, putamen and caudate regions; and between the R putamen and contralateral putamen & pallidum
Yamada et al., 2019	23 IRBD 20 HC	ROI-to-ROI (sensorimotor regions)	HC>IRBD-MMI	↕FC between the R/L anterior putamen and R superior parietal lobule; and between L caudate and R superior parietal lobule
			HC>IRBD-nMI	↕FC between the putamen and R superior parietal lobule
			IRBD-MMI>IRBD-nMI	↕FC between the cerebellum and primary somatosensory and premotor cortices
Byun et al., 2020	37 IRBD 15 HC	Seed-to-voxel analysis (Thalami as seeds)	HC<IRBD	↕FC between the L thalamus and occipital regions (R cuneus, L fusiform and lingual gyri)
Structural connectivity				
Wen et al., 2017	20 proPD‡ 106 PDnovo‡ 55 HC‡	Deterministic tractography ROI-to-ROI whole brain (NBS) Graph analysis	HC<proPD	↕small worldness and cluster coefficient ↕connectivity between the R supplementary motor area and R putamen, and between the L parahippocampal gyrus and L cerebellum
			PDnovo<proPD	↕small worldness and cluster coefficient
				↕connectivity between the L middle frontal gyrus and L inferior frontal gyrus, between the L parahippocampus and L amygdala, and between the R middle frontal gyrus and R middle temporal gyrus
				10 IRBD 14 HC
HC<IRBD	↕Characteristic path length ↕BC bilateral caudate and several regions in the frontal cortex			

Abbreviations: BG, basal ganglia; FC, functional connectivity; fMRI, functional MRI; HC, healthy control; IRBD, idiopathic REM sleep behavior disorder; IRBD-MMI, IRBD with mild motor impairment; IRBD-nMI, IRBD without mild motor impairment; L, left; NBS, network-based statistics; PD, Parkinson's disease; PDnovo, PD de novo; proPD, prodromal PD (IRBD or/and hyposmia); R, right; SN, substantia nigra; ROI, region of interest. ‡PPMI data (<https://www.ppmi-info.org>).

Table 7. Metabolic studies in IRBD

Reference	Sample	Technique/Tracer	Contrast direction	Brain regions
PET				
Albin et al., 2000	6 RBD 19 HC	[¹¹ C]DTBZ-PET	HC>IRBD	↓ striatal binding in the posterior putamen
Wu et al., 2014	21 IRBD	[¹⁸ F]FDG-PET	RBD related pattern (IRBD vs HC)	↑ the pons, thalamus, supplementary motor area, medial frontal and sensorimotor areas, hippocampus, parahippocampal, supramarginal and inferior temporal gyri, and posterior cerebellum
	21 HC			↓ in occipital and superior temporal regions
	21 PDearly			↑ the pons, globus pallidus, putamen, thalamus
	16 PDmoderate			PDRP expression associated to higher risk of conversion
Holtbernd et al., 2014*	10 RBD 10 HC	[¹⁸ F]FDG-PET in PDRP regions	HC<IRBD	
Ge et al., 2015	21 IRBD 21 HC	[¹⁸ F]FDG-PET	Longitudinal findings	
	HC<IRBD		↑ the hippocampus/parahippocampus, cingulate, supplementary motor area and pons	
	HC>IRBD		↓ the occipital cortex/lingual gyrus	
Meles et al., 2017	21 IRBD	[¹⁸ F]FDG-PET	HC<IRBD	
	19 HC		IRBD<PD	PD related pattern
	20 PD			
	22 DLB		IRBD<DLB	
Meles et al., 2018	Cohort A (21 IRBD, 19 HC)	[¹⁸ F]FDG-PET	IRBD related pattern	↑ Cerebellum, brainstem, thalamus, sensorimotor cortex, and hippocampus
	Cohort B (19 IRBD, 13 HC)			↓ Middle cingulate, temporal, occipital, and parietal cortex
	Cohort C (38 PD, 44 HC)		Overlap IRBD and PD patterns	Both the PDRP and the IRBDRP increased across groups (HC<IRBD<PD)
			HC>IRBD IRBD>PD	Putamen
Knudsen et al., 2018	21 IRBD 10 PD 18 HC	[¹⁸ F]DOPA	HC>IRBD IRBD>PD	
Lee et al., 2019	30 IRBD	[¹⁸ F]FP-CIT-PET	HC>IRBD	Left posterior putamen and bilateral substantia nigra
	30 PD drug naïve 19 HC		IRBD>PD	Bilateral caudate, anterior and posterior putamen and SN
Bedard et al., 2019	5 IRBD 5 HC	[¹⁸ F]FEOBV	HC<IRBD	Brainstem, ventromedial thalamus, cerebellum, paracentral lobule, anterior cingulate and orbitofrontal cortex

Reference	Sample	Technique/Tracer	Contrast direction	Brain regions
Liguori et al., 2019	54 IRBD 28 PD 10 DLB 55 AD 35 HC	$[^{18}\text{F}]\text{FDG-PET}$	HC>IRBD	Precuneus, superior parietal lobule and middle temporal gyrus
			HC<IRBD	Brainstem and temporal and limbic lobe and parietal cortex
			IRBD>PD	Midbrain and pons, cerebellum, lentiform nucleus and claustrum, and fronto-temporal lobes
			IRBD>DLB	Temporal, parietal and occipital lobes
			IRBD<DLB	Frontal and limbic lobes
			IRBD<AD	Limbic, temporal and parietal lobes
Yoon et al., 2019	28 IRBD 21 PDRBD 24 HC	$[^{18}\text{F}]\text{FDG-PET}$	IRBD>PDRBD	Caudate, and anterior and posterior putamen
Miyamoto et al., 2020*	11 IRBDc 13 IRBDnc	FMT-PET	IRBDc<IRBDnc	Bilateral putamen
SPECT				
Eisensehr et al., 2000	5 IRBD 14 PD 7 HC	$[^{123}\text{I}]\text{IBZM}$ $[^{123}\text{I}]\text{IPT}$	HC>IRBD	Reduced striatal $[^{123}\text{I}]\text{IPT}$ binding
Stiasny-Kolster et al., 2005	11 IRBD 10 HC	$[^{123}\text{I}]\text{FP-CIT}$	HC>IRBD	No significant
Mazza et al., 2006	8 IRBD 9 HC	$^{99\text{m}}\text{Tc-ECD}$	HC>IRBD	Frontal and temporo-parietal cortices
			HC<IRBD	Pons and putamen bilaterally and in the R hippocampus
Iranzo et al., 2010b*	43 IRBD 18 HC	$[^{123}\text{I}]\text{FP-CIT}$	HC>IRBD	Striatum
			Longitudinal findings	Tracer uptake reduction identify IRBD at short-term risk of conversion
			HC>IRBD	Putamen
Kim et al., 2010	12 IRBD 14 PD 12 HC	$[^{123}\text{I}]\text{FP-CIT}$	IRBD>PD	Striatum
			IRBD<HC	Striatum
Iranzo et al., 2011	20 IRBD 20 HC	$[^{123}\text{I}]\text{FP-CIT}$	Longitudinal findings	Progressive nigrostriatal dopaminergic dysfunction in IRBD
Hanyu et al., 2011	24 IRBD 18 HC	IMP-SPECT	HC>IRBD	Precuneus, limbic lobe and cerebellum
Vendette et al., 2011	20 IRBD 20 HC	$^{99\text{m}}\text{Tc-ECD}$	HC>IRBD	Frontal cortex and in medial parietal areas
			HC<IRBD	Bilateral pons, putamen, and hippocampus

Reference	Sample	Technique/Tracer	Contrast direction	Brain regions
Vendette et al., 2012	10 IRBD-MCI	^{99m} Tc-ECD	HC>IRBD	Frontal cortex
	10 IRBD-NMCI		HC>IRBD-MCI	Occipital, temporal and parietal
	20 HC		IRBD-NMCI>IRBD-MCI	
			HC<IRBD	R hippocampus and parahippocampal gyri
			HC<IRBD-MCI	Putamen and the left paracentral gyrus
Dang-Vu et al., 2012*	10 IRBDnc 10 IRBDc	^{99m} Tc-ECD	IRBDnc < IRBDc	L hippocampus (at baseline)
Sakurai et al., 2014*	19 IRBD 18 HC	IMP-SPECT	IRBD ₀ >IRBD _{F-up}	R posterior cingulate
Holtbernd et al., 2014*	17 RBD 17 HC	^{99m} Tc-ECD in PDRP regions	HC<IRBD	Pons, the Globus pallidus, putamen, cerebellum
			IRBCnc<IRBDc	Pons
Arnaldi et al., 2015	12 IRBD 16 PD 24 PDRBD	[¹²³ I]FP-CIT	IRBD>PD>PDRBD	Putamen
Zoetmulder et al., 2016	10 IRBD	[¹²³ I]FP-CIT	HC>IRBD	Putamen
	10 PD		IRBD>PD	
	10 RBD 10 HC		IRBD>PDRBD	
Iranzo et al., 2017a	87 IRBD 20 HC	[¹²³ I]FP-CIT	Longitudinal findings	Increased risk of conversion in patients with abnormal uptake in the putamen
Bae et al., 2018	18 IRBD 18 HC 18 PD	[¹²³ I]FP-CIT	HC>IRBDnh IRBDnh>PD	Whole striatum, anterior and posterior putamen
Iranzo et al. 2020	167 rh IRBD	[¹²³ I]FP-CIT	Dominant hemisphere	Lower binding in the L striatum and caudate than in the R ones

Abbreviations: AD, Alzheimer's disease; [¹¹C]DTBZ, [¹¹C]Dihydrotetraabenazine; DLB, Dementia with Lewy bodies; [¹⁸F]DOPA, [¹⁸F]Dihydroxyphenylalanine; [¹⁸F]FDG-PET, [¹⁸F]fluorodeoxyglucose; [¹⁸F]FEOBV, [¹⁸F]Fluoroethoxybenzovesamicol; FMT-PET, 6-[¹⁸F]fluoro-meta-tyrosine; [¹²³I]FP-CIT, 1-123-N-o-Fluoropropyl-2B-Carbomethoxy-3b-(4-Iodophenyl)-Nortropan; HC, healthy control; [¹²³I]IBZM, [¹²³I](S)-2-hydroxy-3-iodo-6-methoxy-(1-ethyl-2-pyrrolidinylmethyl) benzamide; IMP-SPECT, N-isopropyl-p-[¹²³I]Iodoamphetamine; [¹²³I]IPT, (N)-(3-iodopropene-2-yl)-2β-carbomethoxy-3β-(4-chlorophenyl)tropane; IRBD, idiopathic REM sleep behavior disorder; IRBD₀, IRBD at baseline; IRBDc, IRBD patients that converted to a Lewy body disorder; IRBD_{F-up}, IRBD at follow-up; IRBD-MCI, IRBD with mild cognitive impairment; IRBDnc, IRBD patients that did not phenoconvert; IRBDnh, IRBD with nigral hypointensity; IRBD-NMCI, IRBD without mild cognitive impairment; L, left; PD, Parkinson's disease; PDRBD, PD patients with RBD; PDRP, PD related pattern; PiB-PET, [¹¹C]-Pittsburgh compound B positron emission tomography; R, Right; rh, right-handed; ^{99m}Tc-ECD, ^{99m}Tc-Ethylene Cysteinate Dimer. *Longitudinal studies.

Table 8. Neural correlates in IRBD

REFERENCE	RBD duration	Motor status	FFEE/ Attention	Visuospatial	Color	Memory
Vendette et al., 2011					↓Frontal and occipital metabolism	
Dang-Vu et al., 2012		↑Hippocampal perfusion			↑ pons and L hippocampus	
Ge et al., 2015	↑ metabolism in anterior vermis and ↓ in the medial frontal gyrus					
Rahayel et al., 2017	↓ GM volume in the frontal cortices ↓ CTh in the L medial superior frontal and supramarginal cortex and precuneus	↓ Caudate and ↑ cerebellum volumes				
Rahayel et al., 2018b			↓ CTh in the medial superior, dorsolateral paracentral, sensorimotor, fusiform, lingual, and cuneus	↓ CTh in the frontal, temporal, posterior lingual and fusiform, insular, precuneus, and cuneus and lateral occipital ↓ GM volume in the R lateral occipital cortex ↑ surface expansion in the R hippocampus	↓ CTh in the R cuneus. Abnormal surface expansion in the R hippocampus	↓ CTh in the temporal (pole, anterior superior, posterior lingual and fusiform), insular, and cuneus. ↑ surface expansion in the R hippocampus
Bezdicek et al., 2018						↓ DA uptake in striatum and putamen ↓ CTh in the L superior temporal, L caudal middle frontal, R superior frontal and R lateral occipital gyri
Pereira et al., 2019		↓ CTh of the L superior frontal and fusiform and R precentral gyri		↓ CTh L fusiform and R supramarginal		
Yamada et al., 2019		↓ uptake in the striatum				
Sun et al., 2019 Byun et al., 2020	↑ iron in the SN					Thalami-fusiform FC

Abbreviations: CTh, cortical thickness; FC, functional connectivity; GM, gray matter; L, left; R, right; SN substantia nigra.

1.2. Olfactory dysfunction

The estimated prevalence of smell dysfunction in a population-based study (n=3519) was 13.5% and was found to increase with age (Liu et al., 2016). Furthermore, a more rapid decrement of olfaction has been systematically reported in several neurodegenerative disorders. Particularly, there is a large body of literature highlighting the relation between Synucleinopathies and smell dysfunction. In this section, I will review the link between olfactory impairment and Synucleinopathies, with a special focus on olfactory dysfunction on premotor stages of PD. I will present the known neural correlates of hyposmia in PD and its premotor stages. Finally, I will introduce the most commonly used smell tests.

1.2.1. Olfaction in Synucleinopathies

Research on olfaction in Synucleinopathies has a long tradition, especially in PD. Olfactory impairment is observed in PD de novo patients and can, therefore, occur regardless of dopaminergic treatment (Doty et al., 1992; Tissingh et al., 2001). In a multicenter study, the prevalence of olfactory deficits in PD was estimated to be 96.7% when compared with young subjects, and 74.5% when adjusted for age (Haehner et al., 2009). The degree of olfactory impairment can range from normal to severe hyposmia. In the same study, Haehner, et al. (2009), identified 45% of PD patients as anosmics, 51.7% as hyposmics, whereas only 3.3% PD patients were normosmics (Haehner et al., 2009). It has been suggested that normosmic patients could be a unique clinical phenotype with fewer motor deficits and a more benign disease course (Lee et al., 2015). In contrast, severe olfactory dysfunctions have been associated with worse cognitive performance, more cognitive decline over time, and a higher risk of progression to MCI and dementia. (Baba et al., 2012; Damholdt et al., 2011; Fullard et al., 2016; J.-W. Park et al., 2018; Postuma and Gagnon, 2010). Smell function has also been investigated as a potential marker of disease progression. In this context, most studies did not show correlation between olfaction and disease duration (Doty et al., 1988; Haehner et al., 2014; Hawkes et al., 1997; Herting et al., 2008), suggesting that olfactory impairment is independent of disease severity and duration, making it unlikely to be a reliable marker of disease progression (Fullard et al., 2017) (**Table 9** shows the most important uses of olfactory testing in PD).

Olfaction in differential diagnosis

The importance of olfaction in PD is such that hyposmia can distinguish patients from healthy individuals (Doty et al., 1995; Double et al., 2003; Suzuki et al., 2011) and has demonstrated to be as sensitive as DaTscan in the diagnosis of PD (Deeb et al., 2010). Furthermore, smell dysfunction may help in the differential diagnosis of some neurodegenerative disorders. Whereas in DLB previous data showed an olfactory phenotype similar to that of PD (McShane et al., 2001; Olichney et al., 2005; Westervelt et al., 2003; Yoon et al., 2015), olfaction is less affected in other parkinsonian syndromes, such as MSA, corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP) (Krismer et al., 2017; Müller et al., 2002a; Silveira-Moriyama et al., 2010b, 2009; Wenning et al., 1995). For this reason, smell performance can be useful in distinguishing PD from other motor disorders (**Table 9**), such as PSP (Doty et al., 1993; Silveira-Moriyama et al., 2010b; Suzuki et al., 2011; Wenning et al., 1995) and MSA (Goldstein et al., 2008; Kikuchi et al., 2011; Krismer et al., 2017; Suzuki et al., 2011; Wenning et al., 1995).

Olfactory progression over PD

The progression of olfactory deficits in PD is unclear. Only three longitudinal works have attempted to study the olfactory progression in PD (Herting et al., 2008; Meusel et al., 2010; Müller et al., 2002b). Whereas Meusel et al. (2010) reported an overall olfactory decline, Herting et al. (2008) and Müller et al. (2002) found contradictory results, with some patients presenting an olfactory progression and others remaining stable. The main shortcoming of these previous works is that they did not include a control group, which makes it difficult to distinguish disease-related decline from the effects of aging on olfactory function (Sorokowska et al., 2015).

Altogether highlights the strong prevalence of olfactory dysfunction in PD patients, even in the early stages of the disease, supporting the Braak's scheme early implication of the olfactory bulb. Although olfactory changes over time have been slightly detected in PD, the lack of a control group in previous studies precludes to discard aging effects. Finally, olfactory tests may be useful to distinguish among neurodegenerative disorders, as well as to predict conversion in prodromal PD, such as IRBD. The next subsection will present data demonstrating that smell dysfunction is already affected in PD premotor stages.

Uses of olfactory testing	Study findings
Differential diagnosis	
PD versus tauopathies	Odor identification in PSP and CBD is similar to controls. Olfactory deficit can aid in discrimination between PD and tauopathies ^a
PD versus MSA	Olfactory impairment in MSA is intermediate between PD and tauopathies. Olfactory ability should be used cautiously to distinguish PD and MSA ^b
PD, DLB versus AD	PD, DLB and AD all demonstrate olfactory impairment. Hyposmia tends to be more common and more severe in PD and DLB than AD, but significant overlap prevents differentiation based on olfaction alone ^c
Potential biomarker for PD in premotor stages	
	In asymptomatic relatives of PD patients and in prodromal PD (IRBD patients) hyposmia is associated with risk of developing PD ^d
Prognosis in PD	
Motor progression	Some evidence suggests that olfactory impairment is independent of disease severity and duration, making it unlikely to be a reliable marker of disease progression ^e
Non-motor progression	Olfactory impairment has been associated with apathy, autonomic symptoms, anxiety and depression in cross-sectional studies ^f
Cognitive decline	Severe olfactory dysfunctions have been associated with worse cognitive performance, more cognitive decline over time, and higher risk of progression to MCI and dementia ^g

Table 9. Use of olfactory function testing in PD. Adapted from Fullard, et al. 2017.

^a(Doty et al., 1993; Silveira-Moriyama et al., 2010b; Suzuki et al., 2011; Wenning et al., 1995); ^b(Goldstein et al., 2008; Kikuchi et al., 2011; Krismer et al., 2017; Silveira-Moriyama et al., 2009; Suzuki et al., 2011; Wenning et al., 1995); ^c(McShane et al., 2001; Olichney et al., 2005; Westervelt et al., 2003; Yoon et al., 2015); ^d(Berendse and Ponsen, 2009; Haehner et al., 2007; Postuma et al., 2019; Ross et al., 2008); ^e(Doty et al., 1988; Haehner et al., 2014; Hawkes et al., 1997; Herting et al., 2008); ^f(Berendse et al., 2011; Cramer et al., 2010; Goldstein et al., 2010); ^g (Baba et al., 2012; Damholdt et al., 2011; Fullard et al., 2016; J.-W. Park et al., 2018; Postuma and Gagnon, 2010). Abbreviations: AD, Alzheimer's disease; DLB, dementia with Lewy bodies; CBD, corticobasal degeneration; IRBD, idiopathic REM sleep behavior disorder; MCI, mild cognitive impairment; MSA, multiple system atrophy; PD, Parkinson's disease; PSP, progressive supranuclear palsy.

1.2.2. Olfactory dysfunction as a premotor manifestation of PD

The high prevalence of hyposmia in PD has fostered a great interest in olfaction as a potential biomarker for PD. In this sense, olfactory dysfunction has been found to be a preclinical or premotor manifestation in PD (Haehner et al., 2007; Ross et al., 2008). In a study of asymptomatic relatives of PD patients, hyposmia was associated with a 12.5% risk of developing PD within five years (Berendse and Ponsen, 2009). Similarly, patients

diagnosed with idiopathic hyposmia are at higher risk of developing PD (Haehner et al., 2007) and have a greater future decline in language and memory functions (Weintraub et al., 2017).

Hyposmia is a sensitive measure of underlying PD pathology, yet its specificity is low since there are other causes of olfactory impairment. For this reason, the potential of olfaction as a biomarker of conversion is higher when used in combination with other PD markers, namely IRBD, constipation, neuroimaging findings (i.e, abnormal DAT imaging), and cognitive impairment. Hence, the implementation of olfactory testing in a population at high risk for developing a Synucleinopathy, such as IRBD, might help to improve the positive predictive value of olfaction (Fullard et al., 2017). The next subsection does an in-depth review of olfactory dysfunction in IRBD patients.

Olfactory dysfunction in IRBD

Evidence of olfactory dysfunction in premotor stages of Synucleinopathies is also supported by the presence of hyposmia in IRBD patients. Olfactory impairment in IRBD has been reported in 35.7–97.0% of patients compared with 2.5–16.6% of healthy control subjects (Högl et al., 2018). Classification based on olfactory tests, highlights that 5.5-36% of IRBD patients are normosmic (Iwanami et al., 2010; Miyamoto et al., 2010; Yoon et al., 2019), >40-80% have hyposmia (Iwanami et al., 2010; Miyamoto et al., 2010) and >13-42.2% anosmia (Iwanami et al., 2010; Miyamoto et al., 2010; Shin et al., 2013; Yoon et al., 2019). In IRBD olfactory impairment is associated with age (Fantini et al., 2006; Miyamoto et al., 2010), particularly odor discrimination (Miyamoto et al., 2010) and identification worsened with increasing age (Fantini et al., 2006; Miyamoto et al., 2010). However, no significant effect for gender has been shown, suggesting that both men and women are affected by the same extent of olfactory loss (Fantini et al., 2006; Wan et al., 2016). **Table 10** at the end of the current section, present the most important findings relating to IRBD and olfactory performance.

Olfactory dysfunction in IRBD patients includes impairment in odor threshold (Iranzo et al., 2013; Mahlknecht et al., 2015; Miyamoto et al., 2010; Stiasny-Kolster et al., 2005), discrimination (Mahlknecht et al., 2015; Miyamoto et al., 2010; Stiasny-Kolster et al., 2005) and identification. With smell identification as the most systematically reported olfactory dysfunction in IRBD (Aguirre-Mardones et al., 2015; Barber et al., 2017; Dušek

et al., 2019; Fantini et al., 2006; Huang et al., 2016; Iranzo et al., 2013; Mahlknecht et al., 2015; Miyamoto et al., 2011, 2010; Pereira et al., 2019; Shin et al., 2019, 2013; Stiasny-Kolster et al., 2005). In keeping with the idea of larger identification impairment in IRBD, some previous works found that odor identification can discriminate with a high sensitivity 83.3-90% and specificity 81.5-83.3% between IRBD and healthy individuals (Huang et al., 2016; Miyamoto et al., 2011).

Olfactory dysfunction in these patients has been found even 20 years before motor onset (Fereshtehnejad et al., 2019). But interestingly, longitudinal works showed that olfaction changes only slightly over time (5 years follow-up) (Postuma et al., 2011), and that the decline in IRBD is similar to that observed in healthy elder controls after a 4-year follow-up (Iranzo et al., 2013) (**Figure 24**). The notion that olfactory impairment may be stable across time in IRBD, is supported by the fact that most cross-sectional findings did not show a significant relationship between smell impairment and disease severity or duration (Aguirre-Mardones et al., 2015; Iranzo et al., 2013; Miyamoto et al., 2011, 2010, 2009; Shin et al., 2013). Overall, it is suggested that the lack of olfactory changes over IRBD might be explained by the topographical progression of alfa-synuclein pathology proposed by Braak staging, being the olfactory bulbs affected in premotor stages of Synucleinopathies.

With the above caveats in mind, hyposmia has gained attention as a potential biomarker of conversion in those individuals at prodromal stages of the disease. In this sense, in IRBD patients, hyposmia has demonstrated to be the most specific feature to predict phenoconversion among all non-motor symptoms (Fereshtehnejad et al., 2019). Particularly, previous works have found IRBD patients with olfactory dysfunction to have an increased short-term risk of developing manifested PD and DLB (Mahlknecht et al., 2015; Postuma et al., 2011). It is worth mentioning that the presence of olfactory dysfunction in IRBD is also associated with larger cognitive impairment (Sasai et al., 2013; Shin et al., 2019).

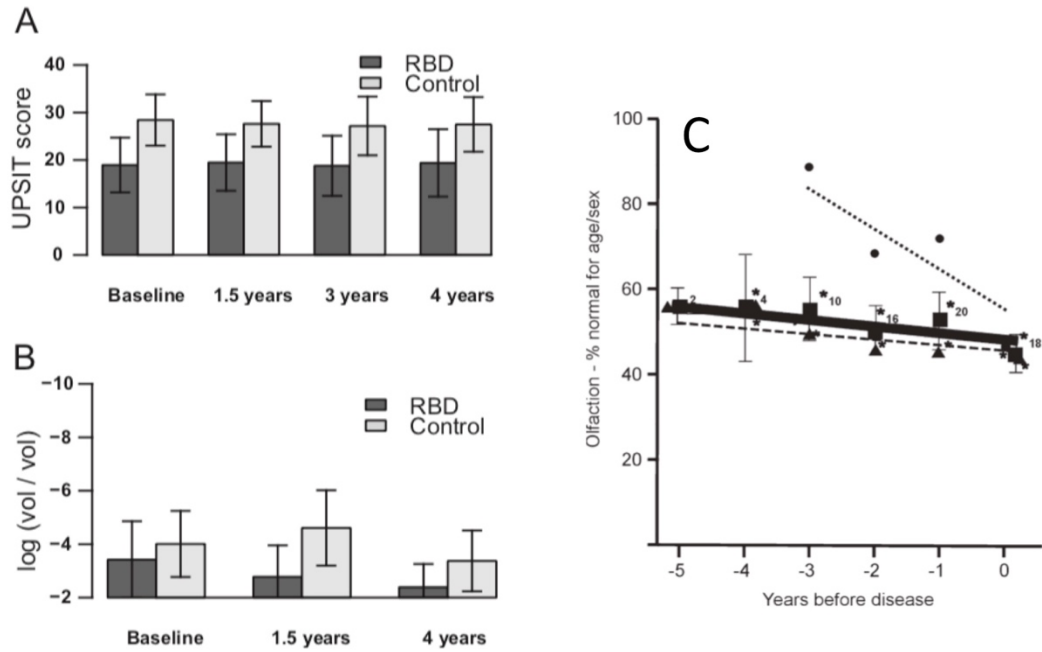


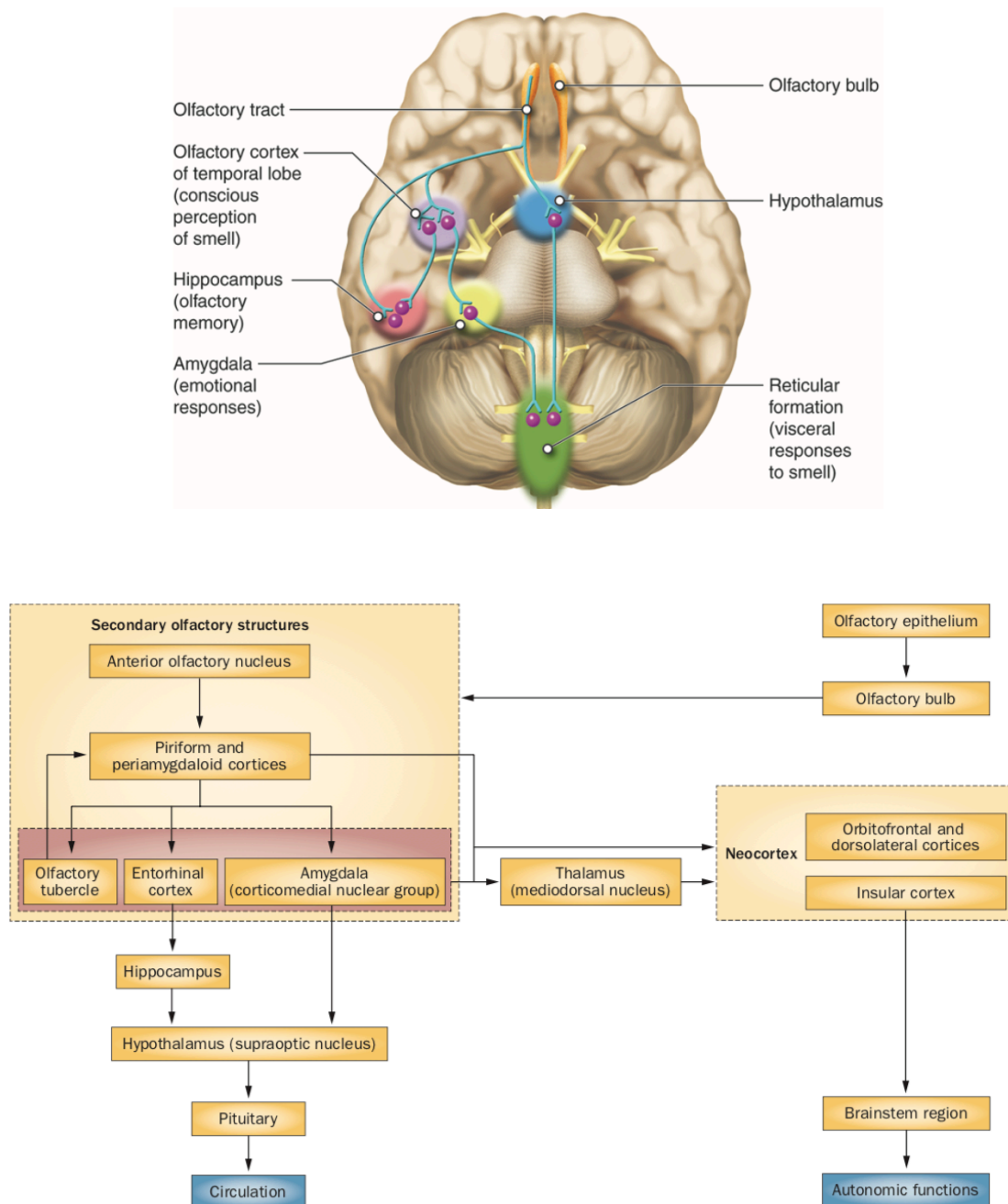
Figure 24. Progression of olfactory deficit in IRBD. **A.** Odor identification UPSIT score (Iranzo et al., 2013); **B.** Odor detection threshold STT score (Iranzo et al., 2013); **C.** Olfactory testing in preclinical intervals of disease. Year 0 refers to examination at time of disease diagnosis, and years -1 to -5 to examinations before disease diagnosis. Square= entire disease group; triangle= dementia/ PD-dementia; circle= tremor-onset PD. Error bars are for the disease-group as a whole (standard error). The number beside each data point represents the number of assessments at that time point for the disease group as a whole. * significantly different than age/sex-adjusted expected values (1-sample t test). The solid line represents the regression line for the entire disease group, and dotted lines represent regression lines for each disease subgroup (dementia/PD-dementia and tremor- onset PD). (Postuma et al., 2011)

1.2.3. Neural correlates of olfactory dysfunction

The mechanisms underpinning olfactory loss in PD are still uncertain but may involve neuropathological alterations in the olfactory system (see **Panel 5**). In this sense, anatomical correlates of olfactory impairment in PD has been associated with atrophy of the olfactory bulb (Chen et al., 2014; Sengoku et al., 2015; Wang et al., 2011), piriform (Ibarretxe-Bilbao et al., 2010; E.-Y. Lee et al., 2014; Wattendorf et al., 2009; Wu et al., 2011), and orbitofrontal cortices (E.-Y. Lee et al., 2014; Wu et al., 2011). The gray matter correlates of olfactory deficits differ according to the disease stage; in early PD patients, significant correlations are seen in the right piriform cortex, while in moderate and advanced patients the right amygdala is implicated (Wattendorf et al., 2009) (**Figure 25**). According to Braak stages (Braak et al., 2003), the orbitofrontal cortex, a tertiary olfactory structure, is affected relatively early, and prior to posterior parietal regions. Changes in the orbitofrontal cortex are therefore expected in early disease stages. Finally, there are no previous follow-up studies including MRI data, so the neural correlates of smell decline remain unknown.

Neuroanatomical substrates of olfactory impairment in IRBD is largely based on nuclear neuroimaging data. Olfactory identification loss was related to reduced regional cerebral blood flow in the anterior parahippocampal gyri, a region that is known to be involved in olfactory functions, but also in the right occipital gyrus, left superior temporal, posterior cerebellum (Vendette et al., 2011), the medial frontal gyrus (Dang-Vu et al., 2012) and with higher expression of the PD (Meles et al., 2017) and PDRBD related pattern (Yoon et al., 2019). Moreover, previous studies have identified correlations between smell loss and putaminal (Dušek et al., 2019), amygdala (Shin et al., 2019), striatal, and SN uptake (Lee et al., 2019). As for structural data, smell identification has been associated with cortical thinning in bilateral lateral occipital cortices and the right cuneus (Rahayel et al., 2018b), orbitofrontal and precentral regions (Pereira et al., 2019) (**Figure 26**).

Panel 5 Olfactory system



Olfactory receptors are first order neurons located in the nasal epithelium on the superior nasal cavity. From these olfactory cells, information is sent to neurons located in the olfactory bulb, located in the ventral surface of the frontal lobe. From the olfactory bulb, axons project to structures within the limbic cortex, including the cortex in the temporal lobe associated with long-term memory formation. Figure at the top depicts regions that receive information from the olfactory bulb (<https://courses.lumenlearning.com/austinncc-ap1/chapter/special-senses-smell-olfaction/>). The scheme represents the major afferent projections and structures of the olfactory system. (extracted from Doty, 2012).

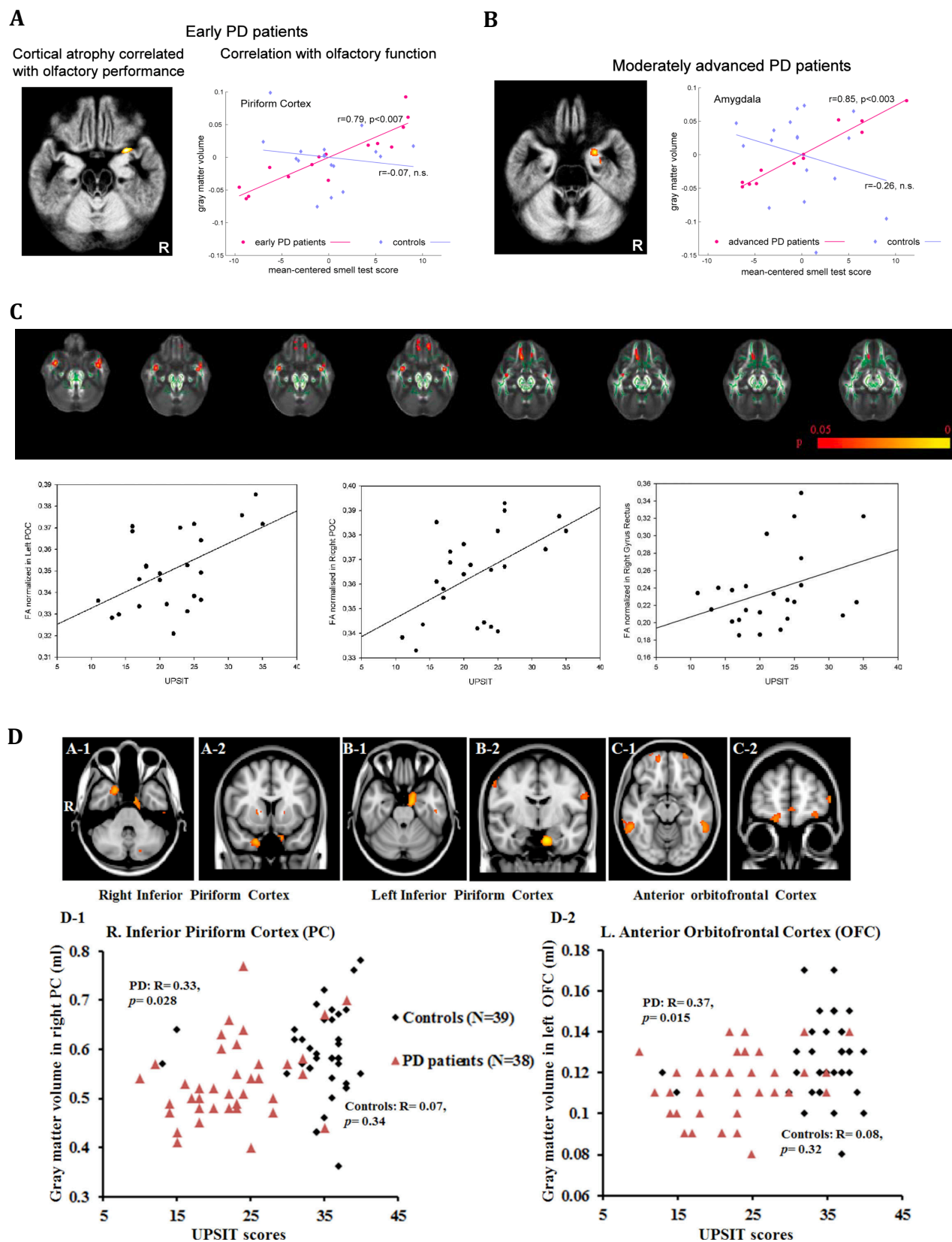


Figure 25. Neural correlates of olfaction in PD. **A.** Olfactory-associated cortical atrophy in early PD patients and in, **B.** moderately advanced PD patients. The correlation plots show that the relative GM volume decreases with lower scores of olfactory function (Wattendorf et al., 2009);

C. PD patients with anosmia demonstrated significantly lower fractional anisotropy in the WM adjacent to gyrus rectus and to the primary olfactory cortex than HC. Regression plots show correlation between FA values in WM adjacent to primary olfactory cortex and UPSIT scores in PD patients (Ibarretxe-Bilbao et al., 2010); D. GM volume loss in PD subjects relative to HC. Scatter plots of GM volume with UPSIT scores for both PD and controls: PD GM volume in the right inferior piriform cortex (D-1) and left anterior orbitofrontal cortex (D-2) correlated with UPSIT scores (E. Y. Lee et al., 2014). Abbreviations: FA, fractional anisotropy; GM, gray matter; PD, Parkinson's disease; R, right; UPSIT, University of Pennsylvania Smell Identification Test; WM, white matter.

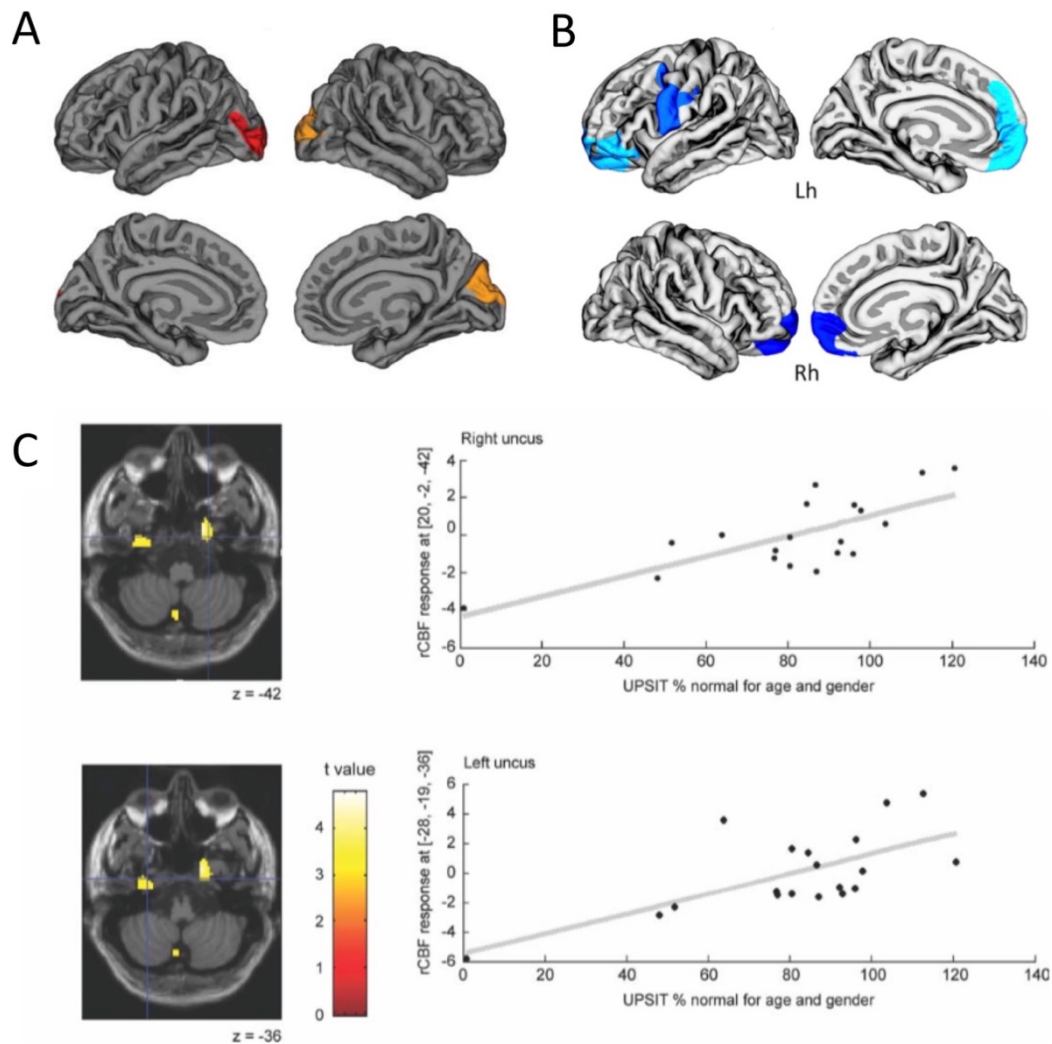


Figure 26. Neural correlates of olfaction in IRBD. **A-B.** Correlation between UPSIT and cortical thickness **A.** UPSIT scores correlate with the bilateral lateral occipital cortices and the right cuneus (Rahayel et al., 2018b); **B.** UPSIT scores correlate with the left medial orbitofrontal, left precentral and right medial orbitofrontal thinning (Pereira et al., 2019); **C.** Correlation between regional cerebral blood flow and UPSIT scores (Vendette et al., 2011).

Hyposmia in the context of the Braak Staging System for PD

As mentioned in the first section of the introduction (see **Figure 3**), according to Braak staging for PD, Lewy pathology begins in the olfactory bulb and dorsal motor nucleus of

the vagus (Braak et al., 2003), which is consistent with the premotor onset of hyposmia. This hypothesis is supported by the fact that alpha-synuclein pathology has been systematically found in olfactory regions in PD patients (**Panel 5** depicts olfactory system regions). Particularly, this pathology has been found in the olfactory bulb, the anterior olfactory nucleus, the amygdala, the piriform cortex, the entorhinal cortex and the orbitofrontal cortex of PD (Beach et al., 2009b; Harding et al., 2002b; Pearce et al., 1995). Lewy body pathology in the olfactory bulb has also been detected in PD premotor stages, such as IRBD (Iranzo, et al., 2014a). Moreover, Beach et al., found out that when only one structure was affected in patients with incidental Lewy bodies, it was most commonly the olfactory bulb (Beach et al., 2009a). These findings support the hypothesis that early alpha-synuclein pathology in PD involves the olfactory bulb and associated structures and may progress from the periphery to central structures.

1.2.4.Measuring olfaction

There are different ways of testing smell functions. Broadly, it could be divided into electrophysiological (odor-related potentials) and psychophysical tests. This last category encompasses tests that measure odor identification, threshold detection, discrimination, and memory of odorants (Doty, 2012a). Odor identification tests, such as the University of Pennsylvania Smell Identification Test (UPSIT) (Doty, 1995) and Sniffin Identification tests (Hummel et al., 2007), are by far the most commonly implemented tests to evaluate olfaction worldwide (Doty, 2012a). Such tests are used in both research and clinical settings to assess olfactory dysfunction in neurodegenerative disorders. Indeed, UPSIT and Sniffin' Stick tests have been traditionally used as diagnostic tools in PD (Doty, 2012a), and their accuracy for this disease is high (Doty et al., 1995; Mahlknecht et al., 2016; Rodríguez-Violante et al., 2014a). The neural correlates of such tests in IRBD and PD are presented in the subsection **1.2.3 Neural correlates of olfactory dysfunction**.

The main drawback of these tools is that performance in smell identification tests are subject to cultural variation. For this reason, the UPSIT has been adapted to several countries (Ogihara et al., 2011; Picillo et al., 2014; Rodríguez-Violante et al., 2011; Silveira-Moriyama et al., 2010a, 2008; Yücepur et al., 2012). Even though changes in some specific items have improved sensitivity and specificity, it is hard to generalize the results

to other populations (Picillo et al., 2014; Rodríguez-Violante et al., 2014a; Silveira-Moriyama et al., 2008). Furthermore, previous works found a decreased identification of some UPSIT items in healthy populations (Kondo H, Matsuda T, Hashiba M, 1998; Picillo et al., 2014; Rodríguez-Violante et al., 2014b; Silveira-Moriyama et al., 2008). Given that cross-cultural aspects may influence UPSIT results (Rodríguez-Violante et al., 2014a), it could be of interest to perform a more detailed analysis of the UPSIT to identify those with less capability to detect olfactory deficits in a given population due to cultural bias.

Olfactory functions in normal subjects are clearly influenced by age (Mckinnon et al., 2010) and gender (Doty et al., 1982, 1978, 1975). Loss of olfactory identification ability is seen from the fifth decade onwards but is most remarkable between the seventh and eighth decades of life. In PD patients, it has also been found that UPSIT scores decreased as a function of age (Doty et al., 1995). Sex-related differences in the UPSIT have also been described in PD, with a lower performance for men (Doty et al., 1995). These results suggest that age and sex should be taken into consideration when using olfactory measures as a tool for PD diagnosis. In this setting, Doty and colleagues (Doty et al., 1995) proposed optimal cut-off scores that best discriminate between PD and healthy subjects, according to different age groups and according to gender. As far as we know, these results have not been validated for PD in the UPSIT Spanish version.

Because olfactory impairment manifests years before to the motor onset, olfactory testing has the potential to serve as an early diagnostic marker of PD (Deeb et al., 2010; Rodríguez-Violante et al., 2011; Silveira-Moriyama et al., 2008), particularly in IRBD patients. As mentioned above, the UPSIT and the Sniffin' Stick test are the most frequently used in PD research and clinics. Therefore, there is an especial interest in knowing not only the performance but also the diagnostic accuracy in distinguishing IRBD patients from controls and the neural correlates of such tests. Improving the accuracy of olfactory clinical testing in IRBD could help identify patients at a higher risk of conversion to a neurodegenerative disease, and thus define target populations for disease-prevention trials.

Summary

The strong prevalence of olfactory dysfunction in the early stages of PD is in accordance with the Braak's scheme early implication of the olfactory bulb. Evidence of olfactory dysfunction in premotor stages of Synucleinopathies is supported by the presence of hyposmia in IRBD patients, even 20 years before motor onset. The importance of olfaction in PD and IRBD is such that hyposmia can differentiate patients from healthy individuals. Furthermore, olfactory tests may be useful to distinguish among motor neurodegenerative disorders, as well as to predict conversion in prodromal PD. Longitudinal works in IRBD and PD showed that olfaction changes only slightly over time across the Lewy body continuum, which might be explained by the topographical progression of alfa-synuclein pathology proposed by Braak staging, being the olfactory bulbs affected in premotor stages of Synucleinopathies, with later progression to the central structures. Improving the accuracy of olfactory clinical testing in IRBD could help identify patients at a higher risk of conversion to a neurodegenerative disease, and thus define target populations for disease-prevention trials.

Table 10. Studies with olfactory data in IRBD

Reference	Sample	Test	Smell impairment	Classification	SE(SP)/Cut-off	Neural correlates
Stiasny-Kolster et al., 2005	30 RBD (IRBD=6) 30 HC	Sniffin	↑threshold ↓discrimination ↓identification			
Fantini et al., 2006	54 IRBD 54 HC	B-SIT	↓identification		61% (83%)/-	
Miyamoto et al., 2009	48 IRBD 21 PD 34 OSAS	OSIT-J	↓identification		IRBD vs OSAS 88.2% (83.3%)/8.5	
Miyamoto et al., 2010	73 IRBD middle-aged 33 IRBD older-aged 28 HC	Sniffin OSIT-J	↑threshold ↓discrimination ↓identification (better discriminates IRBD middle-aged vs HC)	IRBD middle-aged: <u>Sniffin' Sticks</u> : mild hyposmia 15.1% moderate 23.3% severe 32.9% anosmia 28.8% <u>OSIT-J</u> : normosmia 5.5% hyposmia 53.4% anosmia 42.4%	IRBD middle-aged vs HC Sniffin-Th 75% (74%)/5.8 Sniffin-D 78.6% (64.4%)/9.5 Sniffin-I 96.4% (78.1%)/8.5 OSIT-J 89.3% (94.5%)/8.5	
Iwanami et al., 2010	34 IRBD 17 PD 21 HC	OSIT-J	no group comparison	IRBD: normosmia 20.6% hyposmia 41.2% anosmia 38.2%		
Miyamoto et al., 2011	30 IRBD 38 PD 20 HC	OSIT-J	↓identification		IRBD vs HC 90% (83.3%)/8.5	
Postuma et al., 2011*	21 IRBDc 41 IRBDnc	UPSIT	IRBDc<IRBDnc (at baseline) ↓identification			
Vendette et al. 2011	20 IRBD 20 HC	CC-SIT	no group comparison			rCBF in the anterior parahippocampal gyri, the R occipital gyrus, L superior temporal and posterior cerebellum

Reference	Sample	Test	Smell impairment	Classification	SE(SP)/Cut-off	Neural correlates
Dang-Vu et al., 2012	20 IRBD	CC-SIT				rCBF in the medial frontal gyrus
Iranzo et al., 2013*	19 IRBD 19 HC	UPSIT STT	IRBD<HC			
			At 4 years follow-up No group differences in the rate of change			
Shin et al., 2013	15 IRBD 30 PD 30 HC	CC-SIT	IRBD=PD<HC	IRBD		
			↓identification	Hyposmia 80% Anosmia 13.3%		No correlation with SN echosignal in IRBD and PD
Sasai et al., 2013	32 IRBD 17 HC	Sniffin	no group comparison between HC and IRBD			
Aguirre-Maradones et al., 2015	44 IRBD 40 HC	UPSIT	IRBD<HC		Cut-off <18 more frequent in IRBD (35.7%) than HC (2.5%)	
			↓identification			
Mahlknecht et al., 2015*	34 IRBD (9 IRBDc; 25 IRBDnc)	Sniffin	IRBD<HC			
			↓discrimination ↓identification			
			IRBD _c <IRBD _{nc}			
			↓discrimination ↓identification (best predictor of conversion)		Sniffin total score < 18 was associated with increased risk of conversion	
Wan et al., 2016	41 IRBD 35 PDNRBD 42 PDRBD 63 HC	Sniffin	At 2.4 years			
			82.4% accuracy predicting conversion			
			HC>IRBD=PD=PDRBD			
Huang et al., 2016	54 IRBD 54 PD 54 HC	Sniffin-12	Impaired olfaction (Sniffin total score)			
			HC>IRBD>PD	IRBD vs HC		
			↓identification	83.3% (81.5%)/7.5 IRBD vs PD 51.9% (87%)/4.5		
Meles et al., 2017	21 IRBD 19 HC	Sniffin	IRBD<HC			PDRP was higher expressed in IRBD with hyposmia
Barber et al., 2017	171 IRBD 296 HC 119 PD untreated	Sniffin-I	Impaired olfaction (Sniffin total score)			
			HC>IRBD=PD=PDRBD			

Reference	Sample	Test	Smell impairment	Classification	SE(SP)/Cut-off	Neural correlates
Rahayel et al., 2018b	52 IRBD (17 IRBD-MCI; 35 IRBD-NMCI)	UPSIT	No differences between IRBD-MCI and IRBD-NMCI			CTh in the L and R lateral occipital cortices and R cuneus
	41 HC					
Shin et al., 2019	25 IRBD 13 HC	B-SIT	IRBD < HC	↓identification		Amygdala uptake
Yoon et al., 2019	28 IRBD	BTT		IRBD: ↑threshold ↓identification		In IRBD the PDRBD-RP was negatively correlated with olfaction
	21 PDRBD 24 HC	CC-SIT	F test	Normosmia 36% Anosmia 29%		
Pereira et al., 2019*	27 IRBD‡		HC>IRBD=PD	↓identification		UPSIT correlates with CTh in the L medial orbitofrontal, L precentral and R medial orbitofrontal thinning
	151 PD‡	UPSIT				
	31 HC‡		IRBDc=IRBDnc			
Dusek et al., 2019	74 IRBD 39 HC	UPSIT	IRBD < HC	↓identification		Putaminal binding ratio
Lee et al., 2019	30 IRBD	B-SIT				
	30 PD	BTT				
	19 HC					IRBD with hyposmia had uptake reduction in the anterior and posterior putamen, caudate and SN compared to HC

Abbreviations: B-SIT, Brief University of Pennsylvania Smell Identification Test; BTT, the buthanol threshold test; CC-SIT, University of Pennsylvania Smell Identification Test 12 items; CTh, cortical thickness; HC, healthy controls; IRBD, idiopathic REM sleep behavior disorder; IRBDc, IRBD converters; IRBD-MCI, IRBD with MCI; IRBDnc, IRBD non-converters; IRBD-NMCI, IRBD without MCI; L, left; MCI, mild cognitive impairment; OSAS, obstructive sleep apnea syndrome; OSIT-J, Odor stick Identification Test for Japanese; PD, Parkinson's disease; PDRP, PD related pattern; PDNRBD, PD patient without RBD; R, right; RBD, REM sleep behavior; SE, sensitivity; rCBF, regional cerebral blood flow; SN, substantia nigra; Sniffin, Sniffin' Sticks test; Sniffin-12, Sniffin' Stick screening 12 test; Sniffin-D, Sniffin' Sticks discrimination test; Sniffin-I, Sniffin' Sticks identification test; Sniffin-Th, Sniffin' Sticks threshold test; SP, specificity; SIT, Smell Threshold Test. ‡ Parkinson's Progressions Markers Initiative data (<https://www.ppmi-info.org>);

* Longitudinal studies

CHAPTER 2

Objectives and Hypotheses

2.1. Objectives

MAIN OBJECTIVES:

The present Doctoral Thesis is contextualized in the use of MRI to characterize structural and functional brain changes underpinning cognitive impairment in Idiopathic REM sleep behavior disorder, as well as the study of olfactory dysfunction as an early perceptual dysfunction in IRBD and PD.

The main objectives of this Thesis are:

- 1 To characterize structural and functional brain substrates underlying Idiopathic REM sleep behavior disorder as well as to relate these findings to cognitive performance,
- 2 To study olfactory dysfunction as a preclinical and clinical biomarker of alpha-Synucleinopathies, and its progression throughout the disease,
- 3 To investigate progressive brain degeneration in IRBD, and to investigate how these changes relate to cognitive decline.

SPECIFIC OBJECTIVES:

- 1 To characterize cognitive impairment in IRBD patients and its correlation with cortical and subcortical parameters of MRI brain atrophy.
- 2 To characterize functional connectivity and their relation to impaired cognition in IRBD.
- 3 To assess the sensitivity and specificity of olfactory tests in IRBD and PD.
- 4 To investigate olfactory dysfunction and its progression in IRBD and PD, as well as its neural correlates.
- 5 To describe cortical degeneration in IRBD.
- 6 To explore changes over time in cognitive functions in IRBD and their relationship with the progression of brain atrophy.

2.2. Hypotheses

MAIN HYPOTHESES:

The main hypotheses of the present Doctoral Thesis are:

- 1 IRBD will be characterized by specific changes in brain structure and functional connectivity that will be associated with olfactory and cognitive impairment,
- 2 IRBD patients will show structural brain changes over time and cognitive decline superior to that seen in normal aging,
- 3 Smell dysfunction will be identified in IRBD and PD, and it is expected to progress slightly with disease course.

SPECIFIC HYPOTHESES:

- 1 IRBD patients will show clinical, cognitive, and olfactory impairment compared to healthy controls, which is expected to be similar to that previously described in PD.
- 2 We expect cortical and subcortical brain atrophy in IRBD patients compared to controls. Specific MRI changes in this condition will be consistent with findings in Synucleinopathies.
- 3 Functional connectivity will be reduced in IRBD patients compared to controls, similar to that found in PD and DLB.
- 4 IRBD will present distinct neural correlates for olfactory impairment and other cognitive functions.
- 5 The progression of olfactory impairment is expected to be mild both in PD and IRBD.
- 6 Progressive cortical degeneration in IRBD patients compared to normal aging over time is expected, given that this parasomnia is considered part of the prodromal phase of the Synucleinopathies PD and DLB.
- 7 Given that cortical atrophy is expected throughout the disease course, IRBD patients will show cognitive decline over time with specific neuroanatomical substrates.

CHAPTER 3

Materials and Methods

The present Doctoral Thesis consists of a total of six studies, listed below, comprising samples of patients with IRBD, PD and healthy controls (**Figure 27** depicts the study samples included in the current Thesis).

Study 1

Campabadal, A., Segura, B., Junque, C., Serradell, M., Abos, A., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Compta, Y., Bargallo, N., Iranzo, A., Cortical gray matter and hippocampal atrophy in idiopathic Rapid Eye Movement sleep behavior disorder. *Front Neurol.* 10, 312 (2019) <https://doi:10.3389/fneur.2019.00312>

Study 2

Campabadal. A., Abos, A., Segura, B. Serradell, M., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Compta, Y., Bargallo, N., Junque, C., Iranzo, A., Disruption of posterior brain functional connectivity and its relation to cognitive impairment in idiopathic REM sleep behavior disorder. *Neuroimage Clinical* 25, 102138 (2020). <https://doi.org/10.1016/j.nicl.2019.102138>

Study 3

Campabadal, A., Segura, B., Baggio, H. C., Abos, A., Uribe, C., Garcia-Diaz, A. I., Marti, M.J., Valldeoriola, F., Compta, Y., Bargallo, N., Junque, C., Diagnostic Accuracy, Item Analysis and Age Effects of the UPSIT Spanish Version in Parkinson's Disease. *Arch. Clin. Neuropsychol* acy053 (2018) <https://doi.org/10.1093/arclin/acy053>

Study 4

Campabadal, A., Segura, B., Junque, C., Serradell, M., Abos, A., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Bargallo, N., Iranzo, A., Comparing the accuracy and neuroanatomical correlates of the UPSIT-40 and the Sniffin' Sticks test in REM sleep behavior disorder. *Park. Relat. Disord* 65, 197-202 (2019) <https://doi.org/10.1016/j.parkreldis.2019.06.013>

Study 5

Campabadal, A.*, Inguanzo, A.*, Segura, B., Junque, C., Serradell, M., Abos, A., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Compta, Y., Bargallo, N., Iranzo, A., Cortical Gray Matter progression in Idiopathic REM sleep behavior disorder and its relation to cognitive decline. *Under review*

Study 6

Campabadal, A.*, Uribe, C.*, Segura, B., Baggio, H.C., Abos, A., Garcia-Diaz, A.I., Marti, M.J., Valldeoriola, F., Compta, Y., Bargallo, N., Junque, C., Brain correlates of progressive olfactory loss in Parkinson's disease. *Park. Relat. Disord* **41**, 44–50 (2017) <https://doi.org/10.1016/j.parkreldis.2017.05.005>

Below, a brief description of the principal methodological procedures for each study is provided. A summary of the settings and analyses for each one is given in **Table 11**. Further details are specified along this chapter and in the corresponding manuscripts attached in Chapter 4.

3.1. Study Sample

Study samples 1, 2, and 4

Samples 1, 2, and 4 consist of twenty-one patients with IRBD and twenty-seven healthy participants. One IRBD patient was excluded in *studies 1* and *2* for the presence of MRI artifacts. And one HC in *study 2*, due to excessive head movement. IRBD patients were recruited from our sleep disorders center (Department of Neurology, Hospital Clinic, Barcelona, Catalonia, Spain). Diagnosis of IRBD required a history of dream-enacting behaviors, video-polysomnographic demonstration of REM sleep without atonia, and absence of other neurological diseases (see further details of diagnostic criteria in **Chapter 1**). IRBD patients did not have cognitive or motor complaints at the time of diagnosis. Healthy subjects did not show cognitive, motor, or sleep complaints and were recruited from the *Institut de l'Envel·liment* (Barcelona, Spain).

Study Sample 3

Study sample 3 was mainly selected from a data base of the neuroimaging lab including a larger cohort of healthy elders and Parkinson's disease patients who underwent olfactory evaluation. The study sample included 97 non-demented PD patients and 65 healthy subjects. PD patients were recruited from an outpatient Movement disorders clinic (Parkinson's disease and Movement Disorders Unit, Department of Neurology, Hospital Clinic, Barcelona, Catalonia, Spain). Healthy subjects consisted of individuals who had

previously volunteered who take part in studies of aging at the *Institut de l'Envel·liment* (Barcelona, Spain).

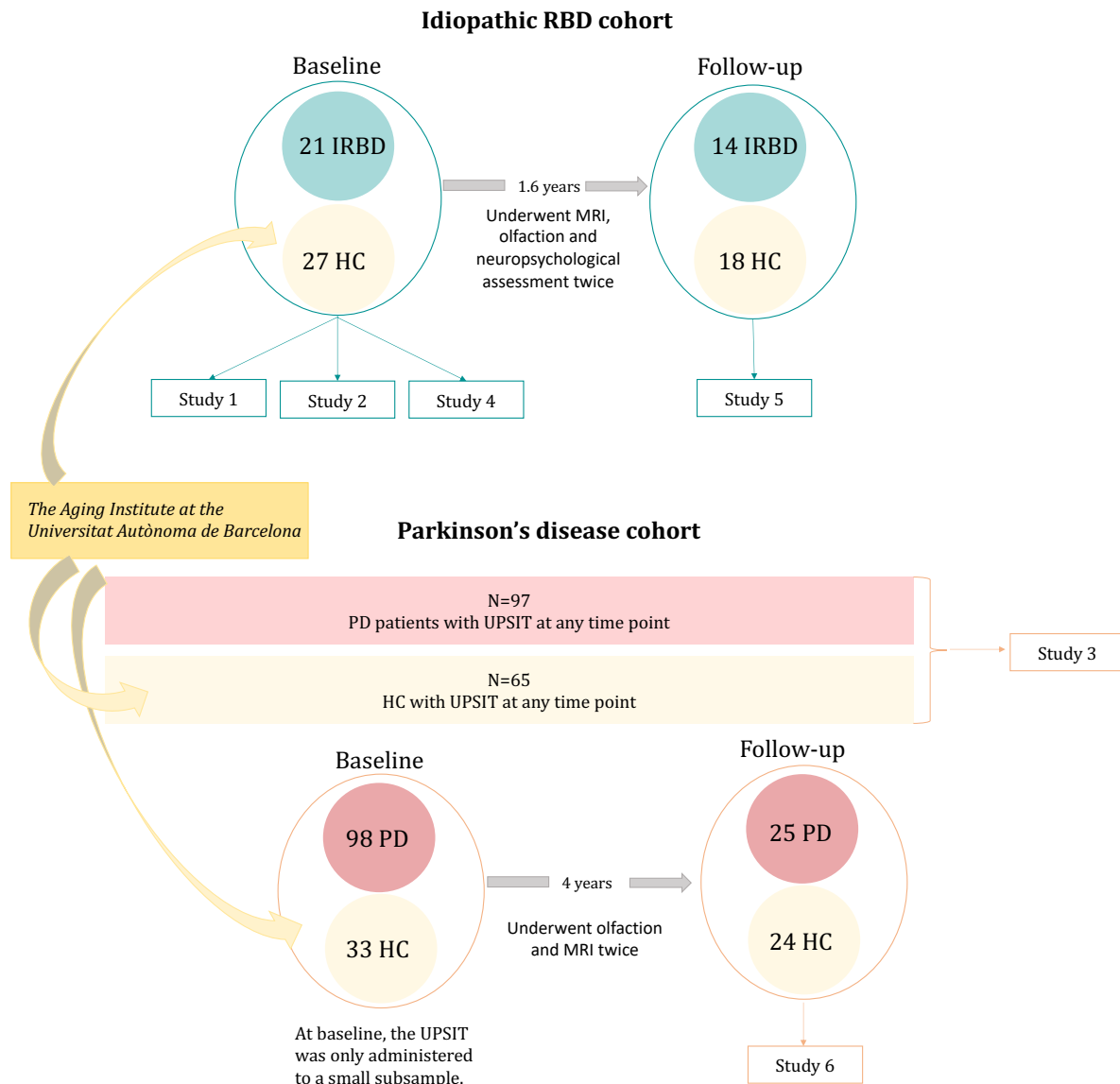


Figure 27. Study sample. The figure placed at the top represents the idiopathic REM sleep behavior disorder (IRBD) cohort. A group of healthy controls (HC), and IRBD patients underwent MRI, olfactory and neuropsychological assessment twice (1.6 years between evaluations). Study samples 1, 2, and 4 included the baseline data, whereas study 5 included those patients that were evaluated twice. Lower figure represents the Parkinson's disease (PD) cohort: The study sample 3, included 97 non-demented PD patients and 65 HC who underwent olfactory evaluation with the UPSIT. Study sample 6, consist of 25 PD and 24 HC who underwent olfactory and MRI evaluation twice (4 years between evaluations). These subjects are part of a cohort of 98 PD and 33 HC, recruited between October 2010 and March 2012 (baseline assessment) and then followed up after four-year period approximately. We only included subjects who underwent olfactory and MRI evaluation at both time points. At baseline, the UPSIT was only administered to a subsample. PD patients were recruited from an outpatient movement disorders clinic (Parkinson's disease and Movement Disorders Unit, Neurology Service, Hospital Clinic, Barcelona, Catalonia, Spain), IRBD patients were recruited from a sleep unit (Multidisciplinary Sleep Unit, Neurology service,

Hospital Clinic, Barcelona, Catalonia, Spain). HC consisted of individuals who had previously volunteered in studies of aging at the aging Institute at the Universitat Autònoma de Barcelona (*Institut de l'envelliment*).

Study Sample 5

Sample 5 included 14 patients with IRBD and 18 healthy subjects who underwent neuropsychological and MRI evaluation at two different time-points; at baseline and after 1.6 ± 0.3 years for patients and 1.6 ± 0.2 years for controls of follow-up ($t = 0.807$; $P = 0.426$). All participants are part of a longitudinal cohort recruited between 2015 and 2017. IRBD patients were recruited from our sleep disorders center (Department of Neurology, Hospital Clinic, Barcelona, Catalonia, Spain), and none of them developed parkinsonism during the follow-up. Healthy subjects did not show cognitive, motor, or sleep complaints and were recruited from the *Institut de l'Envelliment (Barcelona, Spain)*.

Study sample 6

The study sample included 25 PD patients and 24 healthy controls who underwent olfactory and MRI evaluation twice. These subjects are part of a bigger cohort recruited between October 2010 and March 2012. In the present study, we only included subjects who underwent olfactory and MRI evaluation at both time points. In the 2010 cohort, the UPSIT was only administered to a subsample. The mean ($\pm$ standard deviation) follow-up interval was 44.9 ± 5.7 months for patients and 45.9 ± 3.5 months for controls ($F = 0.732$; $P = 0.468$).

Exclusion and Inclusion criteria

The exclusion criteria for all samples consisted of: [1] Presence of psychiatric and/or neurologic comorbidity, [2] low global IQ score estimated by the Vocabulary subtest of the Wechsler Adult Intelligence Scale, 3rd edition (scalar score ≤ 7 points), [3] Mini-mental state examination (MMSE) score < 25 , [4] claustrophobia, [5] MRI movement artifacts, and [6] significant pathological MRI findings other than mild white matter hyperintensities in the FLAIR sequence, and [7] evidence in HC of sleep disorders or mild cognitive impairment.

The specific inclusion criteria for IRBD group (*studies 1, 2, 4, and 5*) were: [1] no cognitive or motor complaints at the time of diagnosis [2] a history of dream-enacting behaviors,

video-polysomnographic demonstration of REM sleep without atonia; [3] absence of other neurological diseases.

Table 11. Summary of main characteristics from each study

Study	Sample size	Design	MRI data	Analysis technique	Neuropsychological and Olfactory assessment
1	20 IRBD 27 HC	Cross-sectional	T1-weighted	▪ CTh ▪ Shape analysis ▪ Subcortical segmentation ▪ Hippocampal subfields segmentation	VFD, JLO, FRT, Phonetic fluency, Semantic fluency, RAVLT, Digit Forward and Backward, Stroop test, SDMT, TMT, BNT
2	20 IRBD 27 HC	Cross-sectional	T1-weighted rs-fMRI	▪ TFNBS ▪ Graph analysis	FRT, Phonetic fluency, RAVLT, Clock Copying Test, Stroop Test, Digit Forward and Backward, SDMT
3	97 PD 65 HC	Cross-sectional	-	-	UPSIT
4	21 IRBD 27 HC	Cross-sectional	T1-weighted	▪ VBM	UPSIT Sniffin' Sticks Extended Test
5	14 IRBD 18 HC	Longitudinal	T1-weighted	▪ CTh	VFD, JLO, FRT, Phonetic fluency, Semantic fluency, RAVLT, Digit Forward and Backward, Stroop test, SDMT, TMT, BNT, UPSIT
6	25 PD 24 HC	Longitudinal	T1-weighted	▪ CTh	UPSIT

Abbreviations: BNT, Boston Naming Test; FRT Short, Facial Recognition test short version; CTh, Cortical Thickness; HC, healthy controls; IRBD, idiopathic rapid eye movement sleep behavior disorder; JLO, Benton's Judgment of Line Orientation test; MMSE, Mini-mental state examination; MRI, Magnetic Resonance Imaging; RAVLT, Rey's Auditory Verbal Learning Test; rs-fMRI, resting state functional MRI; SDMT, Symbol Digits Modalities Test Oral version; TFNBS, Threshold-free network-based statistics; TMT, Trail Making Test; UPSIT, University of Pennsylvania Smell Identification Test; VBM, Voxel-based morphometry; VFD, Visual Form Discrimination.

The specific inclusion criteria for PD group (*studies 3 and 6*) were: [1] the fulfillment of UK PD Society Brain Bank diagnostic criteria for PD (Hughes et al., 1992); [2] no surgical treatment with deep brain stimulation. The exclusion criteria for PD were: [1] Hoehn and Yahr (H&Y) (Hoehn and Yahr, 1967) score > 3 (defined as mild to moderate bilateral disease with some postural instability but physically independent); [2] dementia

according to Movement Disorder Society criteria (Dubois et al., 2007): (a) PD developed prior to the onset of dementia, (b) PD associated with a decreased Global Cognitive Efficiency, (c) cognitive impairment in more than one cognitive domain, and (d) impact on daily living resulting from cognitive deficits, over and above those imposed by motor and autonomic problems.

Specific exclusion criteria for the olfactory assessment (*studies 3, 4, 5, and 6*) were applied: [1] history of nasal bone fracture, [2] diagnosis of rhinitis, or nasal polyps, and [3] upper respiratory tract infections in the 2 weeks prior to testing.

Ethical Statements

Studies 1, 2, 4, and 5

The studies were approved by the Ethics Committee of the University of Barcelona (IRB00003099) and Hospital Clinic (HCB/2014/0224). All subjects provided written informed consent to participate after full explanation of the procedures involved.

Studies 3 and 6

The studies were approved by the Ethics Committee of the University of Barcelona (IRB00003099). All subjects provided written informed consent to participate after full explanation of the procedures involved.

3.2. Clinical and Neuropsychological assessment

In all study samples, neuropsychiatric symptomatology was assessed. Moreover, for IRBD and PD patients motor signs were evaluated. A comprehensive battery of neuropsychological tests was administered for *studies 1, 2, and 5*. In *studies 3, 4, 5, and 6* olfactory testing was included. Further details are specified along the subsequent subsections.

3.2.1. Clinical assessment

Studies 1, 2, 4, and 5

In *studies 1, 2, 4, and 5* the presence of motor symptoms in the IRBD group was evaluated using the International Parkinson and Movement Disorders Society Unified Parkinson's Disease Rating Scale motor section (MDS-UPDRS-III) (Goetz et al., 2008). Neurological examination based on the MDS-UPDRS-III frequently disclosed mild parkinsonian signs, that were, however, not sufficient to diagnose Parkinsonism according to standard criteria. (Postuma et al., 2015a).

To study the presence of RBD-like symptomatology in both patients and controls the Innsbruck REM Sleep Behavior Disorder Inventory (RBD-I) was used (Frauscher et al., 2012).

Studies 3 and 6

In *studies 3 and 6* motor disease severity was evaluated using H&Y staging and the Unified Parkinson's Disease Rating Scale motor section (UPDRS-III) (Fahn and Elton, 1987). All PD patients were taking antiparkinsonian medication consisting of different combinations of L-DOPA, COMT inhibitors, MAO inhibitors, dopamine agonists, and amantadine. In order to standardize doses, levodopa equivalent daily dose (LEDD) was calculated as suggested by Tomlinson and colleagues (Tomlinson et al., 2010). All assessments were done in on state.

Studies 1, 2, 3, and 4

Neuropsychiatric symptomatology was measured by means of Beck Depression Inventory II (Beck A, Steer R, 1996), Starkstein's Apathy Scale (Starkstein et al., 1992), and the Neuropsychiatric Inventory (NPI) (Cummings et al., 1994).

3.2.2. Neuropsychological assessment

In *studies 1, 2, and 5* participants were evaluated with a comprehensive neuropsychological battery (**Table 12**). Attention and working memory were assessed with the Digit Span Forward and Backward, the Symbol Digits Modalities Test (SDMT)-Oral version. Executive functions were evaluated with Trail Making Test (TMT, parts A and B) (in seconds), phonetic (words beginning with the letter “p” in 1 min), and semantic (animals in 1 min) fluencies and the Stroop Color-word Test. Language was assessed by the total number of correct responses in the short version of the Boston Naming Test (BNT). In the memory domain, we assessed total learning recall (sum of correct responses from trial I to trial V), delayed recall (total recall after 20min), and recognition abilities using Rey’s Auditory Verbal Learning Test (RAVLT total, RAVLT recall, and RAVLT recognition, respectively). Visuospatial and visuoperceptual functions were assessed with Benton’s Judgement of Line Orientation (JLO), Visual Form Discrimination (VFD), Clock Copying Test, and Facial Recognition (FRT) tests (Lezak, 2012).

Table 12. Summary of Neuropsychological tests

DOMAIN	TEST
Global Cognition	Mini Mental State Examination (MMSE) (Folstein et al., 1975)
Attention and Working memory	Digit Span Forward and Backward (Wechsler, 1999)
	Symbol Digits Modalities Tests (SDMT) (Smith, 2000)
	Trail Making Test (TMT, in seconds) part A and part B (Lezak et al., 2012)
Executive functions	Phonetic fluency (words beginning with the letter “p” in 1 minute, Lezak et al. 2012)
	Semantic fluency (animals in 1 minute, Lezak et al. 2012)
	Stroop Color-word Test (Stroop, 1935)
Memory	Total learning recall (sum of correct responses from trial I to trial V), delayed recall (total recall after 20 min) and recognition from Rey’s Auditory Verbal Learning Test (RAVLT) (Lezak et al. 2012)
Visuospatial and visuoperceptual functions	Visual Form Discrimination (VFD), (Benton et al., 1994)
	Clock Copying Test (Royall et al., 1998)
	Benton’s Judgement of Line Orientation (JLO), (Benton et al., 1978)
	Facial Recognition test Short form (FRT) (Benton et al., 1994)
Language	Short version of the Boston Naming Test (BNT) (Kaplan et al., 1983)

Expected z scores adjusted for age, sex, and education for each test, and each subject were calculated based on a multiple regression analysis performed in the HC group for the study sample 1. (Aarsland et al., 2009).

In *study 2*, five cognitive domains were obtained using the combination of two neuropsychological tests for each one (computed as the mean of the expected z scores): [1] attention domain, including Digit Span Forward and Backward (WAIS); [2] memory domain, the total learning recall (sum of correct responses from trial I to trial V) and recognition (true recognition after 20 min) from the Rey's Auditory Verbal Learning Test; [3] visuospatial and visuoperceptual (VS/VP) domain, calculated with Benton Facial Recognition and Clock Copying Test; [4] executive function domain, with Stroop Interference and phonetic fluency (words beginning with the letter “p” in 1 min); and [5] mental processing speed domain, using the Symbol Digits Modalities Test-Oral version and Stroop Color test.

3.2.3. Statistical analysis for clinical and neuropsychological measures

In all the studies, statistical analyses and plots of neuropsychological, demographical and clinical data were carried out using the statistical package SPSS 22 and 24 (2013 and 2016; Armonk, NY: IBM Corp.), Matlab (R2019b) and RStudio (2015; available online at: www.rstudio.com). Independent tests (t-test/Mann-Whitney U test) were used to assess group differences between patients and healthy subjects in clinical variables such as neuropsychological tests, and neuropsychiatric symptoms at baseline. Pearson's chi-squared test was applied to assess group differences in categorical variables. In *study 5* and *6*, group-by-time interaction effects in clinical variables between patients and controls were assessed through a repeated-measures general lineal model. Demographical data was included as covariate when needed (further details in the corresponding manuscript). Spearman's correlations were used to study the association among clinical data, and between clinical and neuroimaging data.

3.3. Olfactory assessment

In the present Doctoral Thesis, four of the studies included an olfactory evaluation. Further details regarding measures and analyses are specified in the following subsections and the corresponding manuscripts attached in Chapter 4 (*studies 3, 4, 5, and 6*). **Panel 6** includes a presentation of the olfactory measures used in these studies.

Olfactory assessment *studies 3, 5, and 6*

In *studies 3, 5, and 6*, odor identification was assessed using the Spanish version of the University of Pennsylvania Smell Identification Test (UPSIT) (Doty, 1995).

Olfactory assessment *study 4*

In *study 4*, olfaction was assessed using the Spanish version of the UPSIT (Doty, 1995) and the Sniffin' Sticks Extended Test (Hummel et al., 2007).

3.3.1. Sensitivity, specificity and diagnostic accuracy

One of the aims in *studies 3 and 4*, was to study the diagnostic accuracy of different olfactory tests in a sample of PD (*study 3*) and a sample of IRBD patients (*study 4*). In both studies, the statistical analysis of olfactory data was carried out using the statistical package SPSS-24 (2016; Armonk, NY: IBM Corp.) and RStudio (2015; available online at: www.rstudio.com). Below, a detailed explanation of the procedure used to compute these measures is presented for each study.

Study 3

First, multiple regression analysis with a stepwise method was used to define possible UPSIT score predictors in the whole sample and the PD group. Predictors were chosen based on previous literature (Deeb et al., 2010; Doty, 2012a, 2012b, 1987; Frye et al., 1990; Hubert et al., 1980; Joyner, 1964; Mckinnon et al., 2010) and correlation analysis. Age, PD diagnosis, sex, years of education, and smoking status were introduced as possible predictors for the whole sample regression. For the PD subsample, PD diagnosis was excluded, and clinical measures such as H&Y, UPDRS-III, and LEDD were added.

Secondly, sensitivity, specificity, diagnostic accuracy, positive predictive value, and negative predictive value for PD condition, were calculated for the UPSIT total score, and for each item (**Panel 7** further explains diagnostic measures calculation). Finally,

Pearson's chi-squared test was used to compare the percentage of HC and PD patients who correctly identified each item of the 40 items UPSIT version.

Panel 6 Olfactory measures

The University of Pennsylvania Smell Identification Test (UPSIT-40)

The UPSIT is a standardized multiple-choice scratch-and-sniff test consisting of four test booklets with 10 items each. In accordance with normative instructions, subjects scratch the impregnated area and have to recognize familiar odorants. Then, they are asked to select one of four possible answers for each item. Following normative data presented in the UPSIT manual (Doty et al., 1995), which includes adjustment for age and sex, scores greater than 33 in males and 34 in females are considered to reflect normosmia, and scores lower or equal to 18 reflect anosmia. Microsmia ranges from 33 or 34 (depending on the subject's gender) to 19.

The Sniffin' Sticks Extended Test (Sniffin)

The Sniffin' Sticks Extended Test (www.burghart-mt.de) consists of three subtests:

1. the Sniffin Threshold test (Sniffin-Thr) used to ascertain the patient's olfactory threshold, measuring the lowest concentration of an odorant that the patient can discern. It has 48 Sniffin' Sticks (32 blanks and 16 dilutions of n-butanol)
2. the Sniffin Discrimination test (Sniffin-D), where the patient must differentiate between smells with varying degrees of similarity. It consists of 48 Sniffin' Sticks (48 Sniffin' Sticks = 16 pairs of odorants each with one individual smell)
3. the Sniffin Identification test (Sniffin-I) consists of 16 Sniffin' Sticks with everyday smells which the patients have to identify using a selection card containing four choices.

The results of all subtests are added up to get the total score (Sniffin-Total).



The University of Pennsylvania Smell Identification Test™ (Sensonics) (Image extracted from Doty and Kamath, 2014) and The Sniffin' Sticks test kit (Burghardt®, Wedel, Germany) (Image extracted from Rumeau, Nguyen and Jankowski, 2016)

Study 4

In *study 4*, the Mann-Whitney U test was used to assess group differences between IRBD and controls in clinical and olfactory continuous variables, and Pearson's chi-squared test was applied to assess group differences in categorical variables. Correlations between olfactory measures and clinical scores in IRBD patients were analyzed using Spearman's rho correlation. Finally, stepwise independent binary logistic regressions were computed introducing the diagnosis (either HC or IRBD) as a dependent variable and the olfactory measures as predictive variables. The Bonferroni method was used to correct for multiple comparisons. A receiver operating characteristic (ROC) curve was computed for each olfactory measure. RStudio (2015) was used to calculate statistical differences of the area under the curve (AUC) values from ROC (AUROC) analysis. Sensitivity, specificity, diagnostic accuracy, positive predictive value, and negative predictive value for IRBD were calculated according to the resulting optimal cut-off (see **Panel 7**).

Panel 7 Diagnostic accuracy measures

Altered test performances were considered as positive findings, whereas correct performance defined negatives. True positives (TP) were defined as the number of patients with altered performance in a certain test; true negatives (TN) as the number of HC with correct performance; false positives (FP) were calculated as the number of HC with altered performance; finally, false negatives (FN) were described as the number of patients who had scores greater than the optimal cut-off.

The following diagnostic measures were calculated:

- **Sensitivity**, defined as the probability of testing positive when the disease is present:
$$TP / (TP + FN)$$
- **Specificity**, defined as the probability of testing negative when the disease is absent:
$$TN / (FP + TN)$$
- **Positive predictive value (PPV)**, calculated as the percentage of subjects with a positive test who actually have the disease:
$$TP / (TP + FP)$$
- **Negative predictive value (NPV)**, defined as the percentage of subjects with a negative test who do not have the disease:
$$TN / (TN + FN)$$
- **Overall diagnostic accuracy** was defined as the proportion of correctly classified subjects among all subjects:
$$(TP + TN) / (TP + TN + FP + FN)$$

3.4. MRI acquisition

MRI data were acquired in a 3-Tesla Siemens scanner (Magnetom Trio Tim scanner syngo) using an eight-channel head coil at the *Unitat d'Imatge per Ressonància Magnètica IDIBAPS (Institut d'Investigacions Biomèdiques Agustí Pi i Sunyer), Hospital Clínic de Barcelona, Barcelona*. All the images were inspected visually to ensure that they did not contain MRI artifacts or excessive movement before analysis.

In the present Doctoral Thesis, the scanning protocol included the following MRI sequences:

- Structural T1 images: high-resolution 3-dimensional T1-weighted images acquired in the sagittal plane (TR=2300 ms, TE=2.98 ms, TI=900 ms, 240 slices, FOV=256 mm, 1-mm isotropic voxel) in *studies 1, 2, 4, 5, and 6*.
- FLAIR: axial FLAIR sequence (TR = 9000 ms, TE = 96 ms) in *studies 1, 2, 4, 5, and 6*.
- Resting-state functional images: a resting-state 10-min-long functional gradient-echo echo-planar imaging sequence (240 T2*-weighted, TR=2500 ms, TE=28 ms, flip angle=80°, slice thickness=3 mm, FOV=240 mm, with 3-mm isotropic voxels) was acquired in *study 2*. Subjects were instructed to keep their eyes closed, not to fall asleep, and not to think anything in particular.

3.5. Neuroimaging techniques

After acquiring the MRI data, the images were preprocessed and analyzed. In this section, I will present the neuroimaging techniques used in *studies 1, 2, 4, 5, and 6*. Further technical details are exposed in the corresponding manuscripts attached in Chapter 4.

3.5.1. Structural MRI

In this work, T1-weighted MR images had two main purposes. Firstly, structural images were used to obtain cortical parcellation, subcortical volumetry, and hippocampal subfield segmentation. Secondly, given that structural images have higher spatial resolution compared to other acquisitions, they were also employed in registration and normalization preprocessing steps in functional *study 2*, allowing a better transformation from the subject native space to a standardized space.

In *studies 1, 4, 5, and 6* structural analyses were computed. Specifically, in *study 1* Cortical thickness, hippocampal shape analysis, subcortical and hippocampal subfields segmentation approaches were applied. In *study 4*, voxel-based morphometry was used to study the relationship between smell impairment and GM volume in the olfactory system. In *studies 5 and 6*, longitudinal analysis of cortical thickness was used to study the GM progression in IRBD and PD. Finally, in *study 6*, the percentage of change of the striatal nuclei was calculated.

Cortical thickness

Cortical thickness is calculated as the distance between the gray/white matter boundary and the pial surfaces at each vertex of the cortical mantle (Fischl and Dale, 2000) (**Figure 28**). In *studies 1, 5, and 6*, FreeSurfer software 5.1 version was used to estimate cortical thickness (available at <https://surfer.nmr.mgh.harvard.edu/> since 2011). The 3D cortical surface model used in this estimation is created using intensity and continuity information, as described in detail by the authors (Fischl and Dale, 2000). Independent steps are performed in the initial preprocessing of images for each subject: removal of non-brain tissue, automated Talairach transformation, intensity normalization (Sled et al., 1998), tessellation of the gray matter/white matter boundary, automated topology correction (Ségonne et al., 2007), and accurate surface deformation to optimally place the gray matter/white matter and gray matter/cerebrospinal fluid (CSF) boundaries (Fischl and Dale, 2000). The resulting representation of cortical thickness is calculated as the distance between white and pial surfaces at each vertex of the reconstructed cortical mantle (Sled et al., 1998). In our study, results for each subject were carefully inspected visually to ensure accuracy of registration, skull stripping, segmentation, and cortical surface reconstruction. Cortical thickness maps were smoothed using a circularly symmetric Gaussian kernel across the surface with a full width at half maximum (FWHM) of 15 mm.

Comparisons between groups were assessed using a vertex-by-vertex general linear model introducing demographical covariates when needed (further details in the corresponding manuscript). Vertex-wise correlations between cortical thickness and cognitive measures were computed in the *study 1*. In order to avoid clusters appearing significant purely by chance (i.e., false positives), Monte Carlo Null-Z Simulation with

10,000 iterations was applied to cortical thickness maps to provide clusterwise correction for multiple comparisons; results were thresholded at a corrected p-value of 0.05 (Hagler et al., 2006).

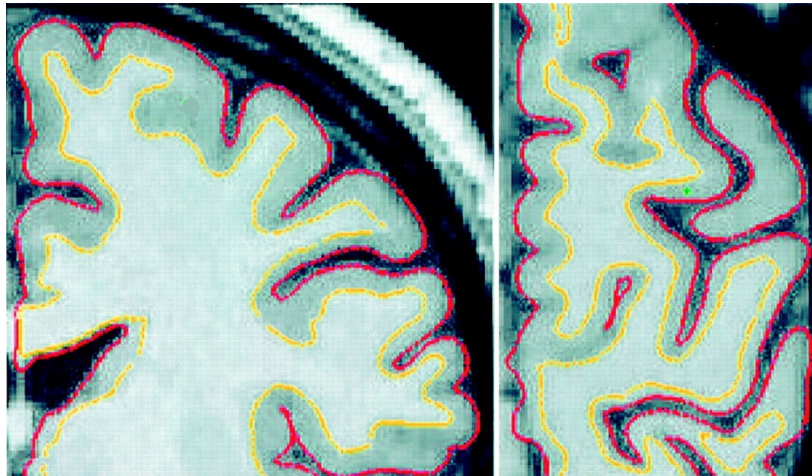


Figure 28. Coronal (Left) and horizontal (Right) slices of the left hemisphere with gray/white (yellow) and pial (red) surfaces overlaid. Adapted from Fischl & Dale (2000).

Longitudinal analysis of Cortical thickness

In *studies 5* and *6* after processing each subject cross-sectionally, in order to perform the longitudinal analyses of the data, within-subject templates (Reuter and Fischl, 2011) and the corresponding longitudinal files were created for each time point and subject. Briefly, a template volume for each subject, using information from all of their time points (time 1 and time 2), and an average image were created using robust, inverse, consistent registration (Reuter et al., 2010). All time points were constructed through unbiased mean images and later aligned. After registration and creation of the templates, images from all time points were mapped to the template location and averaged and processed with the default cross-sectional stream. The symmetrized percent change (SPC) was used for longitudinal analyses of cortical thickness. Cortical thickness SPC is the rate in mm/year with respect to the average thickness $[(\text{Thickness at time point 1} - \text{Thickness at time point 2}) / \text{Interval between assessments}] / [0.5 * (\text{Thickness at time point 1} + \text{Thickness at time point 2})]$. In aging or disease, SPC is expected to be negative in most regions (<https://surfer.nmr.mgh.harvard.edu/fswiki/LongitudinalTwoStageModel>).

Comparisons between groups in the cortical thickness SPC were performed using a vertex-by-vertex general linear model. Time effects in each group and group-by-time interaction effects were computed, SPC was included as a dependent factor and group as an independent factor. Regression models included SPC as an independent factor and

cognitive or olfactory scores as dependent factors. Demographical covariates were included when needed (further details in the corresponding manuscript). In order to avoid clusters appearing significant purely by chance (i.e., false positives), Monte Carlo null-Z simulation with 10,000 iterations was applied to cortical thickness maps to provide clusterwise correction for multiple comparisons. Simulation was applied for negative time effects (time 2 < time 1) in each group, on all other models, simulation was applied for absolute results. Results were thresholded at a corrected p-value <0.05.

Subcortical segmentation

Automated subcortical segmentation performed with FreeSurfer (version 5.1) was used to estimate subcortical volumetry in *studies 1* and *6*. In this procedure, each voxel in the normalized brain volume is assigned one of about forty labels (<http://freesurfer.net/fswiki/SubcorticalSegmentation>). The general linear model was used to assess group differences between patients and healthy subjects in MRI volumetric data. Estimated Total Intracranial Volume (eTIV) was obtained to correct volumetric data for inter-individual differences in brain sizes.

Longitudinal analysis of subcortical volumes

In *study 6*, the SPC of striatal volumes was computed to investigate longitudinal differences between groups. The SPC of UPSIT scores was also calculated and correlated with SPC of striatal volumes. Control of the false-discovery rate to 5% (FDR) was used to correct for multiple testing (Benjamini, 2010).

Hippocampal Subfields segmentation

In *study 1*, in order to characterize GM abnormalities in the hippocampal formation two different techniques were used: hippocampal subfield segmentation and hippocampal shape analysis. For the first approach, FreeSurfer automated hippocampal subfield segmentation [version 6.0 (Iglesias et al., 2015)] was used to estimate individual hippocampal subfield volumes. Segmentation analysis was conducted for the right hippocampus. Since no previous works have studied hippocampal subfields in IRBD patients, an exploratory analysis was undertaken, and results were considered significant if they showed a p-value of $p < 0.05$. All hippocampal subfield measures were introduced in a general linear model with eTIV as a covariate.

Hippocampal Shape Analysis

The second approach to investigate hippocampal abnormalities in *study 1*, was done by the FIRST, FSL's model-based segmentation/registration tool (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST>) (Patenaude et al., 2011). Segmentation of both hippocampi with automated boundary correction was generated. We then used first-utils to run a vertex-wise analysis on the results. FSL's Randomize script (Anderson and Robinson, 2001; Winkler et al., 2014) with 5,000 random permutations of the data was used to study inter-group differences. ETIV was estimated with FSL according to ENIGMA's imaging protocol (<http://enigma.usc.edu/>). Age and eTIV were introduced as covariates in the analyses. A subsequent analysis introducing both age and sex as covariates was computed. All results were thresholded at $p < 0.05$. Correlations with cognitive variables were performed.

Voxel-based morphometry of the olfactory system

Voxel-based morphometry estimates the amount of GM in a voxel through its signal intensity (Good et al., 2001), allowing for voxel-wise comparison of local GM concentrations. In *study 4*, VBM was used to investigate the relationship between olfactory performance and GM volume in the olfactory system. To this end, a region of interest was first defined. Specifically, a mask including the olfactory system regions was created including the amygdalae; hippocampi; thalami; insular cortices; parahippocampal gyri; superior frontal gyrus, orbital part; middle frontal gyrus, orbital part; gyrus rectus; olfactory cortex; and the limbic subregion of the striatum (including the nucleus accumbens and the ventral portion of the caudate nucleus and putamen bilaterally). The Automated Anatomical Labeling atlas was used to create the corresponding masks, except the limbic striatum mask, which was obtained from the Oxford-GSK-Imanova striatal connectivity atlas (available in FSL).

Structural data were analyzed with FSL-VBM (Douaud et al., 2007), an optimized VBM protocol (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM>) (Good et al., 2001) carried out with FSL tools (Smith et al., 2004). First, structural images were brain-extracted and gray-matter-segmented before being registered to the MNI 152 standard space using non-linear registration. The resulting images were averaged to create a study-specific template, to which native gray matter images were non-linearly re-registered. Second, all native gray matter images were non-linearly registered to this study-specific template,

resampled to a voxel size of $2 \times 2 \times 2 \text{ mm}^3$, and modulated to correct for local expansion or contraction due to the non-linear component of the spatial transformation. The olfactory system mask was created to define a search volume for subsequent statistical testing. A voxelwise general linear model with non-parametric permutation testing (5,000 permutations) was used alongside threshold-free cluster enhancement for statistical inference. False-discovery rate (FDR) correction was applied for multiple comparisons correction. Statistical significance was established at FDR-corrected p-values (q-values) < 0.05 .

3.5.2. Functional MRI

Functional MRI measures brain activity by detecting increase blood oxygen level-dependent (BOLD) signal, which is an indirect measure of neuronal activity. Two different modalities are commonly used: task-based fMRI and resting-state fMRI. In task-based fMRI, scanning protocol is done while subjects are performing specific tasks, whereas in rs-fMRI, no specific cognitive demands are required, and the participant is instructed to remain awake and still and not to think anything in particular. In *study 2*, fMRI resting-state modality was used to characterized functional connectivity in IRBD. Functional connectivity gives information about the relationship between BOLD signals obtained from two separate regions of the brain (**Figure 29**) (Bijsterbosch et al., 2017). In the next subsection, I will describe data processing procedures and the different approaches used to study functional connectivity in *study 2*.

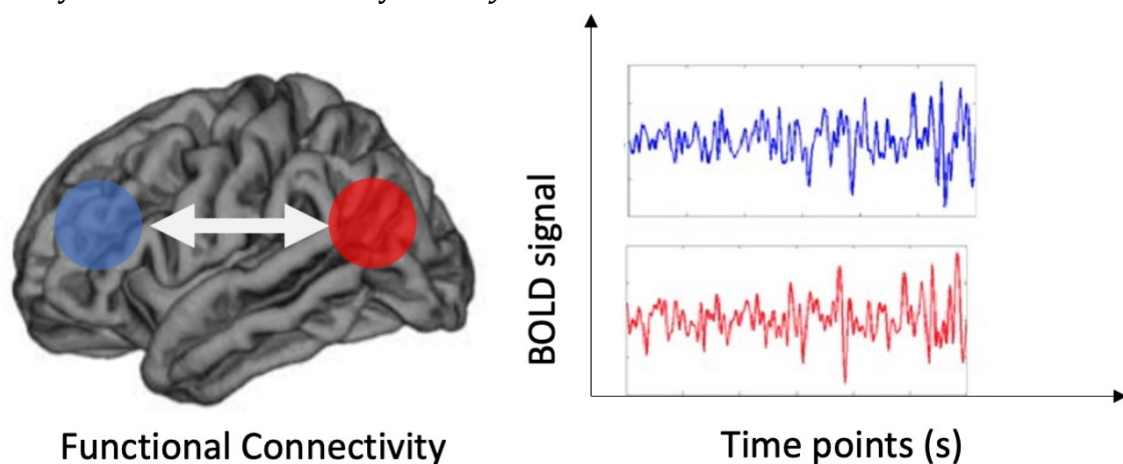


Figure 29. Functional connectivity gives information about the relationship between BOLD signals obtained from two separate regions of the brain (in the picture highlighted in red and blue). The underlying assumption is that if two different regions show similarities in their BOLD signals over time, they are functionally connected (Bijsterbosch et al., 2017).

Data processing

Basic functional image preprocessing was done using AFNI (<https://afni.nimh.nih.gov/>) and FSL (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSL>). First steps included the removal of the first five volumes to allow magnetization stabilization, despiking, motion correction, brain extraction, grand-mean scaling, linear detrending, and temporal filtering (maintaining frequencies above 0.01 Hz).

To remove the effects of head motion and other non-neural sources of signal variation from the functional data, we used an Independent Component Analysis based strategy for Automatic Removal of Motion Artifacts (ICA-AROMA) (Pruim et al., 2015). ICA-AROMA decomposes the data via ICA and automatically identifies which of these components are related to head motion by using four robust and standardized features. As quality control measure to assess the efficacy of ICA-AROMA in reducing relationship between signal variation and motion, we performed correlations between framewise head displacement (Power et al., 2012) and overall signal variation (defined as the voxel-wise root mean square intensity difference between subsequent time points) after regressing the ICA-AROMA components. These two measures should not correlate significantly because signal change should not be explained by head motion. Additionally, average WM and CSF signals obtained from the FreeSurfer masks, and six motion parameters were also regressed out of the functional images. Regarding head motion parameters, an exclusion cut-off was established for mean interframe head motion at $\geq 0.3\text{mm}$ translation or 0.3° rotation; and for maximum interframe head motion at $\geq 1\text{mm}$ translation or 1° rotation. In *study 2*, one HC was excluded due to excessive head movement.

After preprocessing, fMRI data needed to be registered to a common standard space for group analyses. This step was done at the subject level: first, native functional images were linearly registered to their corresponding structural image; secondly, structural images, with better anatomical resolution, were registered to the MNI152 standard template; thirdly, transformation matrices from the previous steps were concatenated, obtaining a transformation matrix to register functional images to the standard space; finally, the combined transformation matrices were applied to the preprocessed functional images to register them to the standard MNI space.

Functional analyses

Network-based statistics (NBS) and graph-derived metrics allow studying the brain as a complex network characterizing the dynamic interactions between different brain regions (Telesford et al., 2011). The main objective in *study 2* was to characterize whole-brain functional connectivity in IRBD using these approaches.

Characterization of brain functional connectivity

In order to test for intergroup differences in interregional connectivity between IRBD and HC, we used Threshold-free network-based statistics (TFNBS) (Baggio et al., 2018), which performs statistical inference on brain graphs. This approach combines NBS (Zalesky et al., 2010), frequently used for statistical analysis of brain graphs, and threshold-free cluster enhancement, a common method in voxel-wise statistical inference (Smith and Nichols, 2009). One of the main characteristics of TFNBS is that it allows generating edge-wise significance values that can be used to select relevant connectivity features. The 246 regions defined in the Brainnetome Atlas (<https://atlas.brainnetome.org/bnatlas.html>) were used for the characterization of global functional connectivity (Fan et al., 2016). Head motion, age, and sex were introduced as covariates in all analyses.

Graph-theory connectivity

The analysis of brain functional connectivity was complemented with topological information derived from graph theory, allowing a description of global (whole-brain) and local (nodal) properties of the network (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010). The Brain Connectivity Toolbox (BCT) was used to extract the topological parameters, including global and local clustering coefficient, global and local node degree, “small-worldness”, path length, local efficiency and betweenness centrality (See **Figure 30** and refer to Rubinov and Sporns, 2010 for detailed definitions and calculations of the graph metrics included in this work). Head motion, age, and sex were introduced as covariates in all analyses. Given that graph metrics were computed using nine density thresholds (maintaining the 5% to 25% strongest edges, at intervals of 2.5%), only those results consistently found in more than one are reported.

Differences between HC and IRBD in connectivity measures were tested with the general lineal model using in-house MATLAB scripts. Statistical significance was established using

Monte Carlo simulations with 10,000 permutations. Two-tailed p-values were calculated as the proportion of values in the null distribution more extreme than those observed in the actual model.

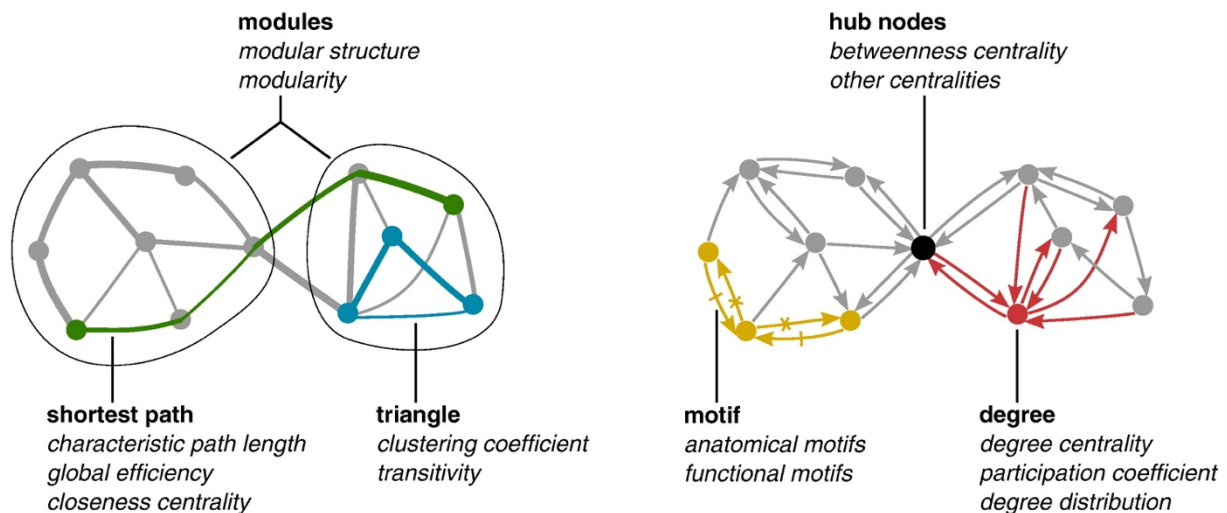


Figure 30. Measures of network topology. An illustration of key complex network measures (in italics) described in Rubinov and Sporns (2010). These measures are typically based on basic properties of network connectivity (in bold type). Thus, measures of integration are based on shortest path lengths (green), while measures of segregation are often based on triangle counts (blue) but also include more sophisticated decomposition into modules (ovals). Measures of centrality may be based on node degree (red) or on the length and number of shortest paths between nodes. Hub nodes (black) often lie on a high number of shortest paths and consequently often have high betweenness centrality. Patterns of local connectivity are quantified by network motifs (yellow). An example three-node and four-link anatomical motif contains six possible functional motifs, of which two are shown—one motif containing dashed links, and one motif containing crossed links. Text and figure extracted from Rubinov and Sporns (2010).

CHAPTER 4

Results

Study 1

Campabadal, A., Segura, B., Junque, C., Serradell, M., Abos, A., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Compta, Y., Bargallo, N., Iranzo, A., Cortical gray matter and hippocampal atrophy in idiopathic Rapid Eye Movement sleep behavior disorder. *Front Neurol* **10**, 312 (2019) <https://doi:10.3389/fneur.2019.00312>



Cortical Gray Matter and Hippocampal Atrophy in Idiopathic Rapid Eye Movement Sleep Behavior Disorder

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Objective: In this study we investigate cortical and subcortical gray matter structure in patients with Idiopathic REM-sleep behavior disorder (IRBD), and their relation to cognitive performance.

Methods: This study includes a sample of 20 patients with polysomnography-confirmed IRBD and 27 healthy controls that underwent neuropsychological and T1-weighted MRI assessment. FreeSurfer was used to estimate cortical thickness, subcortical volumetry (version 5.1), and hippocampal subfields segmentation (version 6.0). FIRST, FSL's model-based segmentation/registration tool was used for hippocampal shape analysis.

Results: Compared with healthy subjects, IRBD patients showed impairment in facial recognition, verbal memory, processing speed, attention, and verbal naming. IRBD patients had cortical thinning in left superior parietal, post-central, and fusiform regions, as well as in right superior frontal and lateral occipital regions. Volumetric and shape analyses found right hippocampal atrophy in IRBD, specifically in posterior regions. Hippocampal subfields exploratory analysis identified significant differences in the right CA1, molecular layer, granule cell layer of dentate gyrus, and CA4 of this patients. No correlations were found between cognitive performance and brain atrophy.

Conclusion: This work confirms the presence of posterior based cognitive dysfunction, as well as cortical and right hippocampal atrophy in IRBD patients.

Keywords: idiopathic REM-sleep behavior disorder, MRI-magnetic resonance imaging, cortical gray matter atrophy, hippocampal atrophy, hippocampal subfields, cognition

INTRODUCTION

Rapid eye movement (REM) sleep behavior disorder (RBD) is a parasomnia characterized by abnormal motor and vocal behaviors associated with unpleasant dreams and increased electromyographic activity during REM sleep (1). Polysomnography with audiovisual recording is needed to confirm the diagnosis of RBD and to exclude other sleep disorders that can mimic its symptoms, including obstructive sleep apnea, nocturnal hallucinations, and confusional arousals (2).

Over the past years, the idiopathic form of RBD (IRBD) has been increasingly recognized as a prodromal phase of some neurodegenerative diseases, mainly of alpha-synucleinopathies such as dementia with Lewy bodies (DLB), Parkinson's disease (PD), and multiple system atrophy (3, 4). The risk for IRBD patients of eventually developing a neurodegenerative disease increases with time (3, 5). IRBD is by far the strongest and most specific clinical predictor of neurodegenerative disease available (6). Therefore, there is growing interest in describing neuroimaging and cognitive biomarkers of brain neurodegeneration in this prodromal disorder.

With an estimated prevalence of 50%, cognitive impairment is frequently present in IRBD patients (7), affecting mainly attention, verbal memory, visuospatial, and executive domains (8). Furthermore, previous works demonstrated cognitive decline in attention and executive functions strongly predict conversion to DLB in IRBD patients (9).

Structural brain imaging techniques have been used to investigate neurodegenerative changes in IRBD. Previous works using diffusion-tensor imaging showed that IRBD patients had decreased fractional anisotropy in the tegmentum of the midbrain, increased mean diffusivity in the pontine reticular formation (10), and microstructural changes in widespread areas including brainstem, substantia nigra, temporal lobe, and visual stream (11). Voxel-based morphometry (VBM) studies revealed that IRBD patients had gray matter volume reduction in the superior frontal sulcus (12), anterior cingulate gyrus, caudate nucleus (13), anterior lobes of the cerebellum, tegmental portion of the pons, and parahippocampal gyrus (14). Studies addressing cortical thickness, in turn, have reported thinning in the frontal cortex, lingual gyrus, and fusiform gyrus (12), as well in medial superior frontal, orbitofrontal, anterior cingulate cortices, and dorsolateral primary motor cortex (13).

In the current work, we aimed to investigate [1] cognitive impairment in a sample of IRBD patients compared to healthy controls, [2] MRI gray matter changes and [3] correlation between cognitive impairment and cortical and subcortical atrophy.

METHODS

Participants

Twenty patients with IRBD without cognitive or motor complaints at the time of diagnosis were recruited from our multidisciplinary sleep unit. Diagnosis of IRBD required a history of dream-enacting behaviors, video-polysomnographic

demonstration of REM sleep without atonia and absence of other neurological diseases (15, 16). Twenty-seven healthy subjects without cognitive, motor, or sleep complaints were recruited from the Institut de l'Envel·liment (Barcelona, Spain).

Exclusion criteria consisted of: [1] Presence of psychiatric and/or neurologic comorbidity, [2] low global IQ score estimated by the Vocabulary subtest of the Wechsler Adult Intelligence Scale, 3rd edition (scalar score ≤ 7 points), [3] MMSE score < 25 , [4] claustrophobia, [5] MRI movement artifacts, and [6] evidence in HC of sleep disorders or mild cognitive impairment.

The study was approved by the Ethics Committee of the University of Barcelona (IRB00003099) and Hospital Clinic (HCB/2014/0224). All subjects provided written informed consent to participate after full explanation of the procedures involved.

Neuropsychological and Clinical Assessment

Participants were evaluated with a neuropsychological battery assessing the main cognitive domains impaired in alpha-synuclein-related neurodegenerative diseases. Attention and working memory were assessed with the Trail Making Test (TMT, parts A and B) (in seconds), Digit Span Forward and Backward, the Stroop Color-word Test, and the Symbol Digits Modalities Test (SDMT)-Oral version. Executive functions were evaluated with phonemic (words beginning with the letter "p" in 1 min) and semantic (animals in 1 min) fluencies. Language was assessed by the total number of correct responses in the short version of the Boston Naming Test (BNT). In the memory domain, we assessed total learning recall (sum of correct responses from trial I to trial V), delayed recall (total recall after 20 min), and recognition abilities using Rey's Auditory Verbal Learning Test (RAVLT total, RAVLT recall, and RAVLT recognition, respectively). Visuospatial and visuo-perceptual functions were assessed with Benton's Judgement of Line Orientation (JLO), Visual Form Discrimination (VFD), and Facial Recognition (FRT) tests (17). Expected z scores adjusted for age, sex, and education for each test and each subject were calculated based on a multiple regression analysis performed in the HC group (18).

Beck Depression Inventory II (19), Starkstein's Apathy Scale (20), and the Neuropsychiatric Inventory (NPI) (21) were used to assess neuropsychiatric symptomatology.

MRI Acquisition

MRI data were acquired with a 3T scanner (MAGNETOM Trio, Siemens, Germany). The scanning protocol included high-resolution 3-dimensional T1-weighted images acquired in the sagittal plane (TR = 2,300 ms, TE = 2.98 ms, TI = 900 ms, 240 slices, FOV = 256 mm; 1 mm isotropic voxel) and an axial FLAIR sequence (TR = 9,000 ms, TE = 96 ms).

MRI Preprocessing and Cortical Thickness Analysis

FreeSurfer software was used to estimate cortical thickness. This study sample is part of an extensive cohort recruited since 2010, for that reason T1-weighted images were preprocessed

with the FreeSurfer 5.1 version (available at <https://surfer.nmr.mgh.harvard.edu/> since 2011). The 3D cortical surface model used in this estimation is created using intensity and continuity information, as described in detail by the authors (22). Independent steps are performed in the initial preprocessing of images for each subject: removal of non-brain tissue, automated Talairach transformation, intensity normalization (23), tessellation of the gray matter/white matter boundary, automated topology correction (24), and accurate surface deformation to optimally place the gray matter/white matter and gray matter/cerebrospinal fluid (CSF) boundaries (22). The resulting representation of cortical thickness is calculated as the distance between white and gray matter surfaces at each vertex of the reconstructed cortical mantle (23). In our study, results for each subject were carefully inspected visually to ensure accuracy of registration, skull stripping, segmentation, and cortical surface reconstruction. Cortical thickness maps were smoothed using a circularly symmetric Gaussian kernel across the surface with a full width at half maximum (FWHM) of 15 mm.

Comparisons between groups were assessed using a vertex-by-vertex general linear model introducing age as a covariate (FreeSurfer 5.1). A subsequent analysis introducing both age and sex as covariates was computed. Vertex-wise correlations between cortical thickness and cognitive measures were computed in the IRBD group. In order to avoid clusters appearing significant purely by chance (i.e., false positives), Monte Carlo Null-Z Simulation with 10,000 iterations was applied to cortical thickness maps to provide clusterwise correction for multiple comparisons; results were thresholded at a corrected p -value of 0.05 (25).

Subcortical Segmentation

Automated subcortical segmentation performed with FreeSurfer (version 5.1) was used to estimate subcortical volumetry. Estimated Total Intracranial Volume (eTIV) was obtained to correct volumetric data for inter-individual differences in head sizes.

TABLE 1 | Demographic and clinical characteristics.

	HC ($n = 27$)	IRBD ($n = 20$)	Test stat/ p -value
Age (years)	66.4 (9.9)	71.3 (7.8)	1.83/0.073
Education (years)	12.19 (4.3)	11.9 (4.9)	0.25/0.804
Sex (male/female)	(13/14)	(14/6)	2.24/0.134
Neuropsychiatric Inventory	1.9 (2.5)	6.4 (5.9)	3.17/0.004
Beck Depression Inventory II	5.1 (4.7)	7.0 (5.0)	1.30/0.201
Starkstein's Apathy Scale	8.9 (5.3)	10.9 (5.6)	1.20/0.237
Disease duration (years)	–	3.1 (3.5)	–
MDS-UPDRSIII	–	2.4 (1.9)	–

HC, healthy controls; IRBD, idiopathic rapid eye movement sleep behavior disorder; MDS-UPDRSIII, Movement Disorder Society Unified Parkinson's Disease Rating Scale motor section. Group differences between HC and IRBD were tested using Student's t -test. Differences in categorical variables were analyzed with Pearson's chi-squared test. Measures are presented as mean (standard deviation) for continuous variables. In bold highlighted those results that reached statistical significance.

Hippocampal Shape Analysis

FIRST, FSL's model-based segmentation/registration tool was used for hippocampal shape analysis (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FIRST>) (26). Segmentation of both hippocampi with automated boundary correction were generated. We then used first-utils to run a vertex-wise analysis on the results. FSL's Randomize script (27, 28) with 5,000 random permutations of the data was used to study inter-group differences. ETIV was estimated with FSL according to ENIGMA's imaging protocol (<http://enigma.usc.edu/>). Age and eTIV were introduced as covariates in the analyses. A subsequent analysis introducing both age and sex as covariates was computed. All results were thresholded at $p < 0.05$. Correlations with cognitive variables were performed.

Segmentation of Hippocampal Subfields

FreeSurfer automated hippocampal subfield segmentation [version 6.0 (29)], was used to estimate individual hippocampal subfield volumes. Segmentation analysis was conducted for the right hippocampus. Since no previous works have studied hippocampal subfields in IRBD patients, an exploratory analysis

TABLE 2 | Group comparison of neuropsychological performance.

	HC ($n = 25$)	IRBD ($n = 20$)	Test stat/ p -value	Effect size
MMSE	29.4 (0.8)	28.2 (1.6)	10.21/0.003*	0.95
VFD	29.6 (2.6)	29.2 (3.5)	0.01/0.914	
JLO	24.7 (3.8)	22.8 (5.2)	1.08/0.303	
FRT Short	23.1 (1.9)	21.4 (2.3)	4.96/0.031*	0.81
Phonetic fluency	15.8 (4.4)	12.9 (4.8)	3.38/0.073	
Semantic fluency	19.5 (3.2)	15.4 (4.9)	9.24/0.004*	0.99
RAVLT total	48.9 (6.8)	41.7 (8.5)	8.44/0.006*	0.94
RAVLT recall	10.4 (2.6)	8.0 (3.5)	6.41/0.015*	0.78
RAVLT recognition	14.6 (0.8)	13.7 (1.5)	6.28/0.016	0.75
Direct Digits	5.2 (1.3)	5.3 (1.5)	0.05/0.827	
Indirect Digits	4.2 (1.1)	4.4 (0.8)	0.44/0.509	
Stroop W	96.6 (14.7)	89.0 (16.8)	1.68/0.202	
Stroop C	64.4 (10.6)	55.9 (11.1)	5.21/0.028	0.78
Stroop WC	34.3 (11.3)	29.5 (9.7)	1.18/0.283	
SDMT	48.4 (9.2)	39.1 (12.5)	6.10/0.018	0.85
TMTA	36.9 (11.4)	53.9 (23.7)	8.95/0.005*	0.91
TMTB	94.1 (49.8)	143.4 (67.5)	5.60/0.023*	0.83
BNT	13.9 (0.9)	13.2 (0.9)	5.30/0.026*	0.78

BNT, Boston Naming Test; FRT Short, Facial Recognition test short form; HC, healthy controls; IRBD, idiopathic rapid eye movement sleep behavior disorder; JLO, Benton's Judgment of Line Orientation test; MMSE, Mini-mental state examination; RAVLT, Rey's Auditory Verbal Learning Test; RAVLT recall, total recall after 20 min; RAVLT recognition, total recognition after 20 min; RAVLT total, sum of correct responses from trial I to trial V; Symbol Digits Modalities Test (SDMT)-Oral version; Stroop W, Stroop Word; Stroop C, Stroop Color; Stroop WC, Stroop Word-Color; TMTA, Trail Making Test part A; TMTB, Trail Making Test part B; VFD, Visual Form Discrimination. Group differences between HC and IRBD were tested using general linear model with age as a covariate. In bold highlighted those results that reached statistical significance. *Comparisons that remain significant ($p < 0.05$) when age, sex and years of education were regressed out. Cohen's d effect size was computed for significant effects. Measures are presented as mean (standard deviation).

was undertaken, and results were considered significant if they showed a p -value of $p < 0.05$. All hippocampal subfield measures were introduced in a general linear model with eTIV as a covariate.

Statistical Analyses

Statistical analyses of neuropsychological, demographic, clinical, and MRI volumetric data were carried out using the statistical package SPSS-24 (2016; Armonk, NY: IBM Corp.). Student's t -test was used to assess group differences between IRBD and healthy subjects in clinical variables. The general linear model was used to assess group differences in neuropsychological variables and MRI volumetric data. Pearson's chi-squared test was applied to assess group differences in categorical variables. Correlations between structural measures and neuropsychological scores were analyzed using Pearson's

correlation. Age, sex, and eTIV were introduced as covariates when needed.

RESULTS

Sociodemographic, Clinical, and Neuropsychological Data

Groups did not differ significantly in any demographical measure. The interval between IRBD diagnosis and time of neuroimaging was 3.1 ± 3.5 years. Inter-group comparisons of clinical, sociodemographic, and neuropsychological variables are shown in Tables 1, 2, respectively.

Cortical Thickness Analysis

Results of whole-brain cortical thickness analyses showed that IRBD patients had cortical thinning compared with HC in left superior parietal, post-central, and fusiform regions, as well as in

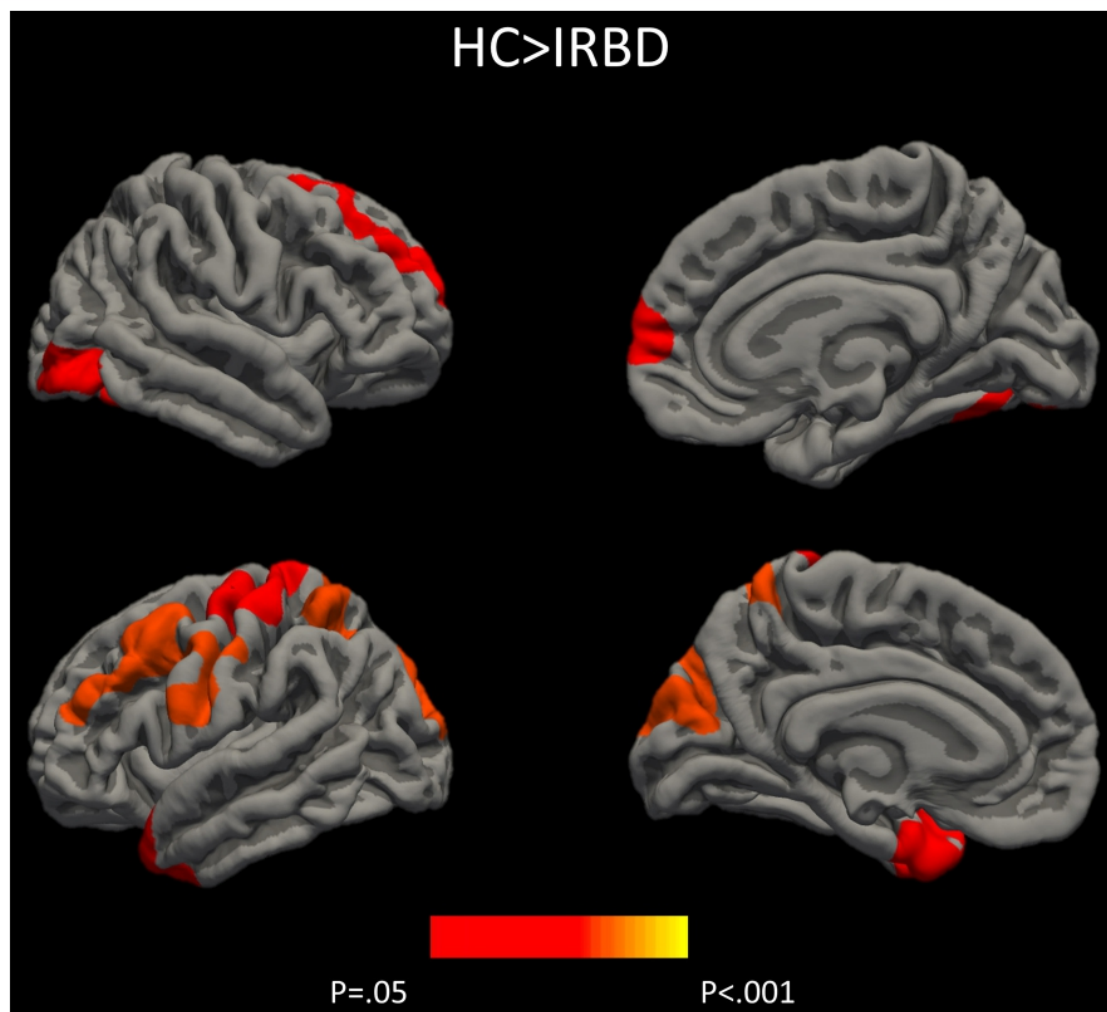


FIGURE 1 | Differences between HC and IRBD patients in cortical thickness. Image show vertex-wise cortical thickness differences between HC and IRBD patients when age was introduced as covariate. Significant clusters are highlighted in warm colors. Results after FWE correction with Monte Carlo simulation and threshold at $p \leq 0.05$. Graphics program: Freeview from FreeSurfer (<https://surfer.nmr.mgh.harvard.edu/fswiki/FreeviewGuide>), and edited with Microsoft PowerPoint®.

right superior frontal and lateral occipital regions. Left superior parietal, left post-central, and right lingual and paracentral regions remained significant after controlling for both, age and sex **Figure 1**, **Table 3**, and **Supplementary Material 1**.

Subcortical Segmentations

Inter-group analysis of subcortical segmentations showed IRBD patients had reduced hippocampal volume ($F = 7.730$; $P = 0.008$; Cohen's d effect size = 0.77). In a subsequent analysis right hippocampal volume achieved significance ($F = 5.086$; $P = 0.029$; Cohen's d effect size = 0.85), whereas no differences were found for the left hippocampus ($F = 2.491$; $P = 0.122$). These results remained significant after controlling for both, age and sex **Table 4**.

Hippocampal Shape Analysis

Shape analysis showed inter-group differences for the right hippocampus in a cluster located in its posterior region, including mainly the CA1, the hippocampal tail, the subiculum, and the dentate gyrus **Figure 2**. A clear tendency to significance was seen when sex was introduced as a covariate ($P = 0.058$).

Segmentation of Hippocampal Subfields

An exploratory analysis of right hippocampal subfields showed that, in comparison with the HC group, IRBD patients had a significant reduction in the *right cornu ammonis 1* (CA1) ($F = 5.685$; $P = 0.021$; Cohen's d effect size = 0.616), *molecular layer* ($F = 5.764$; $P = 0.021$; Cohen's d effect size = 0.645), *granule cell layer of the dentate gyrus* ($F = 4.863$; $P = 0.033$; Cohen's d effect

size = 0.579), and *cornu ammonis 4* (CA4) ($F = 4.890$; $P = 0.032$; Cohen's d effect size = 0.586) **Supplementary Material 2**.

Correlation Analyses

To search for cortical substrates of cognitive changes in patients, we performed cortical thickness analyses. Nevertheless, none cognitive function correlated significantly with cortical thickness maps. Moreover, no significant correlations in the IRBD group were found between measures of hippocampal atrophy and either memory scores or other cognitive variables.

DISCUSSION

To our knowledge this is the first study that combine MRI structural data and cognitive assessment to compare IRBD patients and a group of healthy controls. Our results showed that IRBD patients had posterior based cognitive impairment, cortical atrophy and reduction of the hippocampal volume. In this study we explore the hippocampal structure through different structural MRI techniques and found reductions in right whole-hippocampus volume, mainly in posterior regions.

Similarly to previous neuropsychological results on IRBD, in the current study, patients differed from controls in several cognitive domains (8), namely attention (30, 31), verbal memory (30–33), executive function (30, 34, 35) and semantic fluency (30, 31, 36). Our results showed large effect size for facial recognition, semantic fluency, RAVLT total learning, SDMT, TMTA, and TMTB tests. Interestingly, a previous work found TMT, verbal fluency and Stroop Color Word test, were the best predictors of Lewy bodies dementia in IRBD patients (9). As far as we know, this is the first work assessing facial recognition in IRBD patients and showing lower scores in comparison to HC. In this sense, there are previous literature identifying facial emotion recognition (37) and facial recognition impairment in PD patients (38) suggesting this posterior-based dysfunction could be a cognitive biomarker of PD conversion in IRBD

TABLE 3 | Differences between HC and IRBD patients in cortical thickness.

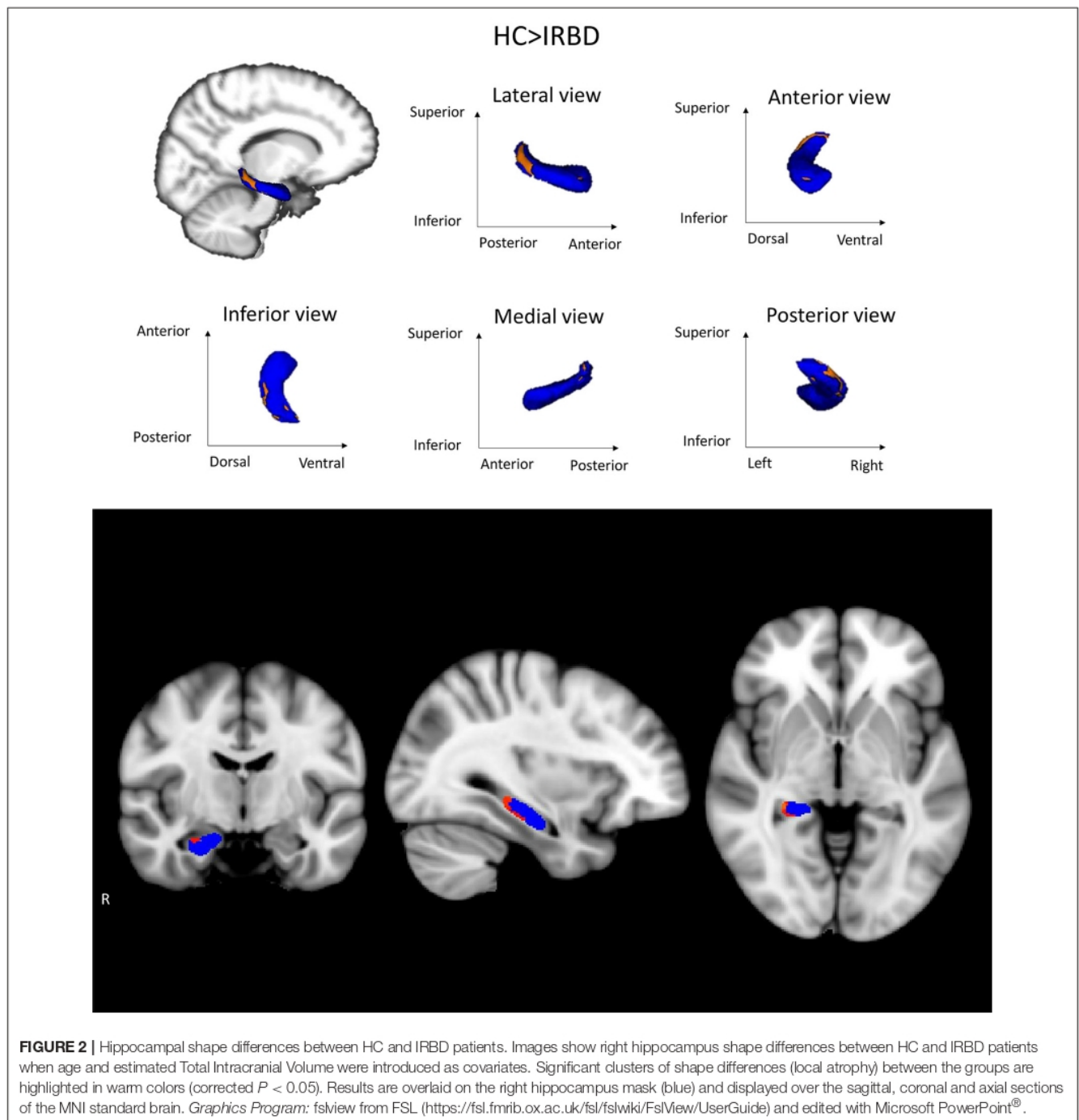
Cluster size (mm ²)	MNI305 space			Clusterwise <i>p</i> -value	Cluster anatomical annotation	
	X	Y	Z			
HC>RBD (AGE AS COVARIATE)						
LH Clusters						
1	4846.8	−22.9	−74.4	29.9	<0.001	Superior parietal
2	2935.7	−52.9	−11.7	23.8	<0.001	Post-central
3	1987.3	−24.1	−31.1	63.3	0.003	Post-central
4	1438.7	−34.0	2.0	−39.4	0.041	Fusiform
RH Clusters						
1	2272.63	21.3	28.4	39.9	0.003	Superior frontal
2	1774.5	43.5	−78.7	−6.4	0.017	Lateral occipital
HC>RBD (AGE AND SEX AS COVARIATES)						
LH Clusters						
1	2863.5	−23.1	−73.5	30.0	<0.001	Superior parietal
2	2672.3	−49.8	−10.3	23.2	<0.001	Post-central
3	1767.1	−32.3	−54.8	61.5	0.007	Superior parietal
RH Clusters						
1	2195.6	9.1	−69.1	−1.1	0.003	Lingual
2	1530.3	8.6	−8.5	66.6	0.045	Paracentral

HC, healthy controls; IRBD, idiopathic rapid eye movement sleep behavior disorder; LH, left hemisphere; RH, right hemisphere. Results after FWE correction with Monte Carlo simulation and threshold at $p \leq 0.05$.

TABLE 4 | Deep gray matter measures (mm³).

	HC ($n = 27$)	RBD ($n = 20$)	Test stat/ p -value	Effect size
Thalamus	12707.1 (1319.2)	12413.8 (1327.6)	1.325/0.256	
Caudate	6609.0 (962.9)	6505.5 (1095.2)	0.337/0.564	
Putamen	9682.2 (1051.0)	9213.5 (1201.5)	2.359/0.132	
Pallidum	3062.9 (363.3)	2968.7 (378.5)	1.208/0.278	
Amygdala	3198.9 (521.1)	2990.1 (431.3)	2.855/0.098	
Accumbens	1051.1 (186.1)	975.5 (182.5)	1.938/0.171	
Brain stem	20318.7 (2455.7)	20364.2 (2270.1)	0.057/0.813	
Hippocampus	8137.0 (941.7)	7447.5 (840.3)	7.730/0.008*	0.77
Right	4082.4 (482.0)	3709.1 (397.3)	5.087/0.029*	0.85
Left	4054.6 (479.9)	3738.4 (479.6)	2.491/0.122	

HC, healthy controls; IRBD, idiopathic rapid eye movement sleep behavior disorder. Measures are presented as means (standard deviation). In bold highlighted those results that reached statistical significance. *Comparisons that were significant ($p < 0.05$) when estimated Total Intracranial Volume, age and sex were introduced as covariates, Cohen's d effect size was computed for significant effects.



patients. Further longitudinal studies are needed to elucidate this issue.

Cortical thickness analysis showed in comparison to HC, IRBD patients had atrophy in left superior parietal, post-central, and fusiform regions, as well as in right superior frontal and lateral occipital regions. When age and sex were introduced as covariates, left superior parietal, left post-central, and right lingual and paracentral regions remained significant. These results agree with those obtained by Rahayel et al. (12, 13) regarding the involvement of dorsolateral prefrontal

and occipito-medial regions. In addition, we observed superior parietal thinning similarly to that described in non-demented PD (39, 40). Interestingly, similar to our results fusiform gyrus and parietal lobe thinning was observed in cross sectional (41, 42) and longitudinal DLB dementia studies (43).

Deep gray matter analysis showed a reduction in the right hippocampus in IRBD patients, there are some works indicating the existence of medial temporal lobe abnormalities in such patients using other neuroimaging approaches. For example, studies using whole-brain VBM approach, reported increased

hippocampal gray matter density (10), but also gray matter reduction in related structures such as the parahippocampal gyrus (14). On the other hand, brain perfusion studies have found an increased metabolic activity of the hippocampus in IRBD patients (30, 44–47). Some of these works have found this effect specifically in the right hippocampus (30, 44). Furthermore, hippocampal perfusion in IRBD has been reported as a predictor of PD or DLB evolution (45).

Exploratory analyses indicated a trend to reduction in IRBD patients in the right CA1 and CA4 subfields, right molecular layer, and right granule cell layer of the dentate gyrus. In agreement with our findings, reductions in the CA1 (48, 49), CA4-DG (50), subiculum, and presubiculum (48) have been found in DLB patients. By contrast, a previous work reported preservation of hippocampal subfields in DLB patients (51). Neuropathological studies in DLB evidenced greater Lewy pathology in the CA2 (52), but also in the entorhinal cortex, CA1, CA3, CA4, and the subiculum (53).

In a longitudinal study including a larger sample of MCI subjects, the hippocampal volume reduction was reported as predictor of evolution to AD rather than DLB (54). In the same line, AD showed higher hippocampal atrophy than PDD and healthy controls, and regional vulnerability differed between AD and PDD specifically in whole right hippocampus and right subiculum (55). Previous literature have found the progression of IRBD patients is mainly to alpha-synucleinopathies such as DLB, PD, and MSA (3, 4), and not to AD. Further recent findings also provide evidence that APOE- $\epsilon 4$ is linked to hippocampal atrophy and learning/memory phenotypes across the AD/DLB spectrum (56). However, in light of previous data, we cannot rule out that some of our patients could evolve to AD, in this sense could be interesting to obtain APOE $\epsilon 4$ genotypic profile.

Finally, it can be difficult to dissociate if hippocampal atrophy and memory impairment are due to sleep problems itself or to sleep problems plus the neurodegenerative process. Sleep deprivation has been related to hippocampal dysfunction and volume reduction (57), so we cannot attribute our results to the neurodegenerative process *per se*. For that reason, studies including other sleep disturbances as control group are needed.

Contrary to our hypothesis, we did not find significant correlations between cognitive performance and brain atrophy. However, there is coherence between the detected brain atrophy by MRI and neuropsychological profile observed in our sample. This could be due to the small sample size, or the lack of linear relationship between structural changes and neuropsychological impairment. A previous work studying IRBD patients reported that cortical thinning was associated with lower performance in cognitive domains, namely attention and executive functions, learning and memory, and visuospatial abilities (58). In this setting, right hippocampus volume has been related to spatial memory abilities (59) and spatial mapping (60), with a greater role of posterior hippocampi (60). The lack of association between hippocampal volume and memory performance in our study might be improved in the future

by using an extensive neuropsychological assessment including visual and spatial memory tests, as well as test paradigms such as the Free and Cued selective Reminding Test to study memory dissociations between recall and recognition in IRBD patients.

Despite the novel findings described above, some limitations of the current study should be acknowledged. First, the relatively small sample size requires caution in generalizing our results, therefore these findings need to be reproduced in larger samples. Second, considering the exploratory nature of the hippocampal subfield analysis, we did not apply correction for multiple comparisons.

In conclusion, we found reductions of mainly posterior cortical thickness and right hippocampal volume in IRBD, alongside evidence of cognitive impairment. This pattern is similar to cognitive decline and atrophy observed in PD and DLB.

AUTHOR CONTRIBUTIONS

CJ contributed in the design of the study. AC, AA, and CU contributed to the analysis of the data and AC, BS, CJ, MS, AA, CU, HB, CG, JS, YC, and AI contributed to the interpretation of the data. AC and CJ contributed to the draft of the article. AC, BS, CJ, MS, AA, CU, HB, CG, JS, YC, NB, and AI revised the manuscript critically for important intellectual content and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fneur.2019.00312/full#supplementary-material>

REFERENCES

- Iranzo A. The REM sleep circuit and how its impairment leads to REM sleep behavior disorder. *Cell Tissue Res.* (2018) 373:245–66. doi: 10.1007/s00441-018-2852-8
- Iranzo A, Santamaria J, Tolosa E. The clinical and pathophysiological relevance of REM sleep behavior disorder in neurodegenerative diseases. *Sleep Med Rev.* (2009) 13:385–401. doi: 10.1016/j.smrv.2008.11.003
- Iranzo A, Fernández-Arcos A, Tolosa E, Serradell M, Molinuevo JL, Valldeoriola F, et al. Neurodegenerative disorder risk in idiopathic REM sleep behavior disorder: study in 174 patients. *PLoS ONE* (2014) 9:e89741. doi: 10.1371/journal.pone.0089741
- Mahlknecht P, Iranzo A, Högl B, Frauscher B, Müller C, Santamaria J, et al. Olfactory dysfunction predicts early transition to a Lewy body disease in idiopathic RBD. *Neurology*. (2015) 84:654–8. doi: 10.1212/WNL.0000000000001265
- Postuma RB, Gagnon JF, Vendette M, Fantini ML, Massicotte-Marquez J, Montplaisir J. Quantifying the risk of neurodegenerative disease in idiopathic REM sleep behavior disorder. *Neurology*. (2009) 72:1296–300. doi: 10.1212/01.wnl.0000340980.19702.6e
- Postuma RB, Aarsland D, Barone P, Burn DJ, Hawkes CH, Oertel W, et al. Identifying prodromal Parkinson's disease: pre-motor disorders in Parkinson's disease. *Mov Disord.* (2012) 27:617–26. doi: 10.1002/mds.24996
- Gagnon J-F, Vendette M, Postuma RB, Desjardins C, Massicotte-Marquez J, Panisset M, et al. Mild cognitive impairment in rapid eye movement sleep behavior disorder and Parkinson's disease. *Ann Neurol.* (2009) 66:39–47. doi: 10.1002/ana.21680
- Högl B, Stefani A, Videnovic A. Idiopathic REM sleep behaviour disorder and neurodegeneration - an update. *Nat Rev Neurol.* (2018) 14:40–56. doi: 10.1038/nrneurol.2017.157
- Marchand DG, Montplaisir J, Postuma RB, Rahayel S, Gagnon J. Detecting the cognitive prodrome of dementia with lewy bodies: a prospective study of REM sleep behavior disorder. *Sleep.* (2016) 40:zsw014. doi: 10.1093/sleep/zsw014
- Scherfner C, Frauscher B, Schocke M, Iranzo A, Gschliesser V, Seppi K, et al. White and gray matter abnormalities in idiopathic rapid eye movement sleep behavior disorder: a diffusion-tensor imaging and voxel-based morphometry study. *Ann Neurol.* (2011) 69:400–7. doi: 10.1002/ana.22245
- Unger MM, Belke M, Menzler K, Heverhagen JT, Keil B, Stiasny-Kolster K, et al. Diffusion tensor imaging in idiopathic REM sleep behavior disorder reveals microstructural changes in the brainstem, substantia nigra, olfactory region, and other brain regions. *Sleep.* (2010) 33:767–73. doi: 10.1093/sleep/33.6.767
- Rahayel S, Montplaisir J, Monchi O, Bedetti C, Postuma RB, Brambati S, et al. Patterns of cortical thinning in idiopathic rapid eye movement sleep behavior disorder. *Mov Disord.* (2015) 30:680–7. doi: 10.1002/mds.25820
- Rahayel S, Postuma RB, Montplaisir J, Bedetti C, Brambati S, Carrier J, et al. Abnormal gray matter shape, thickness, and volume in the motor cortico-subcortical loop in idiopathic rapid eye movement sleep behavior disorder: association with clinical and motor features. *Cereb Cortex.* (2018) 28:658–71. doi: 10.1093/cercor/bhx137
- Hanyu H, Inoue Y, Sakurai H, Kanetaka H, Nakamura M, Miyamoto T, et al. Voxel-based magnetic resonance imaging study of structural brain changes in patients with idiopathic REM sleep behavior disorder. *Parkinsonism Relat Disord.* (2012) 18:136–9. doi: 10.1016/j.parkreldis.2011.08.023
- Boeve BF. REM sleep behavior disorder: updated review of the core features, the RBD-neurodegenerative disease association, evolving concepts, controversies, and future directions. *Ann N Y Acad Sci.* (2010) 184:15–54. doi: 10.1111/j.1749-6632.2009.05115.x
- Iranzo A, Molinuevo JL, Santamaria J, Serradell M, Martí MJ, Valldeoriola F, et al. Rapid-eye-movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study. *Lancet Neurol.* (2006) 5:572–77. doi: 10.1016/S1474-4422(06)70476-8
- Lezak MD. *Neuropsychological Assessment*. Oxford University Press (2012). Available online at: <https://global.oup.com/academic/product/neuropsychological-assessment-9780195395525?cc=es&lang=en&> (accessed May 19, 2017).
- Aarsland D, Bronnick K, Larsen JP, Tysnes OB, Alves G, Norwegian ParkWest Study Group. Cognitive impairment in incident, untreated Parkinson disease: the Norwegian ParkWest Study. *Neurology*. (2009) 72:1121–6. doi: 10.1212/01.wnl.0000338632.00552.cb
- Beck A, Steer R BG. *Manual for the Beck Depression Inventory-II*. San Antonio, TX: Psychological Corporation (1996).
- Starkstein SE, Mayberg HS, Preziosi TJ, Andrezejewski P, Leiguarda R, Robinson RG. Reliability, validity, and clinical correlates of apathy in Parkinson's disease. *J Neuropsychiatry Clin Neurosci.* (1992) 4:134–9.
- Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The neuropsychiatric inventory: comprehensive assessment of psychopathology in dementia. *Neurology*. (1994) 44:2308–14. doi: 10.1212/WNL.44.12.2308
- Fischl B, Dale AM. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc Natl Acad Sci USA.* (2000) 97:11050–5. doi: 10.1073/pnas.200033797
- Sled JG, Zijdenbos AP, Evans AC. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans Med Imaging.* (1998) 17:87–97. doi: 10.1109/42.668698
- Ségonne F, Pacheco J, Fischl B. Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Trans Med Imaging.* (2007) 26:518–29. doi: 10.1109/TMI.2006.887364
- Hagler DJ, Saygin AP, Sereno MI. Smoothing and cluster thresholding for cortical surface-based group analysis of fMRI data. *Neuroimage.* (2006) 33:1093–103. doi: 10.1016/j.neuroimage.2006.07.036
- Patenaude B, Smith SM, Kennedy DN, Jenkinson M. A Bayesian model of shape and appearance for subcortical brain segmentation. *Neuroimage.* (2011) 56:907–22. doi: 10.1016/j.neuroimage.2011.02.046
- Anderson MJ, Robinson J. Permutation tests for linear models. *Aust New Zeal J Stat Stat.* (2001) 43:75–88. doi: 10.1111/1467-842X.00156
- Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. Permutation inference for the general linear model. *Neuroimage.* (2014) 92:381–97. doi: 10.1016/j.neuroimage.2014.01.060
- Iglesias JE, Augustinack JC, Nguyen K, Player CM, Player A, Wright M, et al. A computational atlas of the hippocampal formation using *ex vivo*, ultra-high resolution MRI: application to adaptive segmentation of *in vivo* MRI. *Neuroimage.* (2015) 115:117–37. doi: 10.1016/j.neuroimage.2015.04.042
- Vendette M, Montplaisir J, Gosselin N, Soucy J-P, Postuma RB, Dang-Vu TT, et al. Brain perfusion anomalies in rapid eye movement sleep behavior disorder with mild cognitive impairment. *Mov Disord.* (2012) 27:1255–61. doi: 10.1002/mds.25034
- Terzaghi M, Zucchella C, Rustioni V, Sinforiani E, Manni R. Cognitive performances and mild cognitive impairment in idiopathic rapid eye movement sleep behavior disorder: results of a longitudinal follow-up study. *Sleep.* (2013) 36:1527–32. doi: 10.5665/sleep.3050
- Fantini ML, Farini E, Orrelli P, Zucconi M, Manconi M, Cappa S, et al. Longitudinal study of cognitive function in idiopathic REM sleep behavior disorder. *Sleep.* (2011) 34:619–25. doi: 10.1093/sleep/34.5.619
- Massicotte-Marquez J, Décary A, Gagnon J-F, Vendette M, Mathieu A, Postuma RB, et al. Executive dysfunction and memory impairment in idiopathic REM sleep behavior disorder. *Neurology*. (2008) 70:1250–7. doi: 10.1212/01.wnl.0000286943.79593.a6
- Delazer M, Högl B, Zamarian L, Wenter J, Ehrmann L, Gschliesser V, et al. Decision making and executive functions in REM sleep behavior disorder. *Sleep.* (2012) 35:667–73. doi: 10.5665/sleep.1828
- Youn S, Kim T, Yoon IY, Jeong J, Kim HY, Han JW, et al. Progression of cognitive impairments in idiopathic REM sleep behaviour disorder. *J Neurol Neurosurg Psychiatry.* (2016) 87:890–6. doi: 10.1136/jnnp-2015-311437
- Barber TR, Lawton M, Rolinski M, Hons BA, Evetts S, Baig F, et al. Prodromal Parkinsonism and neurodegenerative risk stratification in REM sleep behavior disorder. *Sleep.* (2017) 40:11–13. doi: 10.1093/sleep/zsx071
- Gray HM, Tickle-Degnen L. A meta-analysis of performance on emotion recognition tasks in parkinson's disease. *Neuropsychology.* (2010) 24:176–91. doi: 10.1037/a0018104
- Pereira JB, Junqué C, Martí MJ, Ramirez-Ruiz B, Bargalló N, Tolosa E. Neuroanatomical substrate of visuospatial and visuo-perceptual impairment in Parkinson's disease. *Mov Disord.* (2009) 24:1193–9. doi: 10.1002/mds.22560
- Segura B, Baggio HC, Martí MJ, Valldeoriola F, Compta Y, García-Díaz AI, et al. Cortical thinning associated with mild cognitive impairment in Parkinson's disease. *Mov Disord.* (2014) 29:1495–503. doi: 10.1002/mds.25982

40. Uribe C, Segura B, Baggio HC, Abos A, Marti MJ, Valdeoriola F, et al. Patterns of cortical thinning in nondemented Parkinson's disease patients. *Mov Disord.* (2016) 31:699–708. doi: 10.1002/mds.26590
41. Watson R, Colloby SJ, Blamire AM, O'Brien JT. Assessment of regional gray matter loss in dementia with lewy bodies: a surface-based MRI analysis. *Am J Geriatr Psychiatry.* (2015) 23:38–46. doi: 10.1016/j.jagp.2014.07.005
42. Blanc F, Colloby SJ, Philippi N, De Pétigny X, Jung B, Demuynck C, et al. Cortical thickness in dementia with lewy bodies and alzheimer's disease: a comparison of prodromal and dementia stages. *PLoS ONE.* (2015) 10:e0127396. doi: 10.1371/journal.pone.0127396
43. Mak E, Su L, Williams GB, Watson R, Firbank MJ, Blamire AM, et al. Progressive cortical thinning and subcortical atrophy in dementia with Lewy bodies and Alzheimer's disease. *Neurobiol Aging.* (2015) 36:1743–50. doi: 10.1016/j.neurobiolaging.2014.12.038
44. Mazza S, Soucy JP, Gravel P, Michaud M, Postuma R, Massicotte-Marquez J, et al. Assessing whole brain perfusion changes in patients with REM sleep behavior disorder. *Neurology.* (2006) 67:1618–22. doi: 10.1212/01.wnl.0000242879.39415.49
45. Dang-Vu TT, Gagnon JF, Vendette M, Soucy JP, Postuma RB, Montplaisir J. Hippocampal perfusion predicts impending neurodegeneration in REM sleep behavior disorder. *Neurology.* (2012) 79:2302–6. doi: 10.1212/WNL.0b013e318278b658
46. Wu P, Yu H, Peng S, Dauvilliers Y, Wang J, Ge J, et al. Consistent abnormalities in metabolic network activity in idiopathic rapid eye movement sleep behaviour disorder. *Brain.* (2014) 137:3122–8. doi: 10.1093/brain/awu290
47. Ge J, Wu P, Peng S, Yu H, Zhang H, Guan Y, et al. Assessing cerebral glucose metabolism in patients with idiopathic rapid eye movement sleep behavior disorder. *J Cereb Blood Flow Metab.* (2015) 35:2062–9. doi: 10.1038/jcbfm.2015.208
48. Sabatoli F, Boccardi M, Galluzzi S, Treves A, Thompson PM, Frisoni GB. Hippocampal shape differences in dementia with Lewy bodies. *Neuroimage.* (2008) 41:699–705. doi: 10.1016/j.neuroimage.2008.02.060
49. Chow N, Aarsland D, Honarpisheh H, Beyer MK, Somme JH, Elashoff D, et al. Comparing hippocampal atrophy in alzheimer's dementia and dementia with lewy bodies. *Dement Geriatr Cogn Disord.* (2012) 34:44–50. doi: 10.1159/000339727
50. Delli Pizzi S, Franciotti R, Bubbico G, Thomas A, Onofri M, Bonanni L. Atrophy of hippocampal subfields and adjacent extrahippocampal structures in dementia with Lewy bodies and Alzheimer's disease. *Neurobiol Aging.* (2016) 40:103–9. doi: 10.1016/j.neurobiolaging.2016.01.010
51. Mak E, Gabel S, Su L, Williams GB, Arnold R, Passamonti L, et al. Multi-modal MRI investigation of volumetric and microstructural changes in the hippocampus and its subfields in mild cognitive impairment, Alzheimer's disease, and dementia with Lewy bodies. *Int Psychogeriatr.* (2017) 29:545–55. doi: 10.1017/S1041610216002143
52. Harding AJ, Lakay B, Halliday GM. Selective hippocampal neuron loss in dementia with Lewy bodies. *Ann Neurol.* (2002) 51:125–8. doi: 10.1002/ana.10071
53. Adamowicz DH, Roy S, Salmon DP, Galasko DR, Hansen LA, Masliah E, et al. Hippocampal α -synuclein in dementia with lewy bodies contributes to memory impairment and is consistent with spread of pathology. *J Neurosci.* (2017) 37:1675–84. doi: 10.1523/JNEUROSCI.3047-16.2016
54. Kantarci K, Lesnick T, Ferman TJ, Przybelski SA, Boeve BF, et al. Hippocampal volumes predict risk of dementia with Lewy bodies in mild cognitive impairment. *Neurology.* (2016) 87:2317–23. doi: 10.1212/WNL.0000000000003371
55. Novellino F, Vasta R, Sarica A, Chiriaco C, Salsone M, Morelli M, et al. Relationship between hippocampal subfields and category cued recall in AD and PDD: a multimodal MRI study. *Neuroscience.* (2018) 371:506–17. doi: 10.1016/j.neuroscience.2017.12.028
56. Saeed U, Mirza SS, MacIntosh BJ, Herrmann N, Keith J, Ramirez J, et al. APOE- ϵ 4 associates with hippocampal volume, learning, and memory across the spectrum of Alzheimer's disease and dementia with Lewy bodies. *Alzheimer's Dement.* (2018) 14:1137–47. doi: 10.1016/j.jalz.2018.04.005
57. Kreutzmann JC, Havekes R, Abel T, Meerlo P. Review sleep deprivation and hippocampal vulnerability: changes in neuronal plasticity, neurogenesis and cognitive function. *Neuroscience.* (2015) 309:173–90. doi: 10.1016/j.neuroscience.2015.04.053
58. Rahayel S, Postuma RB, Montplaisir J, Génier Marchand D, Escudier F, Gaubert M, et al. Cortical and subcortical gray matter bases of cognitive deficits in REM sleep behavior disorder. *Neurology.* (2018) 90:e1759–70. doi: 10.1212/WNL.0000000000005523
59. Ezzati A, Katz MJ, Zammit AR, Lipton ML, Zimmerman ME, Sliwinski MJ, et al. Differential association of left and right hippocampal volumes with verbal episodic and spatial memory in older adults HHS Public Access. *Neuropsychologia.* (2016) 93:380–5. doi: 10.1016/j.neuropsychologia.2016.08.016
60. Brunec IK, Robin J, Patai EZ, Ozubko JD, Javadi A-H, Barens MD, et al. Cognitive mapping style relates to posterior-anterior hippocampal volume ratio. *Hippocampus.* (2019). doi: 10.1002/hipo.23072. [Epub ahead of print].

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

Cortical gray matter and hippocampal atrophy in idiopathic Rapid Eye Movement sleep behavior disorder

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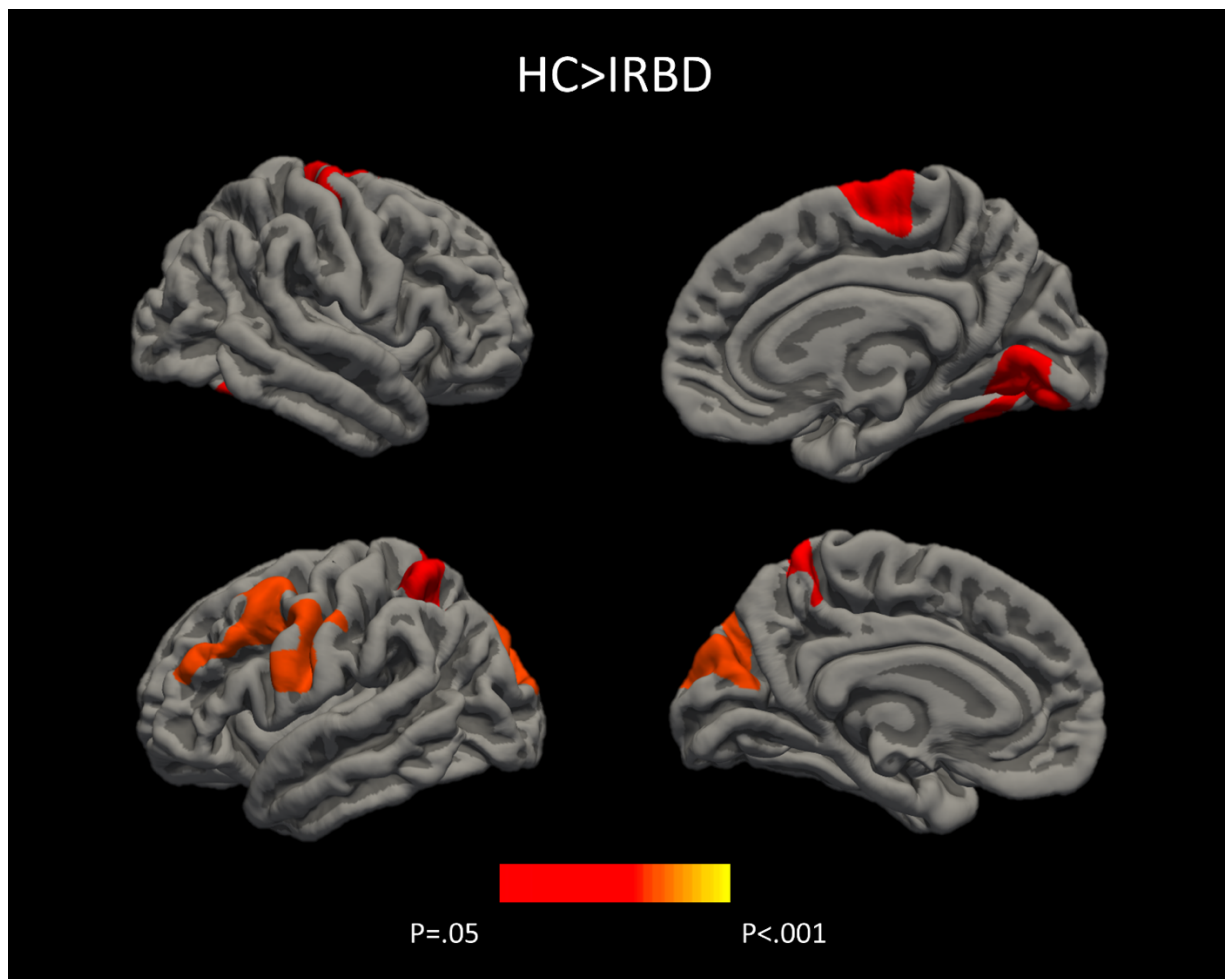
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Supplementary Material 1.



Images show vertex-wise cortical thickness differences between HC and IRBD patients when age and sex were introduced as covariates. Significant clusters are highlighted in warm colors. Results after FWE correction with Monte Carlo simulation and threshold at $p \leq 0.05$.

Graphics program: Freeview from FreeSurfer

(<https://surfer.nmr.mgh.harvard.edu/fswiki/FreeviewGuide>) and edited with Microsoft PowerPoint®.

Supplementary Material 2. Hippocampal subfield volumes (mm³)

	HC (n=27)	IRBD (n=20)	Test stat/p-value	Effect size
Right Hippocampal tail	514.4 (85.9)	475.9 (54.3)	3.819/0.057	
Right subiculum	429.1 (58.1)	400.0 (59.9)	3.316/0.075	
Right CA1	641.0 (79.3)	594.0 (73.2)	5.685/0.021	0.616
Right hippocampal fissure	170.2 (25.5)	173.1 (26.9)	0.098/0.756	
Right presubiculum	289.1 (47.8)	276.7 (46.6)	1.071/0.306	
Right parasubiculum	60.8 (16.1)	65.0 (15.7)	0.665/0.419	
Right molecular layer HP	570.7 (73.9)	524.7 (68.6)	5.764/0.021	0.645
Right granule cell of dentate gyrus	305.8 (41.1)	282.9 (37.8)	4.863/0.033	0.579
Right CA2-CA3	227.1 (33.6)	211.2 (33.1)	3.025/0.089	
Right CA4	264.8 (33.5)	246.3 (29.5)	4.890/0.032	0.586
Right fimbria	78.2 (25.7)	67.6 (27.2)	2.033/0.161	
Right HATA	59.8 (9.1)	56.4 (10.4)	1.951/0.169	

Abbreviations: HC, healthy controls; IRBD, idiopathic rapid eye movement sleep behavior disorder. General lineal model with estimated Total Intracranial Volume as a covariate was used, Cohen's *d* effect size was computed for significant effects. Measures are presented as means (standard deviation). In bold highlighted those results that reached statistical significance.

Study 2

Campabadal. A., Abos, A., Segura, B. Serradell, M., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Compta, Y., Bargallo, N., Junque, C., Iranzo, A., Disruption of posterior brain functional connectivity and its relation to cognitive impairment in idiopathic REM sleep behavior disorder. *Neuroimage Clinical* **25**, 102138 (2020).
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Disruption of posterior brain functional connectivity and its relation to cognitive impairment in idiopathic REM sleep behavior disorder

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ABSTRACT

Background: Resting-state functional MRI has been proposed as a new biomarker of prodromal neurodegenerative disorders, but it has been poorly investigated in the idiopathic form of rapid-eye-movement sleep behavior disorder (IRBD), a clinical harbinger of subsequent synucleinopathy. Particularly, a complex-network approach has not been tested to study brain functional connectivity in IRBD patients.

Objectives: The aim of the current work is to characterize resting-state functional connectivity in IRBD patients using a complex-network approach and to determine its possible relation to cognitive impairment.

Method: Twenty patients with IRBD and 27 matched healthy controls (HC) underwent resting-state functional MRI with a 3T scanner and a comprehensive neuropsychological battery. The functional connectome was studied using threshold-free network-based statistics. Global and local network parameters were calculated based on graph theory and compared between groups. Head motion, age and sex were introduced as covariates in all analyses.

Results: IRBD patients showed reduced cortico-cortical functional connectivity strength in comparison with HC in edges located in posterior regions ($p < 0.05$, FWE corrected). This regional pattern was also shown in an independent analysis comprising posterior areas where a decreased connectivity in 51 edges was found, whereas no significant results were detected when an anterior network was considered ($p < 0.05$, FWE corrected). In the posterior network, the left superior parietal lobule had reduced centrality in IRBD. Functional connectivity strength between left inferior temporal lobe and right superior parietal lobule positively correlated with mental processing speed in IRBD ($r = .633$; $p = .003$). No significant correlations were found in the HC group.

Conclusion: Our findings support the presence of disrupted posterior functional brain connectivity of IRBD patients similar to that found in synucleinopathies. Moreover, connectivity reductions in IRBD were associated with lower performance in mental processing speed domain.

1. Introduction

Rapid-eye-movement (REM) sleep behavior disorder (RBD) is a parasomnia characterized by dream-enacting behaviors, such as shouting, punching, and falling out of bed, related to unpleasant dreams and loss of normal REM-sleep muscle atonia (Schenck and Mahowald, 2002).

The idiopathic form of RBD (IRBD) has been reported as one of the

strongest prodromal biomarkers for alpha-synucleinopathies (Berg et al., 2015), namely Parkinson's disease (PD), dementia with Lewy bodies (DLB) and multiple system atrophy (Iranzo et al., 2014; Mahlknecht et al., 2015). A recent multicenter follow-up Postuma et al. (2019) found that the overall conversion rate from IRBD to an overt neurodegenerative condition was 6.3% per year, with 73.5% converting after a 12-year follow-up. In this setting, there is a need to detect potential new biomarkers of prodromal neurodegeneration in this

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preclinical stage. Since MRI is a non-invasive and widely available technique that could be used for this purpose, there is a growing interest to better characterize brain changes in IRBD patients.

Brain structure in IRBD has previously been studied by means of voxel-based morphometry, cortical thickness and diffusion-tensor imaging, suggesting changes in both cortical (Campabadal et al., 2019; Hanyu et al., 2012; Park et al., 2018; Pereira et al., 2019; Rahayel et al., 2018b, 2018a, 2015; Unger et al., 2010) and subcortical (Ellmore et al., 2010; Hanyu et al., 2012; Park et al., 2018; Rahayel et al., 2018b, 2018a; Scherfler et al., 2011; Unger et al., 2010) structures. Regarding resting-state functional MRI (rs-fMRI), a prior work, using a seed-based approach focused on the substantia nigra, identified altered nigrostriatal and nigrocortical connectivity (Ellmore et al., 2013). Furthermore, a subsequent study found altered basal ganglia connectivity similar to that found in PD patients when using a pre-established template of basal ganglia network (Rolinski et al., 2016). Taking these prior results into account, rs-fMRI has been proposed as a new biomarker of prodromal neurodegeneration in PD (Postuma, 2016).

Network-based statistics (NBS) and graph-derived metrics allow studying the brain as a complex network, characterizing the dynamic interactions between different brain regions (Telesford et al., 2011). These techniques have been used before to detect brain functional connectivity abnormalities in PD (see Hohenfeld et al., 2018 for review) and DLB (Peraza et al., 2015). Nevertheless, as far as we know, there is no previous literature in IRBD studying rs-fMRI following the functional connectome approach. Bearing in mind the findings in α -synucleinopathies, we aimed to characterize brain functional connectivity in IRBD using graph theory analyses and to determine its possible relation to cognitive dysfunctions. We hypothesize that in comparison with healthy subjects, patients will show a reduction in brain functional connectivity, similar to that found in PD and DLB, and that this will be associated with cognitive impairment.

2. Method

2.1. Participants

Twenty patients with IRBD without cognitive or motor complaints at the time of diagnosis, and at the time of the current study, were recruited from our sleep unit. Diagnosis of IRBD required a history of dream-enacting behaviors, video-polysomnographic demonstration of REM sleep without atonia, and absence of other neurological diseases (Boeve, 2010; Iranzo et al., 2006). All patients underwent a full-night polysomnography that demonstrated increased electromyographic activity during REM sleep associated with abnormal disruptive motor behaviors. At the time of the current study, fifteen patients were taking benzodiazepines to reduce RBD symptomatology. Twenty-seven healthy subjects without cognitive, motor, or sleep complaints were recruited from the Institut de l'Envel·liment (Barcelona, Spain).

Exclusion criteria consisted of [1] presence of psychiatric and/or neurologic comorbidity, [2] MMSE score <25, [3] claustrophobia, [4] MRI artifacts, [5] pathological MRI findings other than mild white matter hyperintensities, and [6] evidence in healthy controls (HC) of sleep disorders or mild cognitive impairment by detailed clinical history.

The study was approved by the Ethics Committee of the University of Barcelona (IRB00003099) and Hospital Clinic (HCB/2014/0224). All subjects provided written informed consent to participate after full explanation of the procedures involved.

2.2. Neuropsychological and Clinical Assessment

Participants were evaluated with a comprehensive neuropsychological battery. Expected z scores adjusted for age, sex, and education for each test and each subject were calculated based on a multiple regression analysis performed in the HC group (Aarsland et al., 2009).

Five cognitive domains were obtained using the combination of two neuropsychological tests for each one (computed as the mean of the expected z scores): [1] attention domain, including Digit Span Forward and Backward (WAIS); [2] memory domain, the total learning recall (sum of correct responses from trial I to trial V) and recognition (true recognition after 20 min) from the Rey's Auditory Verbal Learning Test; [3] visuospatial and visuo-perceptual (VS/VP) domain, calculated with Benton Facial Recognition and Clock Copying Test; [4] executive function domain, with Stroop Interference and phonemic fluency (words beginning with the letter "p" in 1 min); and [5] mental processing speed domain, using the Symbol Digits Modalities Test-Oral version (SDMT) and Stroop Color test.

Presence of motor symptoms was evaluated using the International Parkinson and Movement Disorders Society Unified Parkinson's Disease Rating Scale motor section (MDS-UPDRS-III). Neurological examination based on the MDS-UPDRS-III frequently disclosed mild parkinsonian signs, that were, however, not sufficient to diagnose Parkinsonism according to standard criteria. (Postuma et al., 2015)

The Beck Depression Inventory II (BDI) (Beck et al., 1996), Starkstein's Apathy Scale (SAS) (Starkstein et al., 1992), and the Neuropsychiatric Inventory (NPI) (Cummings et al., 1994) were used to assess neuropsychiatric symptomatology.

2.3. MRI Acquisition and Preprocessing

MRI data were acquired with a 3T scanner (MAGNETOM Trio, Siemens, Germany). The scanning protocol included high-resolution 3-dimensional T1-weighted images acquired in the sagittal plane (TR = 2300ms, TE = 2.98ms, TI = 900ms, 240 slices, FOV = 256mm; 1mm isotropic voxel), an axial FLAIR sequence (TR = 9000ms, TE = 96ms) and a resting-state 10-min-long functional gradient-echo echo-planar imaging sequence (240 T2* weighted images, TR = 2.5s, TE = 28 ms, flip angle = 80°, slice thickness = 3mm, FOV = 240mm). Subjects were instructed to keep their eyes closed, not to fall asleep, and not to think anything in particular.

2.4. Resting-State Images

Basic functional image preprocessing, using AFNI tools, included: discarding the first five volumes to allow magnetization stabilization, despiking, motion correction, grand-mean scaling, linear detrending, and temporal filtering (maintaining frequencies above 0.01 Hz).

2.5. Noise Correction and Head Motion

Regarding head motion parameters, an exclusion cut-off was established for mean interframe head motion at ≥ 0.3 mm translation or 0.3° rotation; and for maximum interframe head motion at ≥ 1 mm translation or 1° rotation. We excluded one HC due to excessive head movement. In order to remove the effects of head motion and other non-neural sources of signal variation from the functional data, we used an Independent Component Analysis based strategy for Automatic Removal of Motion Artifacts (ICA-AROMA) (Pruim et al., 2015). ICA-AROMA decomposes the data via ICA and automatically identifies which of these components are related to head motion, by using four robust and standardized features. As quality control measure to assess the efficacy of ICA-AROMA in reducing relationship between signal variation and motion, we performed correlations between framewise head displacement (Power et al., 2012) and overall signal variation (defined as the voxel-wise root mean square intensity difference between subsequent time points) after regressing the ICA-AROMA components. These two measures should not correlate significantly because signal change should not be explained by head motion.

2.6. Characterization of brain functional connectivity

In order to test for intergroup differences in interregional connectivity between IRBD and HC, we used Threshold-free network-based statistics (TFNBS) (Baggio et al., 2018), which performs statistical inference on brain graphs. This approach combines network-based statistics (Zalesky et al., 2010), frequently used for statistical analysis of brain graphs, and threshold-free cluster enhancement, a common method in voxel-wise statistical inference (Smith and Nichols, 2009). One of the main characteristics of TFNBS is that it allows generating edge-wise significance values that can be used to select relevant connectivity features. The 246 regions defined in the Brainnetome Atlas (<https://atlas.brainnetome.org/bnatlas.html>) (Supplementary material 1) were used for the characterization of global functional connectivity (Fan et al., 2016). Head motion, age and sex were introduced as covariates in all analyses.

2.7. Graph theory computation

The analysis of brain functional connectivity was complemented with topological information derived from graph theory, allowing a description of global (whole-brain) and local (nodal) properties of the network (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010). The Brain Connectivity Toolbox (BCT) was used to extract the topological parameters, including global and local clustering coefficient, global and local node degree, “small-worldness”, path length, local efficiency and betweenness centrality (See Rubinov and Sporns, 2010 for detailed definitions and calculations of the graph metrics included in this work). Head motion, age and sex were introduced as covariates in all analyses. Given that graph metrics were computed using nine density thresholds (maintaining the 5% to 25% strongest edges, at intervals of 2.5%), only those results consistently found in more than one are reported.

2.8. Statistical analyses

Statistical analyses of demographic and clinical data were carried out using the statistical package SPSS-24 (2016; Armonk, NY: IBM Corp.). Inter-group comparisons for neuropsychological domains adjusted for age, sex and years of education were performed using Mann-Whitney's U test. Pearson's chi-squared test was applied to assess group differences in categorical variables. Spearman's correlations were used to study the association between clinical and imaging variables. Differences between HC and IRBD in connectivity measures were tested with the general lineal model using in-house MATLAB scripts. Statistical significance was established using Monte Carlo simulations with 10,000 permutations. Two-tailed p-values were calculated as the proportion of values in the null distribution more extreme than those observed in the actual model.

3. Results

3.1. Sociodemographic, clinical and neuropsychological data

Table 1 shows the sociodemographic and clinical characteristics of the groups. No significant intergroup differences were observed for age, sex and years of education. Inter-group comparisons showed IRBD patients had significant greater scores in NPI ($U = 398.0$; $p = .001$), and lower performance in the memory ($U = 134.0$; $p = .008$), VS/VP ($U = 121.0$; $p = .006$) and mental processing speed ($U = 130.0$; $p = .017$) domains. (Table 2).

3.2. Functional connectivity and network graph metrics

Table 1 summarizes head motion parameters. Despite not significant at a $p < .05$ threshold, mean interframe head rotation was higher in the IRBD group ($U = 339.0$; $p = .080$); this measure was therefore

Table 1

Sociodemographic, clinical and head motion comparison between HC and IRBD.

	HC (n = 27)	IRBD (n = 20)	Test stat/p
Sociodemographic and clinical data			
Age (years)	66.5 (13.0)	71.0 (10.0)	343.5/.113
Education (years)	10.0 (8.0)	10.0 (7.0)	257.0/.779
Sex (male/female)	(13/14)	(14/6)	2.24/.134
Neuropsychiatric Inventory	1.0 (4.0)	4.0 (7.0)	398.0/.001
Beck Depression Inventory II	5.0 (8.0)	7.0 (10.0)	286.5/.242
Starkstein's Apathy Scale	8.0 (5.0)	12.0 (9.0)	299.5/.141
IRBD duration (years)	-	2.0 (5.0)	-
MDS-UPDRS-III	-	2.0 (4.0)	-
Mean interframe head motion			
Rotation (degrees)	.026 (.017)	.037 (.024)	339.0/.080
Translation (mm)	.088 (.106)	.104 (.118)	329.0/.126
Maximum interframe head motion			
Rotation (degrees)	.178 (.157)	.181 (.172)	283.0/.610
Translation (mm)	.431 (.252)	.382 (.210)	252.0/.859

Abbreviations: HC, healthy controls; IRBD, idiopathic rapid eye movement sleep behavior disorder; MDS-UPDRS-III, Movement Disorder Society Unified Parkinson's Disease Rating Scale motor section. Data are presented as median (interquartile range) for continuous variables or frequencies for categorical ones. Group differences between HC and IRBD were tested using Mann-Whitney's U test. Pearson's chi-square was applied to assess differences in categorical variables.

Table 2

Group comparison of cognitive domains

Cognitive domains	HC (n = 25)	IRBD (n = 20)	Test stat/ p
Attention	-.232 (.87)	-.272 (.77)	285.0/.424
Memory	.532 (.64)	-.081 (1.39)	134.0/.008
Visuospatial/Visuoperceptual	.345 (.88)	-.529 (1.33)	121.0/.006
Executive functions	-.212 (.91)	-.346 (.64)	193.0/.392
Mental processing speed	-.164 (.87)	-.433 (1.0)	130.0/.017

Abbreviations: HC, healthy controls; IRBD, idiopathic rapid eye movement sleep behavior disorder. Data are presented as median (interquartile range) of the Z scores adjusted for age, years of education and sex. Group differences in cognitive domains were tested using Mann-Whitney's U test.

introduced as a covariate in the analyses that included functional resting-state data.

Fig. 1 shows the six connections with significantly reduced functional connectivity strength in IRBD when compared with HC in the whole-brain analysis ($p < 0.05$, FWE corrected). All connections were found to be cortico-cortical tracts, with a posterior distribution and a left hemispheric predominance. To better study this regional pattern, we performed a supplementary analysis focusing on brain posterior connectivity. To this end, we selected 58 out of the 246 regions extracted from the Brainnetome Atlas, including cuneus, fusiform, superior occipital gyrus, occipital pole, precuneus, inferior parietal and superior parietal subregions. Additionally, in order to have a control network, we also studied brain anterior connectivity with 68 regions including superior frontal gyrus, middle frontal gyrus, inferior frontal gyrus, orbital gyrus, precentral gyrus and paracentral lobule (Supplementary material 1). As shown in Fig. 2 and Supplementary material 2, intergroup-comparison of the posterior network showed reduced connectivity in 51 connections in IRBD ($p < 0.05$, FWE corrected), whereas no group effect was found for the anterior network.

No significant intergroup differences were found in global graph parameters in any network threshold. At the node-level, reduced centrality was observed in IRBD patients compared with HC in the left superior parietal region (specifically, in SPL_L5_2) in 4 out of 9 thresholds in the posterior network. Results were controlled by age, sex and head motion (Fig. 2D).

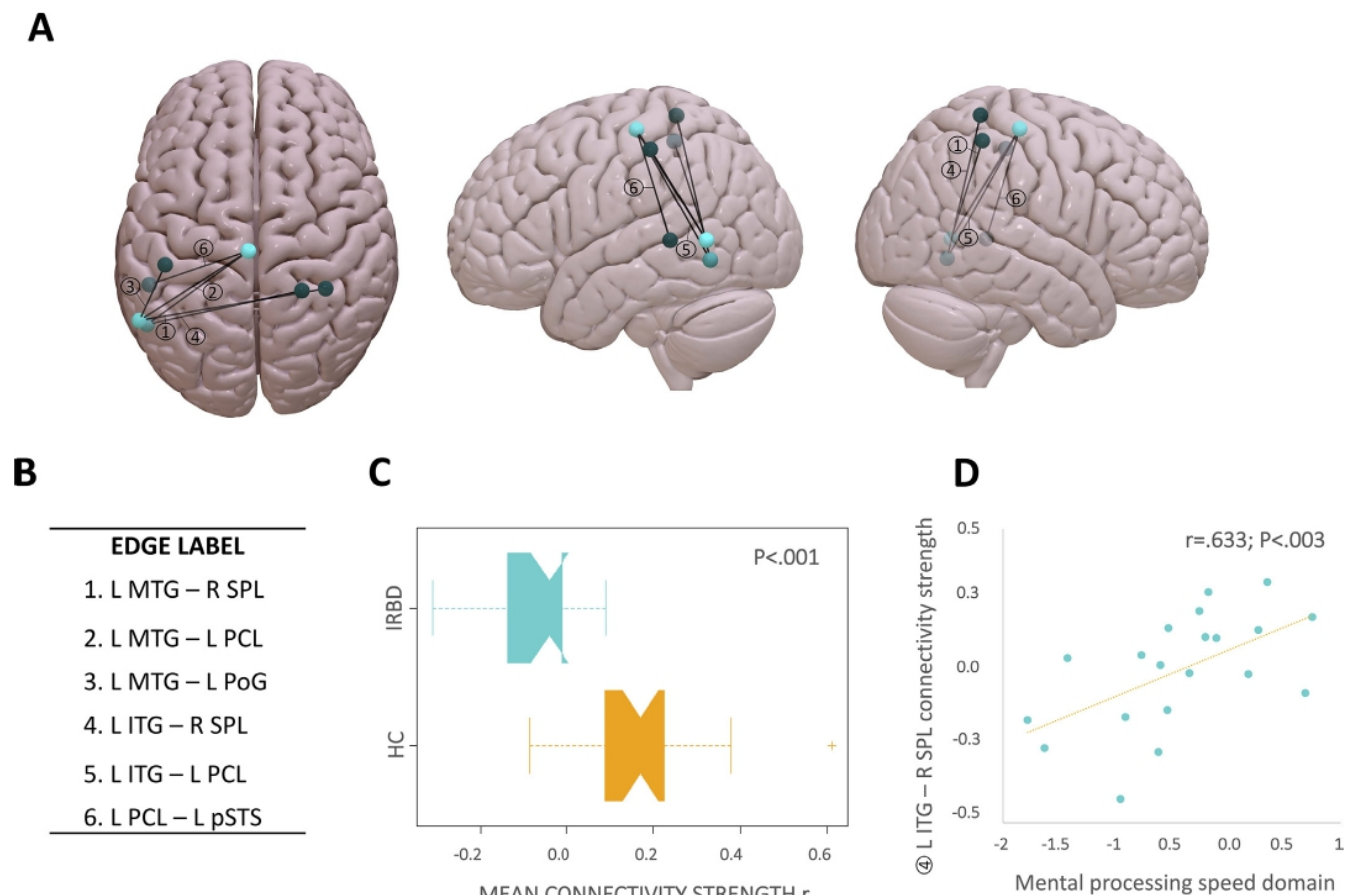


Figure 1. Comparison between idiopathic REM sleep behavior disorder (IRBD) patients and healthy controls (HC) using threshold-free network-based statistics. A. Schematic representation of the reduced functional connectivity strength in IRBD patients compared with HC in the whole-brain analysis consisting of six edges found to be significantly different between groups ($p < 0.05$, family-wise error corrected). Lighter colors represent nodes connected to a greater number of altered connections. Numbers correspond to the edge label (see panel B). B. Labels of the altered edges shown in panel A. Regions were defined based on the Brainnetome Atlas (see Supplementary material 1). Panel C shows the mean connectivity strength distribution (computed for each subject individually) of the six significant connections according to group. D. Significant correlation was found only between the mental processing speed domain and left ITL - right SPL functional connectivity strength (see edge number 4 in brain maps) in IRBD patients. Age, sex and head motion were entered as covariates. Abbreviations: HC, healthy controls; IRBD, idiopathic REM-sleep behavior disorder; ITG, inferior temporal gyrus; L, left; MTG, middle temporal gyrus; PCL, paracentral lobule; PoG, postcentral gyrus; pSTS, posterior superior temporal sulcus; R, right; r , connectivity strength (Pearson's correlation coefficient); SPL, superior parietal lobule. Brain plots were created with Surf Ice (<https://www.nitrc.org/projects/surfice/>).

3.3. Correlation of cognitive parameters with functional connectivity

The mean strength of those edges that showed reduced functional connectivity in the whole-brain analysis (Fig. 1A, 1B) was correlated with the cognitive domains that were impaired in IRBD (Table 2). A positive correlation was found only between the mental processing speed domain and left ITL - right SPL functional connectivity strength in IRBD patients ($r = .633; p = .003$) (Fig. 1D). No significant correlations were found in the HC group.

4. Discussion

As far as we know, this is the first work investigating resting-state functional connectivity in a sample of IRBD patients using complex network analyses. Our data suggest that in comparison with healthy subjects, IRBD patients had reduced resting-state functional connectivity with a predominant posterior distribution. Furthermore, this reduction was associated with cognitive impairment in IRBD patients.

The whole-brain analysis showed reduced posterior cortico-cortical functional connectivity strength in IRBD patients compared with a group of healthy subjects in six edges. Particularly, the left middle

temporal gyrus had reduced functional connectivity with left paracentral lobule, left post central gyrus and right superior parietal lobule. Disrupted connectivity was also found between left inferior temporal gyrus and left paracentral lobule, between left paracentral lobule and left posterior superior temporal sulcus, and also between left inferior temporal gyrus and right superior parietal lobule. In summary, our results showed a connectivity alteration pattern involving associative regions of the temporal and parietal lobes that are known to support complex cognitive functions. To better characterize this posterior-predominant pattern, further independent analyses were performed for anterior and posterior networks. Whereas no significant results were found in the anterior network, posterior complex network characterization showed abnormal connectivity patterns in IRBD with a brain functional connectivity reduction in 51 edges. Although the greater number of significantly altered nodes may be due in part to the lower number of comparisons included in the posterior analysis, the same method was used to analyze the anterior network, yielding no significant results.

As stated in the introduction, only two published studies focused on rs-fMRI data in IRBD, one using an ad hoc developed template (Rolinski et al., 2016) and another performed with a seed-to-seed

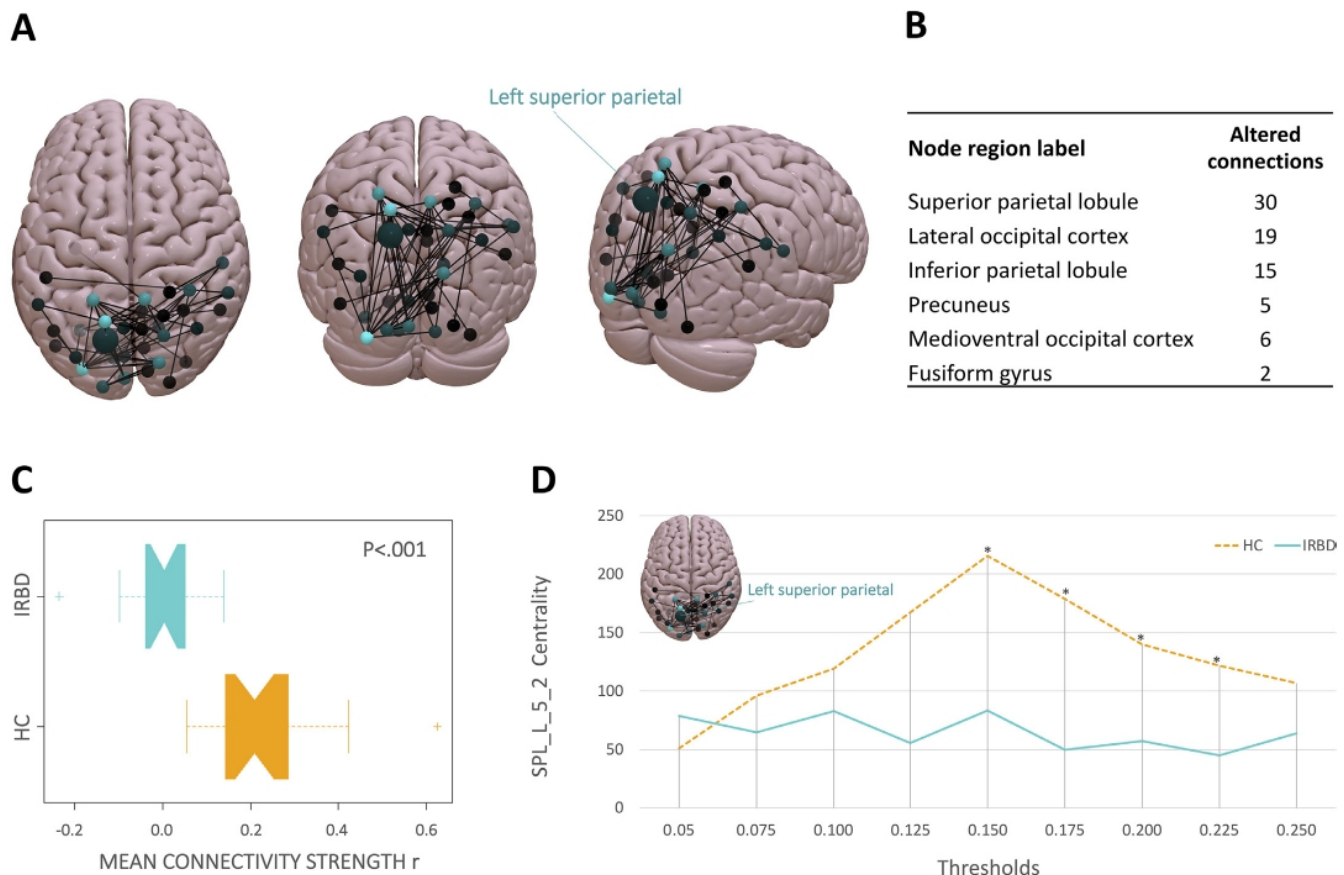


Figure 2. Comparison between idiopathic REM sleep behavior disorder (IRBD) patients and healthy controls (HC) using threshold-free network-based statistics in posterior regions. A. Schematic representation of the reduced functional connectivity strength in IRBD patients compared with HC in the posterior network consisting of 51 edges considered significantly different between groups ($p < 0.05$, family-wise error corrected). Lighter colors represent nodes connected to a greater number of altered connections. The larger sphere represents SPL_L_5_2 (see Supplementary material 1) which showed reduced centrality in IRBD patients. B. Nodes with number of altered edges connected to them. Panel C shows the mean connectivity strength distribution (computed for each subject individually) of the 51 significant connections according to group. Panel D. shows centrality reduction in the left superior parietal in IRBD patients compared with HC. SPL_L_5_2 node centrality (vertical axis) as a function of sparsity thresholds (horizontal axis) for HC and IRBD subgroups. *Indicates significant differences between HC and IRBD. Age, sex and head motion were entered as covariates. Abbreviations: HC, healthy controls; IRBD, idiopathic form of REM-sleep behavior disorder; r , mean connectivity strength of all significant edges (Pearson's correlation coefficient). Brain plots were created with Surf Ice (<https://www.nitrc.org/projects/surface/>).

approach (Ellmore et al., 2013). Both works found altered basal ganglia functional connectivity in these patients. Interestingly, our results demonstrated that when a whole-brain analysis is conducted, reduced cortico-cortical connectivity is also identified. Brain functional connectivity in IRBD should be further addressed with longitudinal cohorts inspecting whether possible involvement of cortical and subcortical structures change with disease progression.

Functional disruption of the parietal cortex in IRBD agrees with previously studies reporting structural decrements in cortical thickness of this region (Rahayel et al., 2018b, 2015; Campabadal et al., 2019). Thus, both structural and functional MRI studies point out a cortical involvement in IRBD congruent with its prodromal symptom of alpha-synucleinopathies. In PD, the posterior atrophy is mainly seen in patients with mild cognitive impairment (Segura et al., 2014). Although our patients did not have cognitive complaints at the time of the study, compared to controls they showed impairment in memory, visuospatial/visuoperceptual and speed of mental processing domains. This neuropsychological pattern is consistent with previous literature where IRBD patients without cognitive complaints and without parkinsonism frequently showed abnormalities when neuropsychological batteries were undergoing (Campabadal et al., 2019; Fantini et al., 2011; Ferini-Strambi et al., 2004; Massicotte-Marquez et al., 2008; Terzaghi et al., 2013, 2008; Vendette et al., 2012).

In the current study, we found evidence of the potential role of altered brain functional connectivity in cognitive impairment. In particular, a positive correlation between mental processing speed and temporo-parietal functional connectivity was found in the IRBD group, but not in healthy controls. It is interesting to note that mental processing speed domain was calculated using the Stroop Color and SDMT-oral version, tests that are known to have a strong posterior cortical component (Forn et al., 2009; Garcia-Diaz et al., 2018a, 2018b; Lezak, 2012). Specifically, and in line with our results, SDMT has been found to be associated with cortical atrophy in temporo-parietal regions in PD patients (Garcia-Diaz et al., 2018a). It could be speculated that the relationship among these tests and the loss of co-activation between right superior parietal and left inferior temporal regions could be reflecting integration abnormalities between the right dorsal-stream, required for visuospatial processing, and regions of the left ventral-stream, that may be involved in visuo-verbal decodification required in both tasks. Such findings could be underlying specific vulnerability of long-distance connections in IRBD patients. In this sense, further research is needed to elucidate possible changes in the organization and topology of white matter tracts in IRBD. Finally, it is worth noting that only mental speed processing domain significantly correlated with brain functional connectivity. It can be speculated that SDMT and Stroop Color Word test require not only mental speed processing, but

also integration of attention, visuospatial, and visuoperceptual functions, possibly making them more sensitive tests when it comes to find brain dysfunctions in IRBD patients.

Results obtained in global graph parameters were less outstanding than those obtained using the TFNBS approach (Baggio et al., 2018). No group effect was found in global graph parameters in any network. We only found decreased centrality for a node located in the left superior parietal lobule. This result is consistent with a prior work with DLB patients where decreased centrality in this same region, among others, was found (Peraza et al., 2015). Neuropathological explanations for this regional centrality reduction in IRBD remain speculative at this point but could include disease-specific regional preferences of Lewy body pathology that have been evidenced previously (Hansen et al., 2019). It is worth noting that our results highlighted brain connectivity reduction with left-hemispheric predominance in IRBD patients. This finding is in line with prior studies showing left asymmetries in motor symptoms and in nigrostriatal function in right-handed PD patients (Barrett et al., 2011; Scherfler et al., 2012; Uitti et al., 2005). Interestingly, in keeping with these works in our IRBD sample eighteen out of twenty patients were right-handed (90%). Based on this, it could be hypothesized that functional brain connectivity has an asymmetric vulnerability at premotor stages that might be related to hemispheric dominance.

Some limitations to the present study should be pointed out. Firstly, IRBD sample is relatively small; thus, we cannot assume that the set of functional connections identified in our study would generalize to other datasets. Future multicenter studies should be carried out to replicate our findings. Secondly, some patients were evaluated under the influence of benzodiazepines. Mainly brain functional connectivity increases (Kiviniemi et al., 2005; Pflanz et al., 2015) have been reported in response to benzodiazepines administration. Unfortunately, our sample does not allow us to study differences between medicated and non-medicated patients. Thirdly, another possible limitation of our work is that, despite the inclusion of a variety of tests in the main cognitive domains defined in recent guidelines (Litvan et al., 2012), we used MMSE instead of MoCa, a less sensitive cognitive screening test in alphasynucleopathies.

5. Conclusion

In the current work, our results indicate that modelling the brain as a functional connectome is a useful approach to detect changes in the organization of brain functional connectivity in IRBD, as a clinical model of PD and DLB prodrome. Overall, our findings support the presence of disrupted posterior functional connectivity in IRBD patients, suggesting a posterior pattern that is in line with previous literature in DLB and PD. In addition, connectivity reductions between the left inferior temporal lobe and the right superior parietal lobule were associated with lower performance in speed of processing domain in IRBD patients.

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Author Contributions

CJ contributed in the design of the study. AC, AA, and CU contributed to the analysis of the data and AC, BS, CJ, MS, AA, CU, HB, CG, JS, YC, and AI contributed to the interpretation of the data. AC and CJ contributed to the draft of the article. AC, BS, CJ, MS, AA, CU, HB, CG, JS, YC, NB, and AI revised the manuscript critically for important intellectual content and approved the final version of the manuscript.

Declaration of Competing Interest

The authors report no conflicts of interest relevant to this study.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.nicl.2019.102138](https://doi.org/10.1016/j.nicl.2019.102138).

References

- Aarsland, D., Bronnick, K., Larsen, J.P., Tysnes, O.B., Alves, G., Norwegian ParkWest Study Group, 2009. Cognitive impairment in incident, untreated Parkinson disease: The Norwegian ParkWest Study. *Neurology* 72, 1121–1126. <https://doi.org/10.1212/01.wnl.0000338632.00552.ch>.
- Baggio, H.C., Abos, A., Segura, B., Campabadal, A., Garcia-Diaz, A., Uribe, C., Compta, Y., Martí, M.J., Valldeoriola, F., Junque, C., 2018. Statistical inference in brain graphs using threshold-free network-based statistics. *Hum. Brain Mapp.* 39. <https://doi.org/10.1002/hbm.24007>.
- Barrett, M.J., Wylie, S.A., Harrison, M.B., Wooten, G.F., 2011. Handedness and motor symptom asymmetry in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 82, 1122–1124. <https://doi.org/10.1136/jnnp.2010.209783>.
- Beck, A., Steer, R., B.G., 1996. Manual for the Beck Depression Inventory-II. Psychological Corporation, San Antonio, Texas.
- Berg, D., Postuma, R.B., Adler, C.H., Bloem, B.R., Chan, P., Dubois, B., Gasser, T., Goetz, C.G., Halliday, G., Joseph, L., Lang, A.E., Liepelt-Scarfone, I., Litvan, I., Marek, K., Obeso, J., Oertel, W., Olanow, C.W., Poewe, W., Stern, M., Deuschl, G., 2015. MDS research criteria for prodromal Parkinson's disease. *Mov. Disord.* 30, 1600–1611. <https://doi.org/10.1002/mds.26431>.
- Boeve, B.F., 2010. REM Sleep Behavior Disorder: Updated Review of the Core Features, the RBD-Neurodegenerative Disease Association, Evolving Concepts, Controversies, and Future Directions. *Ann. N. Y. Acad. Sci.* 15–54. <https://doi.org/10.1111/j.1749-6632.2009.05115.x.REM>.
- Bullmore, E., Sporns, O., 2009. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat. Rev. Neurosci.* 10, 186–198. <https://doi.org/10.1038/nrn2575>.
- Campabadal, A., Segura, B., Junque, C., Serradell, M., Abos, A., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Compta, Y., Bargallo, N., Iranzo, A., 2019. Cortical Gray Matter and Hippocampal Atrophy in Idiopathic Rapid Eye Movement Sleep Behavior Disorder. *Front. Neurol.* 10, 1–9. <https://doi.org/10.3389/fneur.2019.00312>.
- Cummings, J.L., Mega, M., Gray, K., Rosenberg-Thompson, S., Carusi, D.A., Gornbein, J., 1994. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. *Neurology* 44, 2308–2314.
- Ellmore, T.M., Castriotta, R.J., Hendley, K.L., Aalbers, B.M., Furr-Stimming, E., Hood, A.J., Suescun, J., Beurlet, M.R., Hendley, R.T., Schiess, M.C., 2013. Altered nigrostriatal and nigrocollicular functional connectivity in rapid eye movement sleep behavior disorder. *Sleep* 36, 1885–1892. <https://doi.org/10.5665/sleep.3222>.
- Ellmore, T.M., Hood, A.J., Castriotta, R.J., Stimming, E.F., Bick, R.J., Schiess, M.C., 2010. Reduced volume of the putamen in REM sleep behavior disorder patients. *Park. Relat. Disord.* 16, 645–649. <https://doi.org/10.1016/j.parkreldis.2010.08.014>.
- Fan, L., Li, H., Zhuo, J., Zhang, Y., Wang, J., Chen, L., Yang, Z., Chu, C., Xie, S., Laird, A.R., Fox, P.T., Eickhoff, S.B., Yu, C., Jiang, T., 2016. The Human Brainnetome Atlas: A New Brain Atlas Based on Connectional Architecture. *Cereb. Cortex* 26, 3508–3526. <https://doi.org/10.1093/cercor/bhw157>.
- Fantini, M.L., Farini, E., Ortelli, P., Zucconi, M., Manconi, M., Cappa, S., Ferini-Strambi, L., 2011. Longitudinal study of cognitive function in idiopathic REM sleep behavior disorder. *Sleep* 34, 619–625. <https://doi.org/10.1093/sleep/34.5.619>.
- Ferini-Strambi, L., Di Gioia, M.R., Castronovo, V., Oldani, A., Zucconi, M., Cappa, S.F., 2004. Neuropsychological assessment in idiopathic REM sleep behavior disorder (RBD): does the idiopathic form of RBD really exist? *Neurology* 62, 41–45.

- Forn, C., Belloch, V., Bustamante, J.C., Garbin, G., Parcet-Ibars, M.À., Sanjuan, A., Ventura, N., Ávila, C., 2009. A Symbol Digit Modalities Test version suitable for functional MRI studies. *Neurosci. Lett.* 456, 11–14. <https://doi.org/10.1016/j.neulet.2009.03.081>.
- García-Díaz, A.I., Segura, B., Baggio, H.C., Martí, M.J., Valdeoriola, F., Compta, Y., Bargallo, N., Uribe, C., Campabadal, A., Abos, A., Junque, C., 2018a. Structural Brain Correlations of Visuospatial and Visuo-perceptual Tests in Parkinson's Disease. *J. Int. Neuropsychol. Soc.* 24. <https://doi.org/10.1017/S1355617717000583>.
- García-Díaz, A.I., Segura, B., Baggio, H.C., Uribe, C., Campabadal, A., Abos, A., Martí, M.J., Valdeoriola, F., Compta, Y., Bargallo, N., Junque, C., 2018b. Cortical thinning correlates of changes in visuospatial and visuo-perceptual performance in Parkinson's disease: A 4-year follow-up. *Park. Relat. Disord.* 46. <https://doi.org/10.1016/j.parkreldis.2017.11.003>.
- Hansen, D., Ling, H., Lashley, T., Holton, J.L., Warner, T.T., 2019. Review: Clinical, neuropathological and genetic features of Lewy body dementias. *Neuropathol. Appl. Neurobiol.* 1–20. <https://doi.org/10.1111/nan.12554>.
- Hanyu, H., Inoue, Y., Sakurai, H., Kanetaka, H., Nakamura, M., Miyamoto, T., Sasai, T., Iwamoto, T., 2012. Voxel-based magnetic resonance imaging study of structural brain changes in patients with idiopathic REM sleep behavior disorder. *Parkinsonism Relat. Disord.* 18, 136–139. <https://doi.org/10.1016/j.parkreldis.2011.08.023>.
- Hohenfeld, C., Werner, C.J., Reetz, K., 2018. Resting-state connectivity in neurodegenerative disorders: Is there potential for an imaging biomarker? *NeuroImage Clin.* 18, 849–870. <https://doi.org/10.1016/j.nicl.2018.03.013>.
- Iranzo, A., Fernández-Arcos, A., Tolosa, E., Serradell, M., Molinuevo, J.L., Valdeoriola, F., Gelpi, E., Vilaseca, I., Sánchez-Valle, R., Lladó, A., Gaig, C., Santamaría, J., 2014. Neurodegenerative disorder risk in idiopathic REM sleep behavior disorder: Study in 174 patients. *PLoS One* 9. <https://doi.org/10.1371/journal.pone.0089741>.
- Iranzo, A., Molinuevo, J.L., Santamaría, J., Serradell, M., Martí, M.J., Valdeoriola, F., Tolosa, E., 2006. Rapid-eye-movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study. *Lancet Neurol.* 5, 572–577. [https://doi.org/10.1016/S1474-4422\(06\)70476-8](https://doi.org/10.1016/S1474-4422(06)70476-8).
- Kiviniemi, V.J., Haanpää, H., Kantola, J.H., Jauhainen, J., Vainionpää, V., Alahuhta, S., Tervonen, O., 2005. Midazolam sedation increases fluctuation and synchrony of the resting brain BOLD signal. *Magn. Reson. Imaging* 23, 531–537. <https://doi.org/10.1016/j.mri.2005.02.009>.
- Lezak, M.D., 2012. *Neuropsychological assessment*. Oxford University Press.
- Litvan, I., Goldman, J.G., Troster, A.I., Schmand, B.A., Weintraub, D., Petersen, R.C., Mollenhauer, B., Adler, C.H., Marder, K., Williams-Gray, C.H., Aarsland, D., Kulisevsky, J., Rodriguez-Oroz, M.C., Burn, D.J., Barker, R.A., Emre, M., 2012. Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines. *Mov. Disord.* 27, 349–356. <https://doi.org/10.1002/mds.24893>.
- Mahlknecht, P., Iranzo, A., Högl, B., Frauscher, B., Müller, C., Santamaría, J., Tolosa, E., Serradell, M., Mitterling, T., Gschliesser, V., Goebel, G., Brugger, F., Scherfler, C., Poewe, W., Seppi, K., 2015. Olfactory dysfunction predicts early transition to a Lewy body disease in idiopathic RBD. *Neurology* 84, 654–658. <https://doi.org/10.1212/WNL.0000000000001265>.
- Massicotte-Marquez, J., Décar, A., Gagnon, J.-F., Vendette, M., Mathieu, A., Postuma, R.B., Carrier, J., Montplaisir, J., Petersen, R.C., 2008. Executive dysfunction and memory impairment in idiopathic REM sleep behavior disorder. *Neurology* 70, 1250–1257. <https://doi.org/10.1212/01.wnl.0000286943.79593.a6>.
- Park, K.M., Lee, H.J., Lee, B.I., Kim, S.E., 2018. Alterations of the brain network in idiopathic rapid eye movement sleep behavior disorder: structural connectivity analysis. *Sleep Breath* 23, 587–593. <https://doi.org/10.1007/s11325-018-1737-0>.
- Pérez, L.R., Taylor, J.P., Kaiser, M., 2015. Divergent brain functional network alterations in dementia with Lewy bodies and Alzheimer's disease. *Neurobiol. Aging* 36, 2458–2467. <https://doi.org/10.1016/j.neurobiolaging.2015.05.015>.
- Pereira, J.B., Weintraub, D., Chahine, L., Aarsland, D., Hansson, O., Westman, E., 2019. Cortical thinning in patients with REM sleep behavior disorder is associated with clinical progression. *npj Park. Dis.* 3 (5), 7. <https://doi.org/10.1038/s41531-019-0079-3>.
- Pflanz, C.P., Pringle, A., Filippini, N., Warren, M., Gottwald, J., Cowen, P.J., Harmer, C.J., 2015. Effects of seven-day diazepam administration on resting-state functional connectivity in healthy volunteers: A randomized, double-blind study. *Psychopharmacology (Berl)* 232, 2139–2147. <https://doi.org/10.1007/s00213-014-3844-3>.
- Postuma, R.B., 2016. Resting state MRI: a new marker of prodromal neurodegeneration? *Brain* 139, 2106–2108. <https://doi.org/10.1093/brain/aww131>.
- Postuma, R.B., Berg, D., Stern, M., Poewe, W., Marek, K., Litvan, I., 2015. CME MDS Clinical Diagnostic Criteria for Parkinson's Disease Centrality of Motor Syndrome — Parkinsonism and PD Criteria Benchmark — The Expert Examination 30. 10.1002/mds.26424.
- Postuma, R.B., Iranzo, A., Hu, M., Högl, B., Boeve, B.F., Manni, R., Oertel, W.H., Arnulf, I., Ferini-Strambi, L., Puligheddu, M., Antelmi, E., Cohen De Cock, V., Arnaldi, D., Mollenhauer, B., Videnovic, A., Sonka, K., Jung, K.Y., Kunz, D., Dauvilliers, Y., Provini, F., Lewis, S.J., Buskova, J., Pavlova, M., Heidebreder, A., Montplaisir, J.Y., Santamaría, J., Barber, T.R., Stefani, A., Louis, S.E.K., Terzaghi, M., Janzen, A., Leu-Semenescu, S., Plazzi, G., Nobili, F., Sixel-Doering, F., Dusek, P., Bes, F., Cortelli, P., Ehgoetz Martens, K., Gagnon, J.F., Gaig, C., Zucconi, M., Trenkwalder, C., Gan-Or, Z., Lo, C., Rolinski, M., Mahlkecht, P., Holzkecht, E., Boeve, A.R., Teigen, L.N., Toscano, G., Mayer, G., Morbelli, S., Dawson, B., Pelletier, A., 2019. Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behaviour disorder: A multicentre study. *Brain* 142, 744–759. <https://doi.org/10.1093/brain/awz030>.
- Power, J.D., Barnes, K.A., Snyder, A.Z., Schlaggar, B.L., Petersen, S.E., 2012. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage* 59, 2142–2154. <https://doi.org/10.1016/j.neuroimage.2011.10.018>.
- Pruim, R.H.R., Mennes, M., van Rooij, D., Llera, A., Buitelaar, J.K., Beckmann, C.F., 2015. ICA-AROMA: A robust ICA-based strategy for removing motion artifacts from fMRI data. *Neuroimage* 112, 267–277. <https://doi.org/10.1016/j.neuroimage.2015.02.064>.
- Rahayel, S., Montplaisir, J., Monchi, O., Bedetti, C., Postuma, R.B., Brambati, S., Carrier, J., Joubert, S., Latreille, V., Jubault, T., Gagnon, J.F., 2015. Patterns of cortical thinning in idiopathic rapid eye movement sleep behavior disorder. *Mov. Disord.* 30, 680–687. <https://doi.org/10.1002/mds.25820>.
- Rahayel, S., Postuma, R.B., Montplaisir, J., Bedetti, C., Brambati, S., Carrier, J., Monchi, O., Bourgouin, P.A., Gaubert, M., Gagnon, J.F., 2018a. Abnormal Gray Matter Shape, Thickness, and Volume in the Motor Cortico-Subcortical Loop in Idiopathic Rapid Eye Movement Sleep Behavior Disorder: Association with Clinical and Motor Features. *Cereb. Cortex* 28, 658–671. <https://doi.org/10.1093/cercor/bhx137>.
- Rahayel, S., Postuma, R.B., Montplaisir, J., Génier Marchand, D., Escudier, F., Gaubert, M., Bourgouin, P.-A., Carrier, J., Monchi, O., Joubert, S., Blanc, F., Gagnon, J.-F., 2018b. Cortical and subcortical gray matter bases of cognitive deficits in REM sleep behavior disorder. *Neurology* 90, e1759–e1770. [https://doi.org/10.1016/S0306-4522\(99\)00316-4](https://doi.org/10.1016/S0306-4522(99)00316-4).
- Rolinski, M., Griffanti, L., Piccini, P., Roussakis, A.A., Szewczyk-krolkowski, K., Menke, R.A., Quinnell, T., Zaiwalla, Z., Klein, J.C., Mackay, C.E., Hu, M.T.M., 2016. Basal ganglia dysfunction in idiopathic REM sleep behaviour disorder parallels that in early Parkinson's disease. *Brain* 139, 2224–2234. <https://doi.org/10.1093/aww131>.
- Rubinov, M., Sporns, O., 2010. Complex network measures of brain connectivity: Uses and interpretations. *Neuroimage* 52, 1059–1069. <https://doi.org/10.1016/j.neuroimage.2009.10.003>.
- Schenck, C.H., Mahowald, M.W., 2002. REM sleep behavior disorder: clinical, developmental, and neuroscience perspectives 16 years after its formal identification in SLEEP. *Sleep* 25, 120–138.
- Scherfler, C., Frauscher, B., Schocke, M., Iranzo, A., Gschliesser, V., Seppi, K., Santamaría, J., Tolosa, E., Högl, B., Poewe, W., 2011. White and gray matter abnormalities in idiopathic rapid eye movement sleep behavior disorder: A diffusion-tensor imaging and voxel-based morphometry study. *Ann. Neurol.* 69, 400–407. <https://doi.org/10.1002/ana.22245>.
- Scherfler, C., Seppi, K., Mair, K.J., Donnemiller, E., Virgolini, I., Wenning, G.K., Poewe, W., 2012. Left hemispheric predominance of nigrostriatal dysfunction in Parkinson's disease. *Brain* 135, 3348–3354. <https://doi.org/10.1093/brain/aww253>.
- Segura, B., Baggio, H.C., Martí, M.J., Valdeoriola, F., Compta, Y., García-Díaz, A.I., Vendrell, P., Bargallo, N., Tolosa, E., Junque, C., 2014. Cortical thinning associated with mild cognitive impairment in Parkinson's disease. *Mov. Disord.* 29, 1495–1503. <https://doi.org/10.1002/mds.25982>.
- Smith, S.M., Nichols, T.E., 2009. Threshold-free cluster enhancement: Addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage* 44, 83–98. <https://doi.org/10.1016/j.neuroimage.2008.03.061>.
- Starkstein, S.E., Mayberg, H.S., Preziosi, T.J., Andrezejewski, P., Leiguarda, R., Robinson, R.G., 1992. Reliability, validity, and clinical correlates of apathy in Parkinson's disease. *J. Neuropsychiatry Clin. Neurosci.* 4, 134–139. <https://doi.org/10.1176/jnp.4.2.134>.
- Telesford, Q.K., Simpson, S.L., Burdette, J.H., Hayasaka, S., Laurienti, P.J., 2011. The Brain as a Complex System: Using Network Science as a Tool for Understanding the Brain. *Brain Connect.* 1, 295–308. <https://doi.org/10.1089/brain.2011.0055>.
- Terzaghi, M., Sinforiani, E., Zucchella, C., Zambrelli, E., Pasotti, C., Rustioni, V., Manni, R., 2008. Cognitive performance in REM sleep behaviour disorder: a possible early marker of neurodegenerative disease? *Sleep. Med.* 9, 343–351. <https://doi.org/10.1016/j.sleep.2007.06.013>.
- Terzaghi, M., Zucchella, C., Rustioni, V., Sinforiani, E., Manni, R., 2013. Cognitive Performances and Mild Cognitive Impairment in Idiopathic Rapid Eye Movement Sleep Behavior Disorder: Results of a Longitudinal Follow-Up Study. *Sleep* 36, 1527–1532. <https://doi.org/10.5665/sleep.3050>.
- Uitti, R.J., Baba, Y., Whaley, N.R., Wszolek, Z.K., Putzke, J.D., 2005. Parkinson disease: Handness predicts asymmetry. *Neurology* 64, 1925–1930. <https://doi.org/10.1212/01.WNL.0000163993.82388.C8>.
- Unger, M.M., Belke, M., Menzler, K., Heverhagen, J.T., Keil, B., Stiasny-Kolster, K., Rosenow, F., Diederich, N.J., Mayer, G., Möller, J.C., Oertel, W.H., Knake, S., 2010. Diffusion tensor imaging in idiopathic REM sleep behavior disorder reveals microstructural changes in the brainstem, substantia nigra, olfactory region, and other brain regions. *Sleep* 33, 767–773. <https://doi.org/10.1093/sleep/33.6.767>.
- Vendette, M., Montplaisir, J., Gosselin, N., Soucy, J.-P., Postuma, R.B., Dang-Vu, T.T., Gagnon, J.-F., 2012. Brain perfusion anomalies in rapid eye movement sleep behavior disorder with mild cognitive impairment. *Mov. Disord.* 27, 1255–1261. <https://doi.org/10.1002/mds.25034>.
- Zalesky, A., Fornito, A., Bullmore, E.T., 2010. Network-based statistic: Identifying differences in brain networks. *Neuroimage* 53, 1197–1207. <https://doi.org/10.1016/j.neuroimage.2010.06.041>.

Supplementary material

Disruption of posterior brain functional connectivity and its relation to cognitive impairment in idiopathic REM sleep behavior disorder

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Supplementary material 1. Brainnetome atlas nodes and corresponding anatomical regions.**Cingulate**

CG_L(R)_7_1, CG_L(R)_7_2, CG_L(R)_7_3, CG_L(R)_7_4, CG_L(R)_7_5, CG_L(R)_7_6, CG_L(R)_7_7

Frontal

SFG_L(R)_7_1, SFG_L(R)_7_2, SFG_L(R)_7_3, SFG_L(R)_7_4, SFG_L(R)_7_5, SFG_L(R)_7_6, SFG_L(R)_7_7, MFG_L(R)_7_1, MFG_L(R)_7_2, MFG_L(R)_7_3, MFG_L(R)_7_4, MFG_L(R)_7_5, MFG_L(R)_7_6, MFG_L(R)_7_7, IFG_L(R)_6_1, IFG_L(R)_6_2, IFG_L(R)_6_3, IFG_L(R)_6_4, IFG_L(R)_6_5, IFG_L(R)_6_6, OrG_L(R)_6_1, OrG_L(R)_6_2, OrG_L(R)_6_3, OrG_L(R)_6_4, OrG_L(R)_6_5, OrG_L(R)_6_6, PrG_L(R)_6_1, PrG_L(R)_6_2, PrG_L(R)_6_3, PrG_L(R)_6_4, PrG_L(R)_6_5, PrG_L(R)_6_6, PCL_L(R)_2_1, PCL_L(R)_2_2

Insula

INS_L(R)_6_1, INS_L(R)_6_2, INS_L(R)_6_3, INS_L(R)_6_4, INS_L(R)_6_5, INS_L(R)_6_6

Medial temporal

FuG_L(R)_3_1, FuG_L(R)_3_2, FuG_L(R)_3_3, PhG_L(R)_6_1, PhG_L(R)_6_2, PhG_L(R)_6_3, PhG_L(R)_6_4, PhG_L(R)_6_5, PhG_L(R)_6_6, Amyg_L(R)_2_1, Amyg_L(R)_2_2, Hipp_L(R)_2_1, Hipp_L(R)_2_2

Occipital

MVOcC_L(R)_5_1, MVOcC_L(R)_5_2, MVOcC_L(R)_5_3, MVOcC_L(R)_5_4, MVOcC_L(R)_5_5, LOcC_L(R)_4_1, LOcC_L(R)_4_2, LOcC_L(R)_4_3, LOcC_L(R)_4_4, LOcC_L(R)_2_1, LOcC_L(R)_2_2

Parietal

SPL_L(R)_5_1, SPL_L(R)_5_2, SPL_L(R)_5_3, SPL_L(R)_5_4, SPL_L(R)_5_5, IPL_L(R)_6_1, IPL_L(R)_6_2, IPL_L(R)_6_3, IPL_L(R)_6_4, IPL_L(R)_6_5, IPL_L(R)_6_6, Pkun_L(R)_4_1, Pkun_L(R)_4_2, Pkun_L(R)_4_3, Pkun_L(R)_4_4, PoG_L(R)_4_1, PoG_L(R)_4_2, PoG_L(R)_4_3, PoG_L(R)_4_4

Basal ganglia

BG_L(R)_6_1, BG_L(R)_6_2, BG_L(R)_6_3, BG_L(R)_6_4, BG_L(R)_6_5, BG_L(R)_6_6

Temporal

STG_L(R)_6_1, STG_L(R)_6_2, STG_L(R)_6_3, STG_L(R)_6_4, STG_L(R)_6_5, STG_L(R)_6_6, MTG_L(R)_4_1, MTG_L(R)_4_2, MTG_L(R)_4_3, MTG_L(R)_4_4, ITG_L(R)_7_1, ITG_L(R)_7_2, ITG_L(R)_7_3, ITG_L(R)_7_4, ITG_L(R)_7_5, ITG_L(R)_7_6, ITG_L(R)_7_7, pSTS_L(R)_2_1, pSTS_L(R)_2_2

Thalamus

Tha_L(R)_8_1, Tha_L(R)_8_2, Tha_L(R)_8_3, Tha_L(R)_8_4, Tha_L(R)_8_5, Tha_L(R)_8_6, Tha_L(R)_8_7, Tha_L(R)_8_8

Abbreviations: CG, cingulate gyrus; SFG, superior frontal gyrus; MFG, middle frontal gyrus; IFG, inferior frontal gyrus; OrG, orbital gyrus; PrG, precentral gyrus; PCL, paracentral lobule; INS, insula; FuG, fusiform gyrus; PhG, parahippocampal gyrus; Amyg, amygdala; Hipp, hippocampus; MVOcC, medioventral occipital cortex; LOcC, lateral occipital cortex; SPL, superior parietal lobule; IPL, inferior parietal lobule; Pkun, precuneus; PoG, postcentral gyrus; BG, basal ganglia; STG, superior temporal gyrus; MTG, middle temporal gyrus; ITG, inferior temporal gyrus; pSTS, posterior superior temporal sulcus; Tha, thalamus.

(see also <http://cercor.oxfordjournals.org/content/26/8/3508.full.pdf>).

Supplementary material 2. Node pairs that showed a significant reduction in IRBD patients when compared with HC. (FWE corrected, $P < 0.05$).

POSTERIOR NETWORK (HC > IRBD)	
FuG_L_3_2	IPL_L_6_1
IPL_L_6_1	IPL_L_6_5
IPL_L_6_3	IPL_R_6_4
MVOcC_L_5_3	LOcC_L_4_3
MVOcC_R_5_3	LOcC_L_4_3
MVOcC_R_5_1	LOcC_L_4_4
IPL_L_6_4	LOcC_L_4_4
IPL_R_6_2	LOcC_L_4_4
IPL_R_6_4	LOcC_L_4_4
IPL_R_6_4	Pcun_L_4_1
IPL_R_6_6	Pcun_L_4_1
MVOcC_L_5_2	Pcun_R_4_2
MVOcC_L_5_4	Pcun_R_4_2
MVOcC_R_5_2	Pcun_R_4_2
LOcC_L_4_4	Pcun_R_4_2
FuG_L_3_2	Pcun_R_4_3
Pcun_R_4_2	Pcun_R_4_3
MVOcC_L_5_1	LOcC_R_2_1
MVOcC_L_5_3	LOcC_R_2_1
MVOcC_R_5_1	LOcC_R_2_1
LOcC_L_4_2	LOcC_R_2_1
LOcC_L_4_3	LOcC_R_2_1
LOcC_L_4_4	LOcC_R_2_1
MVOcC_L_5_2	SPL_L_5_1
MVOcC_L_5_3	SPL_L_5_1
FuG_L_3_2	SPL_L_5_1
IPL_L_6_4	SPL_L_5_1
IPL_R_6_5	SPL_L_5_1
Pcun_R_4_1	SPL_L_5_1
Pcun_R_4_2	SPL_L_5_1
Pcun_R_4_4	SPL_L_5_1
MVOcC_L_5_3	SPL_L_5_2
LOcC_L_4_4	SPL_L_5_2
SPL_L_5_1	SPL_L_5_2
MVOcC_L_5_1	SPL_L_5_4
MVOcC_L_5_3	SPL_L_5_4
MVOcC_R_5_1	SPL_L_5_4
IPL_R_6_2	SPL_L_5_4
LOcC_L_4_4	SPL_L_5_4
LOcC_R_4_4	SPL_L_5_4
FuG_L_3_1	SPL_R_5_1
IPL_r_6_6	SPL_R_5_2
LOcC_L_4_4	SPL_R_5_2
Pcun_R_4_2	SPL_R_5_2
SPL_L_5_4	SPL_R_5_2

MVOcC_L_5_1	SPL_R_5_3
LOcC_L_4_3	SPL_R_5_3
LOcC_L_4_4	SPL_R_5_3
LOcC_R_4_3	SPL_R_5_3
IPL_R_6_6	SPL_R_5_4
LOcC_L_4_4	SPL_R_5_5

TFNBS posterior analysis results, controlling for age, sex and motion. Node pairs that showed a significant reduction in IRBD patients when compared with HC. (FWE corrected, $P < 0.05$). FuG, fusiform gyrus; HC, healthy controls; IPL, inferior parietal lobule; IRBD, Idiopathic REM sleep behavior disorder; LOcC, lateral occipital cortex; MVOcC, medioventral occipital cortex; Pcun, precuneus; SPL, superior parietal lobule.

Study 3

Campabadal, A., Segura, B., Baggio, H. C., Abos, A., Uribe, C., Garcia-Diaz, A. I., Marti, M.J., Valldeoriola, F., Compta, Y., Bargallo, N., Junque, C., Diagnostic Accuracy, Item Analysis and Age Effects of the UPSIT Spanish Version in Parkinson's Disease. *Arch. Clin. Neuropsychol* acy053 (2019) <https://doi.org/10.1093/arclin/acy053>

Diagnostic Accuracy, Item Analysis and Age Effects of the UPSIT Spanish Version in Parkinson's Disease

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Abstract

Objective: The University of Pennsylvania Smell Identification Test (UPSIT) is the most commonly used test to detect olfactory impairment in Parkinson's disease (PD), but the cut-off score for clinical purposes is often difficult to establish because of age and sex effects. The current work aims to study the sensitivity and specificity of the UPSIT Spanish version and its accuracy in discriminating PD patients at different age groups from healthy controls (HC), and to perform an item analysis.

Method: Ninety-seven non-demented PD patients and 65 HC were assessed with the UPSIT Spanish version. Sensitivity, specificity, and diagnostic accuracy for PD were calculated. Multiple regression analysis was used to define predictors of UPSIT scores.

Results: Using the normative cut-off score for anosmia (≤ 18), the UPSIT showed a sensitivity of 54.6% with a specificity of 100.0% for PD. We found that, using the UPSIT cut-off score of ≤ 25 , sensitivity was 81.4% and specificity 84.6% (area under the receiver operating characteristic curve = 0.908). Diagnosis and age were good predictors of UPSIT scores ($B = -10.948$; $p < .001$; $B = -0.203$; $p < .001$). When optimal cut-off scores were considered according to age ranges (≤ 60 , 61–70, and ≥ 71), sensitivity and specificity values were $>80.0\%$ for all age groups.

Conclusions: In the Spanish UPSIT version, sensitivity and specificity are improved when specific cut-off scores for different age groups are computed.

Keywords: Parkinson's disease; Elderly/geriatrics/aging; Olfaction; Assessment; Movement disorders

Introduction

Olfactory impairment has been found to be a prodromal manifestation in Parkinson's disease (PD) (Berendse, Roos, Raijmakers, & Doty, 2011; Doty, 2012a, 2012b) and other neurodegenerative disorders (Doty, 2012a, 2012b). The prevalence of olfactory deficits in PD is estimated to be about 90% (Fullard et al., 2016; Haehner et al., 2009). Olfactory dysfunction substantially affects well-being and quality of life, reducing enjoyment from food, beverages, personal care products, and the natural environment. Moreover, decreased smell function increases the risk of danger from fire, environmental toxins, leaking natural gas, and spoiled food (Doty, 2017).

Odor identification tests, such as the University of Pennsylvania Smell Identification Test (UPSIT) (Doty, 1995), are used in both research and clinical settings to assess olfactory dysfunction in PD patients. The UPSIT has demonstrated high

capability in differentiating PD patients from healthy subjects (Bohnen, Studenski, Constantine, & Moore, 2008), and has been used as a tool for the diagnosis of PD (Doty, Bromley, & Stern, 1995; Silveira-Moriyama et al., 2008).

It is well known that aging per se is associated with olfactory decline; McKinnon and colleagues (2010), using data collected from 732 subjects, estimated that the decrease in UPSIT scores is around 3.2 points for every 10 years of age (McKinnon et al., 2010). Loss of olfactory identification ability is seen from the fifth decade onwards but is most remarkable between the seventh and eighth decades of life. In PD patients, it has also been found that UPSIT scores decreased as a function of age (Doty et al., 1995).

Olfactory functions in normal subjects are clearly influenced by gender (Doty, Ford, Preti, & Huggins, 1975; Doty, Green, Ram, & Yankell, 1982; Doty, Orndorff, Leyden, & Kligman, 1978). Sex-related differences in the UPSIT have also been described in PD, with lower performance for men (Doty et al., 1995). These results suggest that age and sex should be taken into consideration when using olfactory measures as a tool for PD diagnosis. In this setting, Doty and colleagues (Doty et al., 1995) proposed optimal cut-off scores that best discriminate between PD and healthy subjects, according to different age groups and according to gender. As far as we know, these results have not been validated in the UPSIT Spanish version.

Considering that performance in smell identification tests are subject to cultural variation, the UPSIT has been adapted to several countries (Ogihara, Kobayashi, Nishida, Kitano, & Takeuchi, 2011; Picillo et al., 2014; Rodriguez-Violante, Lees, Cervantes-Arriaga, Corona, & Silveira-Moriyama, 2011; Silveira-Moriyama et al., 2008, 2010; Yücepur et al., 2012). Even though changes in some specific items have improved sensitivity and specificity, it is hard to generalize the results to other populations (Picillo et al., 2014; Rodriguez-Violante et al., 2014a; Silveira-Moriyama et al., 2008).

Furthermore, previous works found a decreased identification of some UPSIT items in healthy populations (Kondo, Matsuda, Hashiba, & Baba, 1998; Picillo et al., 2014; Rodriguez-Violante et al., 2014b; Silveira-Moriyama et al., 2008). In this sense, the analysis of individual smell scores of UPSIT Spanish version may allow identifying items with greater accuracy in PD diagnosis, and items that are culturally unsuitable.

In the current work, we aim to investigate the sensitivity and specificity of the UPSIT Spanish version, its accuracy in discriminating PD patients from healthy controls (HC), age and sex-related effects on test performance, and individual item reliability. It was hypothesized that: (1) PD patients will have poorer performance than HC; (2) the UPSIT will discriminate PD patients from healthy controls; (3) some UPSIT items will show low percentage of success in control subjects; (4) age will have an effect in UPSIT scores; (5) sex will have an effect in UPSIT scores; and (6) diagnostic accuracy will improve when the effects of age and sex are considered.

Methods

Participants

The study sample included 97 non-demented PD patients and 65 HC who underwent olfactory evaluation.

PD patients were recruited from an outpatient movement disorders clinic (Parkinson's disease and Movement Disorders Unit, Department of Neurology, Hospital Clinic, Barcelona, Catalonia, Spain). HC consisted of individuals who had previously volunteered who take part in studies of aging at the "*Fundació Salut i Envel·liment, Universitat Autònoma de Barcelona*" (Barcelona, Spain).

Exclusion criteria for both groups were: (1) significant psychiatric, neurological, or systemic comorbidity; (2) low average premorbid intelligence quotient estimated by the Vocabulary subtest of the Wechsler Adult Intelligence Scale, third edition (scaled score ≤ 7); and (3) Mini Mental State Examination (MMSE) score < 25 . Specific exclusion criteria for the olfaction test were: (1) history of nasal bone fracture, (2) diagnosis of rhinitis or nasal polyps, and (3) upper respiratory tract infections in the 2 weeks prior to testing.

The inclusion criteria for PD were: (1) the fulfillment of UK PD Society Brain Bank diagnostic criteria for PD (Hughes, Daniel, Kilford, & Lees, 1992); (2) no surgical treatment with deep brain stimulation. Exclusion criteria for PD were: (1) Hoehn and Yahr (H&Y) (Hoehn & Yahr, 1967) score > 3 (defined as mild to moderate bilateral disease with some postural instability but physically independent); (2) dementia according to Movement Disorder Society criteria (Dubois et al., 2007): (a) PD developed prior to the onset of dementia, (b) PD associated with a decreased Global Cognitive Efficiency, (c) cognitive impairment in more than one cognitive domain, and (d) impact on daily living resulting from cognitive deficits, over and above those imposed by motor and autonomic problems (Dubois et al., 2007).

All PD patients were taking antiparkinsonian medication consisting of different combinations of L-DOPA, COMT inhibitors, MAO inhibitors, dopamine agonists, and amantadine. In order to standardize doses, levodopa equivalent daily dose (LEDD) was calculated as suggested by Tomlinson and colleagues (2010). All assessments were done in the on state.

Motor disease severity was evaluated using H&Y staging and the Unified Parkinson's Disease Rating Scale motor section (UPDRS-III).

The study was approved by the Ethics Committee of the University of Barcelona (IRB00003099). All subjects provided written informed consent to participate after full explanation of the procedures involved.

Olfactory and Clinical Assessment

Odor identification was assessed using the Spanish version of the University of Pennsylvania Smell Identification Test (UPSIT-40) (Doty, 1995).

The UPSIT is a standardized multiple-choice scratch-and-sniff test consisting of four test booklets with 10 items each. In accordance with normative instructions, subjects scratch the impregnated area and are asked to select one of four possible answers for each item. Following normative data presented in the UPSIT manual (Doty et al., 1995), which includes adjustment for age and sex, scores greater than 33 in males and 34 in females are considered to reflect normosmia, and scores lower or equal to 18 reflect anosmia. Microsmia ranges from 33 or 34 (depending on the subject's gender) to 19.

In order to achieve higher cultural familiarity, the Smell Identification Test™ (UPSIT) Spanish version commercialized by Sensonics (<https://sensonics.com>) substitutes some items with respect to the original UPSIT. Specifically, in item 13, *talcum powder* replaces *liquorice*; in item 20, *apple* replaces *gingerbread*; in item 24, *rubber tire* replaces *root beer*; in item 27, *raspberry* replaces *lime*; and in item 29, *walnut* replaces *wintergreen*.

The Beck Depression Inventory II (Beck, Steer, & Brown, 1996), Starkstein's Apathy Scale (Starkstein et al., 1992), and the Neuropsychiatric Inventory (Cummings et al., 1994) were administered to all subjects to explore the presence of psychiatric symptoms.

Demographic, Clinical, and Olfactory Data Analysis

Statistical analysis of demographic, clinical, and olfactory data was performed using IBM SPSS Statistics 24.0.0 (2016; Armonk, NY: IBM Corp). Group differences in demographic and clinical variables between HC and PD patients were analyzed with independent Student's *t*-tests for quantitative measures or with Pearson's chi-squared test for categorical measures. Bonferroni correction was used to correct for multiple testing. A multiple regression analysis with stepwise method was used to define possible UPSIT score predictors in the whole sample and in the PD group. Predictors were chosen based on previous literature (Deeb et al., 2010; Doty et al., 1978; Doty, 2012a, 2012b; Frye, Schwartz, & Doty, 1990; Hubert, Fabsitz, Feinleib, & Brown, 1980; Joyner, 1964; McKinnon et al., 2010) and correlation analysis. Age, PD diagnosis, sex, years of education, and smoking status were introduced as possible predictors for the whole sample regression. For the PD subsample, PD diagnosis was excluded, and clinical measures such as H&Y, UPDRS-III, and LEDD were added.

UPSIT Item Analysis

Sensitivity, specificity, diagnostic accuracy, positive predictive value, and negative predictive value for PD were calculated for the UPSIT-40 total score, and for each item. Pearson's chi-squared test was used to compare the percentage of HC and PD patients who correctly identified each item of the 40-items UPSIT version.

Incorrect answers were considered as positive findings (i.e., altered performance), whereas correct answers defined negatives. True positives (TP) were defined as the number of PD patients who got a wrong response in a certain item; true negatives (TN) as the number of HC who got a correct response; false positives (FP) were calculated as the number of HC who got a wrong response and, finally, false negatives (FN) were described as the number of PD patients who had a correct answer. The following diagnostic measures were calculated: (1) sensitivity, defined as the probability of testing positive when PD is present: $TP/(TP + FN)$; (2) specificity, defined as the probability of testing negative when PD is absent: $TN/(FP + TN)$; (3) positive predictive value (PPV), calculated as the percentage of subjects with a positive test who actually have PD: $TP/(TP + FP)$; (4) negative predictive value (NPV), defined as the percentage of subjects with a negative test who do not have PD: $TN/(TN + FN)$; finally, (5) overall diagnostic accuracy was defined as the proportion of correctly classified subjects among all subjects: $(TP + TN)/(TP + TN + FP + FN)$.

Results

Demographic and Clinical Characteristics

There were no differences between groups in sex ($\chi^2 = 3.447$; $p = .063$), age ($t = -1.2798$; $p = .437$), years of education ($t = 0.639$; $p = .429$), or estimated IQ scores ($t = 0.041$; $p = .977$). Regarding psychiatric symptoms, there were significant differences between groups in Beck Depression Inventory II ($t = -4.1329$; $p = .001$), Starkstein's Apathy Scale ($t = -4.5463$; $p < .001$), and in Cummings' Neuropsychiatric Inventory scores ($t = -6.6537$; $p < .001$) (see Supplementary material online, Table S1).

Olfactory Performance

PD patients correctly identified 18.6 ± 6.8 UPSIT items and HC 29.8 ± 4.2 ($t = 11.961$; $p < .001$). Considering the normative cut-off score of ≤ 18 , 54.6% of PD patients and no HC were identified as anosmic (see Supplementary material online, Table S2).

UPSIT Diagnostic Measures

For the normative cut-off score of ≤ 18 to define anosmia, UPSIT total score had a sensitivity of 54.6% with a specificity of 100.0%, a PPV of 100.0%, a NPV of 59.6%, and a diagnostic accuracy of 72.8% for PD. The receiver operating characteristic (ROC) curve showed that the optimal cut-off score for UPSIT-40 was ≤ 25 with a sensitivity of 81.4%, a specificity of 84.6%, a PPV of 88.8%, a NPV of 75.3% and a diagnostic accuracy of 82.7% (area under the ROC curve = 0.908) (Table 1).

When considering the whole sample, UPSIT total score correlated negatively with age ($r = -.300$; $p < .001$). In the HC group, significant correlations were found between UPSIT total score and age ($r = -.406$; $p = .001$) and years of education ($r = .296$; $p = .017$). In the PD patient group, a negative correlation was found between UPSIT total score and age ($r = -.366$; $p < .001$). Sex did not achieve significant correlation with UPSIT total score, either in HC ($r = .221$; $p = .076$) or in PD ($r = -.016$; $p = .875$). Diagnosis ($B = -10.948$; $t = -11.333$; $p < .001$) and age ($B = -0.203$; $t = -4.370$; $p < .001$) were found to be the best predictors of UPSIT scores when the whole sample was considered. For the PD patient sample, clinical measures were included in the regression analysis, and only age was identified as a significant UPSIT score predictor ($B = -0.250$; $t = -3.545$; $p < .001$). According to these data, we plotted ROC curves for three age groups (≤ 60 , 61–70, and ≥ 71 years). Table 2 shows cut-off scores with higher sensitivity and specificity for each age group. Sensitivity and specificity for PD diagnosis considering age groups for each UPSIT cut-off score are represented in Fig. 1. In addition, we plotted ROC curves considering the same three groups divided by gender (Table 3).

Table 1. Diagnostic accuracy measures for UPSIT-40

UPSIT version	TP	TN	FP	FN	Diagnostic accuracy	Sensitivity	Specificity	PPV	NPV
UPSIT-40 (cut-off ≤ 18)	53	65	0	44	72.8	54.6	100.0	100.0	59.6
UPSIT-40 (cut-off ≤ 25)	79	55	10	18	82.7	81.4	84.6	88.8	75.3

Note: FN = false negative; FP = false positive; HC = healthy controls; NPV = negative predictive value; PD = Parkinson's disease; PPV = positive predictive value; TN = true negative; TP = true positive; UPSIT = University of Pennsylvania Smell Identification Test; UPSIT-40 (cut-off ≤ 18) = normative cut-off for anosmia; UPSIT-40 (cut-off ≤ 25) = optimal cut-off for this study sample.

Table 2. Optimal cut-off scores and associated sensitivity and specificity for PD diagnosis for three age groups

Age group	Cut-off score	Sensitivity (%)	Specificity (%)	AUROC
≤ 60 years	29	83.3	87.5	0.905
61–70 years	25	82.1	81.0	0.903
≥ 71 years	22	85.7	85.0	0.954

Note: AUROC = area under the receiver operating characteristic curve; HC = healthy controls; PD = Parkinson's disease. Optimal cut-off scores and associated sensitivity and specificity were calculated through ROC curve analysis for three age groups: ≤ 60 years (PD = 30; HC = 24), 61–70 years (PD = 39; HC = 21), and ≥ 71 years (PD = 28; HC = 20).

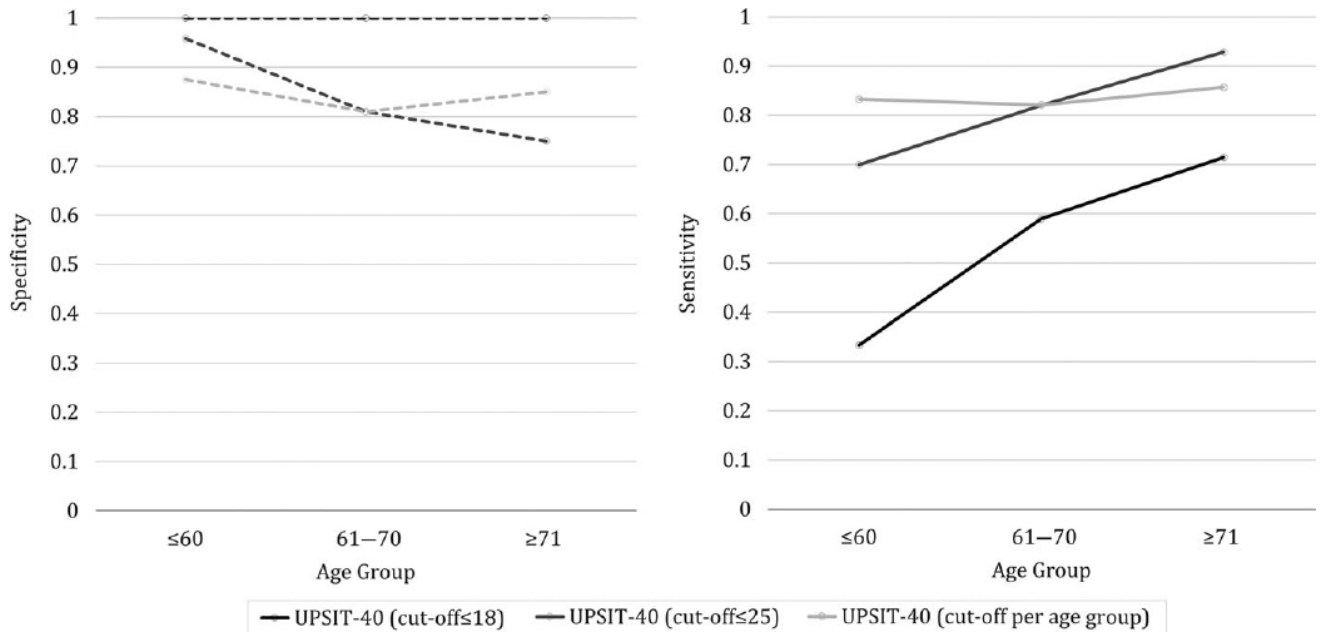


Fig. 1. UPSIT sensitivity and specificity for PD diagnosis considering age group. UPSIT: *University of Pennsylvania Smell Identification Test*; UPSIT-40 (cut-off ≤18): normative cut-off; UPSIT-40 (cut-off ≤25): optimal cut-off for this study sample; UPSIT-40 (cut-off per age group): optimal cut-off score for each age group. Age groups and corresponding group sizes: ≤60 years (PD = 30; HC = 24), 61–70 years (PD = 39; HC = 21), ≥71 years (PD = 28; HC = 20).

Table 3. Optimal cut-off scores and associated sensitivity and specificity for PD diagnosis considering age and gender measures

Age group	Sample size		Cut-off score		Sensitivity (%)		Specificity (%)		AUROC	
	M	F	M	F	M	F	M	F	M	F
Whole sample	32/62	33/35	24	27	80.6	88.6	81.2	87.9	0.891	0.922
≤60 years	9/25	15/5	29	30	84.0	80.0	77.8	80.0	0.889	0.847
61–70 years	12/23	9/16	22	26	82.6	87.5	91.7	88.9	0.897	0.917
≥71 years	11/14	9/14	24	27	100.0	92.9	81.8	77.8	0.987	0.929

Note: Sample size for male and female subgroups is presented as, HC/PD.

UPSIT Item Analysis

Diagnostic measures and group differences for each item are described in Table 4 and Fig. 2.

Items that did not show significant differences between groups in the chi-squared test after Bonferroni correction were: *Bubble gum* ($\chi^2 = 9.727$; $p = .002$), *cherry* ($\chi^2 = 0.063$; $p = .802$), *fruit juice* ($\chi^2 = 8.645$; $p = .003$), *cheese* ($\chi^2 = 3.580$; $p = .058$), *cedar* ($\chi^2 = 1.849$; $p = .174$), *peach* ($\chi^2 = 7.183$; $p = .007$), *pickle* ($\chi^2 = 0.404$; $p = .507$), *orange* ($\chi^2 = 3.836$; $p = .050$), *walnut* ($\chi^2 = 0.059$; $p = .808$), *lemon* ($\chi^2 = 1.652$; $p = .199$), and *soap* ($\chi^2 = 9.556$; $p = .002$).

Items with less than 50.0% correct answers in HC were: *cedar* (44.6%), *lemon* (40.0%), *cherry* (33.8%), *pickle* (26.2%), and *walnut* (23.1%). In particular, according to age grouping, items with an error rate above 50.0% for HC under 60 years old were *dill pickle* (33.3%), *walnut* (20.8%), and *lemon* (37.5%); for the middle-age group, *pizza* (38.1%), *dill pickle* (23.8%), *walnut* (9.5%), and *cherry* (28.6%); lastly, for HC above 71, *pizza* (40.0%), *cedar* (30.0%), *dill pickle* (20.0%), *orange* (40.0%), *walnut* (40.0%), *lemon* (35.0%), and *cherry* (10.0%).

Items with sensitivity above 75.0% in the PD patient group were *walnut* (75.3%), *pickle* (78.4%), and *pizza* (78.4%). Sensitivity below 25.0% was seen for *onion* (23.7%). Items with a specificity above 90.0% were *chocolate* (93.8%), *coconut* (93.8%), *lilac* (95.4%), *onion* (96.9%), *talcum powder* (98.5%), and *smoke* (98.5%). *Walnut* (23.1%) had a specificity below 25.0%. Items with a diagnostic accuracy above 70.0% were *paint thinner* (71.6%), *clove* (72.3%), and *mint* (73.3%), and those below 55.0% were *onion* (53.1%), *cherry* (54.3%), and *walnut* (54.3%).

Table 4. Item analysis of the UPSIT Spanish version in PD

Item	Label	TP	TN	FP	FN	Diagnostic accuracy	Sensitivity	Specificity	PPV	NPV	Chi-square/ <i>p</i> value
1	Pizza	76	33	32	21	67.3	78.4	50.8	70.4	61.1	14.85/<.001
2	Bubble gum	35	56	9	62	56.2	36.1	86.2	79.5	47.5	9.73/.002
3	Menthol	56	54	11	41	67.9	57.7	83.1	83.6	56.8	26.73/<.001
4	Cherry	66	22	43	31	54.3	68.0	33.8	60.6	41.5	0.06/.802
5	Motor oil	58	47	18	38	65.2	60.4	72.3	76.3	55.3	16.65/<.001
6	Mint	61	57	8	35	73.3	63.5	87.7	88.4	62.0	41.54/<.001
7	Banana	42	56	9	54	60.9	43.8	86.2	82.4	50.9	16.01/<.001
8	Clove	63	54	11	34	72.2	64.9	83.1	85.1	61.4	36.18/<.001
9	Leather	38	57	8	59	58.6	39.2	87.7	82.6	49.1	13.82/<.001
10	Coconut	41	61	4	56	63.0	42.3	93.8	91.1	52.1	25.30/<.001
11	Onion	23	63	2	74	53.1	23.7	96.9	92.0	46.0	12.69/<.001
12	Fruit juice	51	46	19	46	59.9	52.6	70.8	72.9	50.0	8.64/.003
13	Talcum powder	27	64	1	70	56.2	27.8	98.5	96.4	47.8	18.82/<.001
14	Cheese	55	38	27	42	57.4	56.7	58.5	67.1	47.5	3.58/.058
15	Cinnamon	50	56	8	47	65.8	51.5	87.5	86.2	54.4	25.51/<.001
16	Gasoline	55	57	8	42	69.1	56.7	87.7	87.3	57.6	32.27/<.001
17	Strawberry	57	46	19	40	63.6	58.8	70.8	75.0	53.5	13.63/<.001
18	Cedar	64	29	36	33	57.4	66.0	44.6	64.0	46.8	1.85/.174
19	Chocolate	31	61	4	66	56.8	32.0	93.8	88.6	48.0	15.30/<.001
20	Apple	51	53	12	46	64.2	52.6	81.5	81.0	53.5	19.06/<.001
21	Lilac	37	62	3	60	61.1	38.1	95.4	92.5	50.8	23.53/<.001
22	Turpentine	66	41	24	30	66.5	68.8	63.1	73.3	57.7	15.93/<.001
23	Peach	44	49	16	53	57.4	45.4	75.4	73.3	48.0	7.18/.007
24	Rubber tire	61	44	21	36	64.8	62.9	67.7	74.4	55.0	14.56/<.001
25	Pickle	76	17	48	21	57.4	78.4	26.2	61.3	44.7	0.40/.507
26	Pineapple	51	56	9	46	66.0	52.6	86.2	85.0	54.9	25.04/<.001
27	Raspberry	32	58	7	65	55.6	33.0	89.2	82.1	47.2	10.51/.001
28	Orange	51	41	24	46	56.8	52.6	63.1	68.0	47.1	3.84/.050
29	Walnut	73	15	50	24	54.3	75.3	23.1	59.3	38.5	0.06/.808
30	Watermelon	40	57	8	57	59.9	41.2	87.7	83.3	50.0	15.62/<.001
31	Paint thinner	58	58	7	39	71.6	59.8	89.2	89.2	59.8	38.93/<.001
32	Grass	55	55	10	42	67.9	56.7	84.6	84.6	56.7	27.65/<.001
33	Smoke	42	64	1	55	65.4	43.3	98.5	97.7	53.8	34.81/<.001
34	Pine	59	44	21	38	63.6	60.8	67.7	73.8	53.7	12.66/<.001
35	Grape	65	41	23	32	65.8	67.0	64.1	73.9	56.2	15.02/<.001
36	Lemon	67	26	39	29	57.8	69.8	40.0	63.2	47.3	1.65/.199
37	Soap	59	41	23	38	62.1	60.8	64.1	72.0	51.9	9.56/.002
38	Natural gas	43	57	8	54	61.7	44.3	87.7	84.3	51.4	18.50/<.001
39	Rose	60	46	19	37	65.4	61.9	70.8	75.9	55.4	16.58/<.001
40	Peanut	44	57	8	53	62.3	45.4	87.7	84.6	51.8	19.51/<.001

Note: Items marked in blue showed no significant differences in the percentage of correct answers between HC and PD patients. FN = false negative; FP = false positive; HC = healthy controls; NPV = negative predictive value; PD = Parkinson's disease; PPV = positive predictive value; TN = true negative; TP = true positive. Pearson's chi-squared tests were used to compare the percentage of HC and PD patients who correctly identified each item. *p*-Values that survive Bonferroni correction for multiple comparisons ($p \leq .00125$) are highlighted in bold.

Discussion

Since olfactory deficits have been found to be a relevant clinical feature in PD (Berendse *et al.* 2011; Doty, 2012a, 2012b), there has been growing interest in incorporating odor tests in routine protocols both in patient assessment and in data collection. Our results show age and sex should be taken into consideration when using UPSIT Spanish version as a tool for identifying olfactory deficits associated with PD.

In the current study, the normative cut-off score of anosmia proposed by the UPSIT manual (≤ 18) demonstrated high specificity (100.0%) and low sensitivity (54.6%). In our sample, the optimal cut-off score was identified as ≤ 25 , with a diagnostic accuracy of 82.7%, a sensitivity of 81.4%, and a specificity of 84.6%. This observed sensitivity is similar to that found in a Mexican population using the same cut-off score (Rodriguez-Violante *et al.* 2014b). In an Italian study, the cut-off score of ≤ 21 differentiated PD and HC with similar sensitivity (82.0%) and specificity (88.2%) (Picillo *et al.*, 2014). Given that cross-

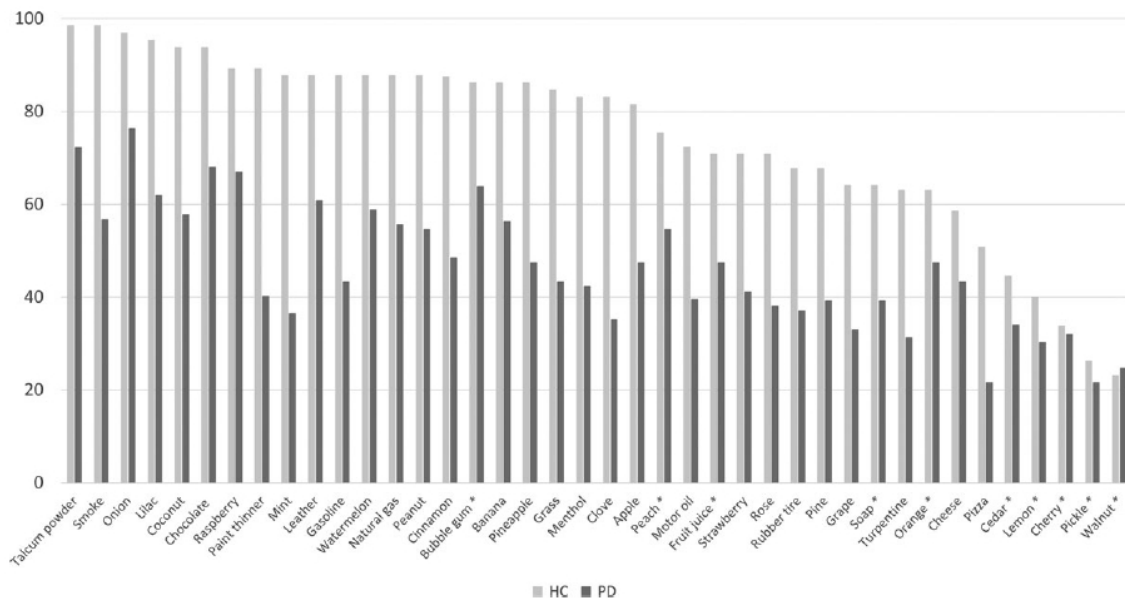


Fig. 2. Percentage of subjects who correctly identified each item of the UPSIT. *Items that did not show significant differences between groups in the chi-squared test after Bonferroni correction. HC: healthy controls; PD: Parkinson's disease.

cultural aspects may influence these results (Rodríguez-Violante *et al.* 2014a), it could be of interest to perform a more detailed analysis of the UPSIT-40 items to identify those with less capability to detect olfactory deficits in a given population due to cultural biases.

We performed an item analysis similar to previous works (Kondo *et al.*, 1998; Picillo *et al.*, 2014; Rodríguez-Violante *et al.* 2014b; Silveira-Moriyama *et al.*, 2008). Our results showed that some items had a rate of correct answers of less than 50.0% in HC (cedar, lemon, cherry, dill pickle, and walnut), specifically, the three age groups coincided on dill pickle and walnut. Such items showed no significant differences in the percentage of success between HC and PD patients. As has been proposed in the literature (Picillo *et al.*, 2014), a low percentage of success in control subjects may be an indicator of culturally unsuitable items. A decreased ability to identify of some odors has been reported as well in Italian (Picillo *et al.*, 2014), Brazilian (Silveira-Moriyama *et al.*, 2008), Mexican (Rodríguez-Violante *et al.* 2014b), and Japanese (Kondo *et al.*, 1998) control subjects. Out of the five items with low identification rates by HC in our study, three items (cherry, cedar, lemon) also showed a low percentage of correct identification in a previous study obtained from an Italian population (Picillo *et al.*, 2014). Rodríguez-Violante *et al.* also found that cherry, walnut, and dill pickle were identified by less than 50.0% of subjects in a Mexican population (Rodríguez-Violante *et al.* 2014b). In Brazil, Silveira-Moriyama *et al.* found an identification rate of less than 50.0% in cedar, dill pickle, and lemon as well (Silveira-Moriyama *et al.*, 2008). In this sense, a large percentage (86.0%) of HC fell in the microsmic range of performance, with only a small number (13.8%) being classified as normosmic. This increased rate of microsmia may reflect, among other reasons, the impact of cultural differences on UPSIT performance. These findings suggest a need to provide and to promote the use of country-specific normative data.

Our results show that UPSIT performance was explained mainly by group, but also by age. Contrary to previous studies (Campabadal *et al.*, 2017; Sharma & Turton, 2012; Silveira-Moriyama *et al.*, 2008), clinical measures such as motor scales and disease duration did not significantly predict smell identification performance in our PD subsample. One explanation could be that olfactory loss is an early symptom that progresses mainly in prodromal stages and may reach maximum severity levels even earlier than motor symptoms, which progress through advanced stages (Doty, Deems, & Stellar, 1988). Further studies in de novo samples could help identify a more accurate cut-off score for PD patients in initial stages, which could eventually have a positive impact on their quality of life.

Age contribution to olfactory deficits is well known both in normal subjects (McKinnon *et al.*, 2010; Sorokowska *et al.*, 2015) and in PD patients (Doty *et al.*, 1995). This is demonstrated by the fact that, in the original English version, discriminatory UPSIT scores are presented for different age groups (Doty *et al.*, 1995). Even though changes in some specific items have been made, these age-related discrimination criteria have not been reviewed for the Spanish version.

Age-group analysis showed that the UPSIT-40 cut-off score of ≤ 25 demonstrated higher sensitivity for all age ranges than the normative cut-off score for anosmia. When cut-off scores for each age group were considered, sensitivity and specificity

values were above 80.0% for all age groups. These values were similar when groups were classified by age and gender. In particular, our results show cut-off scores for the youngest group (≤ 60 years) were 29 for males and 30 for females; for the intermediate age group (61–70 years), they were 22 for males and 26 for females; and for the oldest group (≥ 71 years) they were 24 and 27, respectively. Cut-off scores for all groups were similar but below those proposed by Doty and colleagues (1995). With a sample of 180 PD patients and 612 HC, Doty and colleagues (1995) found that the sensitivity and specificity of the UPSIT in distinguishing between male PD patients and HC under the age of 61 years was 91.0% and 88.0%, respectively (cut-off score 31). For females of the same age, the corresponding values were 79.0% and 85.0% (cut-off score 33). Sensitivity and specificity of the UPSIT-40 for subjects between 61 and 70 years of age were 81.0% and 82.0% for males (cut-off score 25) and 80.0% and 88.0% for females, respectively (cut-off score 30). Finally, for subjects older than 71, sensitivity and specificity for males were 76.0% and 78.0% (cut-off score 22), and 78.0% and 82.0% for females (cut-off score 25) (Doty et al., 1995). Considering that the onset of motor features in PD takes place around the age of 60, there is a need for developing more accurate procedures for detecting olfactory impairment in young PD patients and in the prodromal stages.

Screening for potential early indicators of PD such as olfaction, constipation, or sleep disturbances facilitates the identification of individuals at high risk for PD who could participate in medical trials addressed to prevent or slow disease progression (Ross et al., 2008). Therefore, although the UPSIT has high capability in differentiating PD patients from healthy controls, it should be used within the context of a comprehensive clinical assessment.

In conclusion, our results demonstrate that the sensitivity and specificity of the UPSIT Spanish version were improved when specific cut-off scores for different age and sex groups were considered.

Limitations of this study include the fact that most of our PD patients are in mild to moderate stages of the disease. Since the PD patients of our study were already identified as having PD, our findings cannot address the issue of early diagnosis of PD. Further studies examining early clinical or prodromal stages of PD are needed to address this question. In this study we have excluded patients with low IQ and dementia; thus, our findings are not generalizable to the whole PD population. Finally, our data is preliminary, and the proposed cut-offs need to be replicated before being used clinically.

Supplementary Material

Supplementary material is available at *Archives of Clinical Neuropsychology* online.

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Conflict of interest

Authors AC, BS, HCB, AA, CU, AGD, MJM, FV, NB and CJ report no disclosure. YC has received funding, research support, and/or honoraria in the last 5 years from Union Chimique Belge (UCB pharma), Lundbeck, Medtronic, Abbvie, Novartis, GSK, Boehringer, Pfizer, Merz, Piramal Imaging and Esteve.

References

- Beck, A., Steer, R., & Brown, G. (1996). *Manual for the beck depression inventory-II*. San Antonio, Texas: Psychological Corporation.
- Berendse, H. W., Roos, D. S., Raijmakers, P., & Doty, R. L. (2011). Motor and non-motor correlates of olfactory dysfunction in Parkinson's disease. *Journal of the Neurological Sciences*, 310, 21–24. <https://doi.org/10.1016/j.jns.2011.06.020>
- Bohnen, N. I., Studenski, S. A., Constantine, G. M., & Moore, R. Y. (2008). Diagnostic performance of clinical motor and non-motor tests of Parkinson disease: A matched case-control study. *European Journal of Neurology*, 15, 685–691. <https://doi.org/10.1111/j.1468-1331.2008.02148.x>
- Campabadal, A., Uribe, C., Segura, B., Baggio, H. C., Abos, A., Garcia-Diaz, A. I., et al. (2017). Brain correlates of progressive olfactory loss in Parkinson's disease. *Parkinsonism and Related Disorders*, 41, 44–50. <https://doi.org/10.1016/j.parkreldis.2017.05.005>
- Cummings, J. L., Mega, M., Gray, K., Rosenberg-Thompson, S., Carusi, D. A., & Gornbein, J. (1994). The Neuropsychiatric Inventory: Comprehensive assessment of psychopathology in dementia. *Neurology*, 44, 2308–2314.

- Deeb, J., Shah, M., Muhammed, N., Gunasekera, R., Gannon, K., Findley, L. J., et al. (2010). A basic smell test is as sensitive as a dopamine transporter scan: Comparison of olfaction, taste and DaTSCAN in the diagnosis of Parkinson's disease. *QJM: Monthly Journal of the Association of Physicians*, 103, 941–952. <https://doi.org/10.1093/qjmed/hcq142>
- Doty, R. L. (1995). *The smell identification test administration manual* (3rd ed.). Haddon Heights: Sensonics.
- Doty, R. L. (2012a). Olfaction in Parkinson's disease and related disorders. *Neurobiology of Disease*, 46, 527–552. <https://doi.org/10.1016/j.nbd.2011.10.026>
- Olfaction
- Doty, R. L. (2012b). Olfactory dysfunction in Parkinson disease. *Nature Publishing Group*, 8, 329–339. <https://doi.org/10.1038/nrneurol.2012.80>
- Doty, R. L. (2017). Olfactory dysfunction in neurodegenerative diseases: Is there a common pathological substrate? *The Lancet Neurology*, 16, 478–488. [https://doi.org/10.1016/S1474-4422\(17\)30123-0](https://doi.org/10.1016/S1474-4422(17)30123-0)
- Doty, R. L., Bromley, S. M., & Stern, M. B. (1995). Olfactory testing as an aid in the diagnosis of Parkinson's disease: Development of optimal discrimination criteria. *Neurodegeneration: A Journal for Neurodegenerative Disorders, Neuroprotection, and Neuroregeneration*, 4, 93–97. <http://www.ncbi.nlm.nih.gov/pubmed/7600189>
- Doty, R. L., Deems, D. A., & Stellar, S. (1988). Olfactory dysfunction in Parkinsonism: A general deficit unrelated to neurologic signs, disease stage, or disease duration. *Neurology*, 38, 1237–1244. <http://www.ncbi.nlm.nih.gov/pubmed/3399075>
- Doty, R. L., Ford, M., Preti, G., & Huggins, G. R. (1975). Changes in the intensity and pleasantness of human vaginal odors during the menstrual cycle. *Science (New York, N.Y.)*, 190, 1316–1318. <http://www.ncbi.nlm.nih.gov/pubmed/1239080>
- Doty, R. L., Green, P. A., Ram, C., & Yankell, S. L. (1982). Communication of gender from human breath odors: Relationship to perceived intensity and pleasantness. *Hormones and Behavior*, 16, 13–22. [https://doi.org/10.1016/0018-506X\(82\)90002-2](https://doi.org/10.1016/0018-506X(82)90002-2)
- Doty, R. L., Orndorff, M. M., Leyden, J., & Kligman, A. (1978). Communication of gender from human axillary odors: Relationship to perceived intensity and hedonicity. *Behavioral Biology*, 23, 373–380. <http://www.ncbi.nlm.nih.gov/pubmed/697690>
- Dubois, B., Burn, D., Goetz, C., Aarsland, D., Brown, R. G., Broe, G. A., et al. (2007). Diagnostic procedures for Parkinson's disease dementia: Recommendations from the Movement Disorder Society Task Force. *Movement Disorders*, 22, 2314–2324. <https://doi.org/10.1002/mds.21844>
- Frye, R. E., Schwartz, B. S., & Doty, R. L. (1990). Dose-related effects of cigarette smoking on olfactory function. *JAMA: The Journal of the American Medical Association*, 263, 1233–1236. <http://www.ncbi.nlm.nih.gov/pubmed/2304239>
- Fullard, M. E., Tran, B., Xie, S. X., Toledo, J. B., Scordia, C., Linder, C., et al. (2016). Olfactory impairment predicts cognitive decline in early Parkinson's disease. *Parkinsonism and Related Disorders*, 25, 45–51. <https://doi.org/10.1016/j.parkreldis.2016.02.013>
- Haehner, A., Boesveldt, S., Berendse, H. W., Mackay-Sim, A., Fleischmann, J., Silburn, P. A., et al. (2009). Prevalence of smell loss in Parkinson's disease – A multicenter study. *Parkinsonism and Related Disorders*, 15, 490–494. <https://doi.org/10.1016/j.parkreldis.2008.12.005>
- Hohn, M. M., & Yahr, M. D. (1967). Parkinsonism: Onset, progression and mortality. *Neurology*, 17, 427–442.
- Hubert, H. B., Fabsitz, R. R., Feinleib, M., & Brown, K. S. (1980). Olfactory sensitivity in humans: Genetic versus environmental control. *Science (New York, N.Y.)*, 208, 607–609. <http://www.ncbi.nlm.nih.gov/pubmed/7189296>
- Hughes, A. J., Daniel, S. E., Kilford, L., & Lees, A. J. (1992). Accuracy of clinical diagnosis of idiopathic Parkinson's disease: A clinico-pathological study of 100 cases. *Journal of Neurology, Neurosurgery, and Psychiatry*, 55, 181–184. <https://doi.org/10.1136/jnnp.55.3.181>
- Joyner, R. E. (1964). Effect of cigarette smoking on olfactory acuity. *Archives of Otolaryngology (Chicago, Ill.: 1960)*, 80, 576–579. <http://www.ncbi.nlm.nih.gov/pubmed/14216019>
- Kondo, H., Matsuda, T., Hashiba, M., & Baba, S. (1998). A study of the relationship between the T&T olfactometer and the University of Pennsylvania Smell Identification Test in a Japanese population. *American Journal of Rhinology & Allergy*, 2, 353–358.
- McKinnon, J., Evidente, V., Driver-Dunkley, E., Premkumar, A., Hentz, J., Shill, H., et al. (2010). Olfaction in the elderly: A cross-sectional analysis comparing Parkinson's disease with controls and other disorders. *The International Journal of Neuroscience*, 120, 36–39. <https://doi.org/10.3109/00207450903428954>
- Ogihara, H., Kobayashi, M., Nishida, K., Kitano, M., & Takeuchi, K. (2011). Applicability of the cross-culturally modified University of Pennsylvania Smell Identification Test in a Japanese population. *American Journal of Rhinology & Allergy*, 25, 404–410. <https://doi.org/10.2500/ajra.2011.25.3658>
- Picillo, M., Pellicchia, M. T., Erro, R., Amboni, M., Vitale, C., Iavarone, A., et al. (2014). The use of University of Pennsylvania Smell Identification Test in the diagnosis of Parkinson's disease in Italy. *Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology*, 35, 379–383. <https://doi.org/10.1007/s10072-013-1522-6>
- Rodriguez-Violante, M., Gonzalez-Latapi, P., Camacho-Ordoñez, A., Martínez-Ramírez, D., Morales-Briceño, H., & Cervantes-Arriaga, A. (2014a). Comparing the accuracy of different smell identification tests in Parkinson's disease: Relevance of cultural aspects. *Clinical Neurology and Neurosurgery*, 123, 9–14. <https://doi.org/10.1016/j.clineuro.2014.04.030>
- Rodriguez-Violante, M., Gonzalez-Latapi, P., Camacho-Ordoñez, A., Martínez-Ramírez, D., Morales-Briceño, H., & Cervantes-Arriaga, A. (2014b). Low specificity and sensitivity of smell identification testing for the diagnosis of Parkinson's disease. *Arquivos de Neuro-psiquiatria*, 72, 33–37. <https://doi.org/10.1590/0004-282X20130190>
- Rodriguez-Violante, M., Lees, A. J., Cervantes-Arriaga, A., Corona, T., & Silveira-Moriyama, L. (2011). Use of smell test identification in Parkinson's disease in Mexico: A matched case-control study. *Movement Disorders*, 26, 173–176. <https://doi.org/10.1002/mds.23354>
- Ross, G. W., Petrovitch, H., Abbott, R. D., Tanner, C. M., Popper, J., Masaki, K., et al. (2008). Association of olfactory dysfunction with risk for future Parkinson's disease. *Annals of Neurology*, 63, 167–173. <https://doi.org/10.1002/ana.21291>
- Sharma, J. C., & Turtun, J. (2012). Olfaction, dyskinesia and profile of weight change in Parkinson's disease: Identifying neurodegenerative phenotypes. *Parkinsonism and Related Disorders*, 18, 964–970. <https://doi.org/10.1016/j.parkreldis.2012.05.004>
- Silveira-Moriyama, L., Azevedo, A. M., Ranvaud, R., Barbosa, E. R., Doty, R. L., & Lees, A. J. (2010). Applying a new version of the Brazilian-Portuguese UPSIT smell test in Brazil. *Arquivos de Neuro-psiquiatria*, 68, 700–705. <https://doi.org/10.1590/S0004-282X2010000500005>
- Silveira-Moriyama, L., de Jesus Carvalho, M., Katzenschlager, R., Petrie, A., Ranvaud, R., Barbosa, E. R., et al. (2008). The use of smell identification tests in the diagnosis of Parkinson's disease in Brazil. *Movement Disorders*, 23, 2328–2334. <https://doi.org/10.1002/mds.22241>
- Sorokowska, A., Schriever, V. A., Gudziol, V., Hummel, C., Hähner, A., Iannilli, E., et al. (2015). Changes of olfactory abilities in relation to age: Odor identification in more than 1400 people aged 4 to 80 years. *European Archives of Oto-Rhino-Laryngology*, 272, 1937–1944. <https://doi.org/10.1007/s00405-014-3263-4>

- Starkstein, S. E., Mayberg, H. S., Preziosi, T. J., Andrezejewski, P., Leiguarda, R., & Robinson, R. G. (1992). Reliability, validity, and clinical correlates of apathy in Parkinson's disease. *The Journal of Neuropsychiatry and Clinical Neurosciences*, 4, 134–139. <https://doi.org/10.1176/jnp.4.2.134>
- Tomlinson, C. L., Stowe, R., Patel, S., Rick, C., Gray, R., & Clarke, C. E. (2010). Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Movement Disorders*, 25, 2649–2653. <https://doi.org/10.1002/mds.23429>
- Yücepur, C., Özücer, B., Değirmenci, N., Yıldırım, Y., Veyseller, B., & Özturan, O. (2012). University of Pennsylvania smell identification test: Application to Turkish population. *Kulak Burun Bogaz İhtisas Dergisi: KBB=Journal of Ear, Nose, and Throat*, 22, 77–80. <http://www.ncbi.nlm.nih.gov/pubmed/22548263>

Supplementary material

Diagnostic Accuracy, Item Analysis and Age Effects of the UPSIT Spanish Version in Parkinson's Disease

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Supplementary Table 1. Demographic and clinical characteristics of PD patients and HC

	PD (n=97)	HC (n=65)	Test stats/P-value
Age, yrs., mean (SD)	65.6 (9.6)	64.2 (11.1)	1.28/.437 ^a
Education, yrs., mean (SD)	11.4 (5.3)	12.1 (4.5)	.64/.429 ^a
Vocabulary subtest, mean (SD)	45.2 (9.1)	45.2 (7.9)	.04/.977 ^a
Sex, male, n (%)	62 (63.9)	32 (49.2)	3.45/.063 ^b
Smoking status (nonsmoker/ex-smoker/smoker)	60/6/30	24/5/10	1.77/.413
Disease duration, yrs., mean (SD)	8.7 (5.9)	NA	NA
Age of onset, yrs., mean (SD)	56.7 (11.3)	NA	NA
UPDRS-III, mean (SD)	17.4 (9.5)	NA	NA
Hoehn & Yahr stage, n 1/1.5/2/2.5/3/4	8/2/51/5/24/0	NA	NA
LEDD, mg, mean (SD)	765.9 (452.2)	NA	NA

Abbreviations: HC, healthy controls; LEDD, L-DOPA equivalent daily dose; NA, not applicable; PD, Parkinson's disease; UPDRS part III, Unified Parkinson's Disease Rating Scale motor section. Data are presented as mean (SD), except for sex (number of cases and percentage) and Hoehn and Yahr staging (number of cases).

- a. Independent Student's t-tests were used for continuous variables when comparing HC with all PD patients.
- b. Pearson's chi-squared tests were used for categorical variables.

Supplementary table 2. Olfactory measures in PD and HC.

	PD (n=97)	HC (n=65)	Test stats/ P-value	Effect size
UPSIT-40	18.6 (6.8)	29.8 (4.2)	11.96/<.001 ^a	0.69
<i>Olfactory status according to UPSIT-40 normative data, n (%)</i>				
Normosmia	2 (2.1)	9 (13.8)		
Mild hyposmia	4 (4.1)	25 (38.5)		
Moderate hyposmia	12 (12.4)	22 (33.8)	80.97/<.001 ^b	
Severe hyposmia	26 (26.8)	9 (13.8)		
Anosmia	53 (54.6)	0 (0)		

Abbreviations: HC, healthy controls; PD, Parkinson's disease; UPSIT-40, *University of Pennsylvania Smell Identification Test*. Data are presented as mean (SD) or n (%).

- a. Independent t-tests were used for continuous variables when comparing HC with all PD patients.
- b. Pearson's chi-squared tests were used for categorical variables.

Study 4

Campabadal, A., Segura, B., Junque, C., Serradell, M., Abos, A., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Bargallo, N., Iranzo, A., Comparing the accuracy and neuroanatomical correlates of the UPSIT-40 and the Sniffin' Sticks test in REM sleep behavior disorder. *Park. Relat. Disord* **65**, 197-202 (2019) <https://doi.org/10.1016/j.parkreldis.2019.06.013>
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Comparing the accuracy and neuroanatomical correlates of the UPSIT-40 and the Sniffin' Sticks test in REM sleep behavior disorder

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ABSTRACT

Background: Olfactory impairment increases the risk of developing neurodegenerative diseases in patients with idiopathic REM sleep behavior disorder (IRBD). Knowing the test properties of distinct olfactory measures could contribute to their selection for clinical or research purposes.

Objective: To compare the accuracy in distinguishing IRBD patients from controls with the University of Pennsylvania Smell Identification Test (UPSIT-40) and Sniffin' Sticks Extended test, and to assess the gray-matter volume correlates of these tests.

Method: Twenty-one patients with IRBD and 27 healthy controls were assessed using both olfactory tests. Independent logistic regressions were computed with diagnosis as a dependent variable and olfactory measures as predictive variables. Receiver operating characteristic curves were computed for each olfactory subtest. Diagnostic accuracy for IRBD was calculated according to the resulting optimal cut-off score. Structural MRI data, acquired with a 3T scanner, were analyzed with voxel-based morphometry.

Results: Patients differed from controls in all olfactory measures. The Sniffin-Identification correctly classified 89.1% of cases; the UPSIT-40, 85.4%; the Sniffin-Discrimination, 82.6%; the Sniffin-Total, 81.8%; and the Sniffin-Threshold, 77.3%. Respective AUROC, optimal cut-off, sensitivity, and specificity for each test were: 0.902, ≤ 26 , 85.7%, and 85.2% for the UPSIT-40; 0.884, ≤ 29 , 89.5%, and 76.0% for the Sniffin-Total; 0.922, ≤ 11 , 90.5%, and 88.0% for the Sniffin-Identification; 0.739, ≤ 4 , 73.7%, and 76.0% for the Sniffin-Threshold; and 0.838, ≤ 11 , 85.7%, and 76.0% for the Sniffin-Discrimination. UPSIT-40 scores correlated with gray-matter volumes in orbitofrontal regions in anosmic patients.

Conclusions: UPSIT-40 and Sniffin' Identification showed similar discrimination accuracy, but only the UPSIT-40 showed structural correlates ($p \leq .05$ FDR-corrected).

1. Introduction

Rapid eye movement (REM) sleep behavior disorder (RBD) is a parasomnia characterized by abnormal motor and vocal behaviors associated with unpleasant dreams and increased electromyographic activity during REM sleep [1]. The idiopathic form of RBD (IRBD) is diagnosed when RBD symptomatology occurs in the absence of associated comorbidities or known causing factors. Patients with IRBD are at high risk of eventually developing a neurodegenerative disease, such as

dementia with Lewy bodies (DLB), Parkinson's disease (PD), or multiple system atrophy [2,3]. In this setting, IRBD has been described as the strongest and most specific clinical predictor of neurodegenerative disease available [4]. Therefore, there is a growing interest in finding clinical biomarkers of neurodegeneration in this prodromal disorder.

Since olfactory loss has also been recognized as a premotor symptom of PD [5,6], it has been widely studied as a possible biomarker of neurodegeneration in IRBD [7]. In this sense, olfactory impairment in RBD has been reported in 35.7–97.0% of patients compared with

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2.5–16.6% of healthy control subjects [7]. Furthermore, previous works have found RBD patients with olfactory dysfunction to have an increased short-term risk of developing manifested PD and DLB [3,8]. Therefore, improving the accuracy of olfactory clinical testing in IRBD could help identify patients at a higher risk of conversion to a neurodegenerative disease, and thus define target populations for disease-prevention trials.

The University of Pennsylvania Smell Identification Test (UPSIT-40) and the Sniffin' Stick test are the most frequently used tests to evaluate olfaction worldwide. Both tests have been traditionally used as diagnostic complementary tools in PD [9,10] and their accuracy for this disease is high [11–14]. Furthermore, methods have been proposed to convert between UPSIT-40 and Sniffin' Stick test scores with meta-analytic purposes [15]. Nevertheless, as far as we know, no previous published works have compared in the same study neither the accuracy of UPSIT-40 and Sniffin' Stick tests in distinguishing IRBD patients from controls, nor the neuroanatomical correlates of these tests in IRBD patients.

In the current work, we aimed to compare the accuracy and brain correlates of two smell tests in IRBD patients and to describe its optimal cut-off scores for this disorder. It was hypothesized that: (1) IRBD patients will have worse performance than healthy controls in all olfactory measures; (2) Sensitivity and specificity of smell identification tests for IRBD will be comparable for the tests assessed in manifested PD; and (3) gray matter volume in olfactory regions will be related to the degree of olfactory function.

2. Methods

2.1. Participants

Twenty-one patients with IRBD were recruited from the multi-disciplinary Sleep Unit at Hospital Clinic, Barcelona. Diagnosis of RBD required a history of dream-enacting behaviors, video-polysomnographic demonstration of REM sleep without atonia, associated with abnormal behaviors, absence of motor and cognitive complaints at the time of the recruitment, unremarkable neurological examination, normal brain magnetic resonance imaging and no temporal association between the estimated onset of RBD and the introduction or withdrawal of a medication [16,17]. Twenty-seven healthy subjects (HC) without cognitive, motor, or sleep complaints were recruited from the Institut de l'Entrellament (Barcelona, Spain).

Exclusion criteria consisted of [1]: Presence of psychiatric and/or neurologic comorbidity [2], MMSE score < 25 [3], claustrophobia [4], MRI artifacts [5], pathological MRI findings other than mild white matter hyperintensities, and [6] exclusion of HC with evidence of a clinical history suggestive of abnormal sleep behaviors and cognitive impairment. Specific exclusion criteria for the olfaction test were: (1) history of nasal bone fracture, (2) diagnosis of rhinitis or nasal polyps, and (3) upper respiratory tract infections in the two weeks prior to testing.

The study was approved by the Ethics Committee of the University of Barcelona (IRB00003099) and Hospital Clinic (HCB/2014/0224). All subjects provided written informed consent.

2.2. Olfactory and clinical assessment

Olfaction was assessed using the Spanish version of the UPSIT-40 [9] and the Sniffin' Sticks Extended Test [18]. The UPSIT-40 is a standardized multiple-choice scratch-and-sniff test consisting of four test booklets with 10 items each. In accordance with normative instructions, subjects scratch the impregnated area and are asked to select one of four possible answers for each item.

The Sniffin' Sticks Extended Test (www.burghart-mt.de) consists of three subtests [1]: the Threshold test (Sniffin-Thr) used to ascertain the patient's olfactory threshold. It has 48 Sniffin' Sticks (32 blanks and 16

dilutions of n-butanol) [2]; the Discrimination test (Sniffin-D), where the patient must differentiate between smells with varying degrees of similarity. It consists of 48 Sniffin' Sticks (48 Sniffin' Sticks = 16 pairs of odorants each with one individual smell); and [3] the Identification test (Sniffin-I) that consists of 16 Sniffin' Sticks with everyday smells which the patients have to identify using a selection card containing four choices. The results of all subtests are added up to get the total score (Sniffin-Total).

Presence of motor symptoms was evaluated using the International Parkinson and Movement Disorders Society Unified Parkinson's Disease Rating Scale motor section (MDS-UPDRS-III). Neurological examination based on the MDS-UPDRS-III frequently disclosed mild parkinsonian signs, that were, however, not sufficient to diagnose Parkinsonism according to standard criteria [19].

The Beck Depression Inventory II (BDI), the Starkstein's Apathy Scale (AS), and the Neuropsychiatric Inventory (NPI) were administered to all subjects to explore the presence of psychiatric symptoms. Finally, the Innsbruck REM Sleep Behavior Disorder Inventory (RBD-I) was used to study the presence RBD-like symptomatology in both patients and controls for comparisons.

2.3. MRI acquisition and analyses

MRI data were acquired with a 3T scanner (MAGNETOM Trio, Siemens, Germany). The scanning protocol included high-resolution 3-dimensional T1-weighted images acquired in the sagittal plane (TR = 2300 ms, TE = 2.98 ms, TI = 900 ms, 240 slices, FOV = 256 mm; 1 mm isotropic voxel) and an axial FLAIR sequence (TR = 9000 ms, TE = 96 ms).

2.4. Definition of regions of interest

A mask that defined the olfactory system was created including the amygdalae; hippocampi; thalami; insular cortices; parahippocampal gyri; superior frontal gyrus, orbital part; middle frontal gyrus, orbital part; gyrus rectus; olfactory cortex; and the limbic subregion of the striatum (including the nucleus accumbens and the ventral portion of the caudate nucleus and putamen bilaterally). The Automated Anatomical Labeling atlas was used to create the corresponding masks, except the limbic striatum mask, which was obtained from the Oxford-GSK-Imanova striatal connectivity atlas (available in FSL).

2.5. Gray matter volume analysis: voxel-based morphometry

Structural data was analyzed with FSL-VBM (<http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/FSLVBM>), an optimized voxel-based morphometry (VBM) protocol [20] carried out with FSL tools [21]. First, structural images were brain-extracted and gray-matter-segmented before being registered to the MNI 152 standard space using non-linear registration. The resulting images were averaged to create a study-specific template, to which native gray matter images were non-linearly re-registered. Second, all native gray matter images were non-linearly registered to this study-specific template, resampled to a voxel size of 2x2x2 mm³, and modulated to correct for local expansion or contraction due to the non-linear component of the spatial transformation. A mask encompassing the structures of interest (see *Definition of Regions of interests*) was created to define a search volume for subsequent statistical testing. A voxelwise general linear model with non-parametric permutation testing (5,000 permutations) was used alongside threshold-free cluster enhancement for statistical inference. False-discovery rate (FDR) correction was applied for multiple comparisons correction. Statistical significance was established at FDR-corrected p-values (q-values) < 0.05.

2.6. Statistical analyses of non-MRI analyses

Statistical analyses of demographic, clinical, and olfactory data were carried out using the statistical package SPSS-24 (2016; Armonk, NY: IBM Corp.). The Mann-Whitney *U* test was used to assess group differences between IRBD and healthy subjects in clinical and olfactory continuous variables, and Pearson's chi-squared test was applied to assess group differences in categorical variables. Correlations between olfactory measures and clinical scores in IRBD patients were analyzed using Spearman's rho correlation. Finally, stepwise independent binary logistic regressions were computed introducing the diagnosis (either healthy subject or IRBD) as a dependent variable and the olfactory measures as predictive variables. The Bonferroni method was used to correct for multiple comparisons.

A receiver operating characteristic (ROC) curve was computed for each olfactory measure. RStudio (2015; available online at: www.rstudio.com) was used to calculate statistical differences of area under the curve (AUC) values from ROC (AUROC) analysis. Sensitivity, specificity, diagnostic accuracy, positive predictive value, and negative predictive value for IRBD were calculated according to the resulting optimal cut-off. In this setting, altered test performances were considered as positive findings, whereas correct performance defined negatives. True positives (TP) were defined as the number of IRBD patients with altered performance in a certain test; true negatives (TN) as the number of HC with correct performance; false positives (FP) were calculated as the number of HC with altered performance; finally, false negatives (FN) were described as the number of IRBD patients who had scores greater than the optimal cut-off. The following diagnostic measures were calculated: (1) Sensitivity, defined as the probability of testing positive when IRBD is present: $TP/(TP + FN)$; (2) Specificity, defined as the probability of testing negative when IRBD is absent: $TN/(FP + TN)$; (3) Positive predictive value (PPV), calculated as the percentage of subjects with a positive test who actually have IRBD: $TP/(TP + FP)$; (4) negative predictive value (NPV), defined as the percentage of subjects with a negative test who do not have IRBD: $TN/(TN + FN)$; finally, (5) overall diagnostic accuracy was defined as the proportion of correctly classified subjects among all subjects: $(TP + TN)/(TP + TN + FP + FN)$.

3. Results

3.1. Demographic and clinical characteristics

No significant differences between groups were found in age, years of education, sex, BDI, and AS. Intergroup differences were found in NPI ($U = 418.000$; $p < .001$) and RBD-I scores ($U = 466.000$; $p < .001$) (Table 1).

3.2. Comparison between healthy subjects and IRBD patients in olfactory performance

After adjusting for age, years of education, and sex, intergroup differences were found in UPSIT-40 total score ($U = 64.000$; $p < .001$), Sniffin-D ($U = 82.000$; $p < .001$), Sniffin-I ($U = 48.000$; $p < .001$) and Sniffin-Total ($U = 65.000$; $p < .001$) scores (Table 1).

Following the normative cut-offs of each test, the percentage of HC and IRBD patients identified as normosmic, hyposmic, and anosmic was calculated (Supplementary material 1).

3.3. Correlation with clinical measures

No correlations were found in the IRBD group between olfactory measures and age, years of education, disease duration, NPI, AS, and BDI. The RBD-I was the only clinical measure that correlated with olfactory measures in IRBD patients, specifically with Sniffin-I scores ($r = -.550$; $p = .012$).

3.4. Discrimination between HC and IRBD by means of olfactory testing

Stepwise independent logistic regressions were performed to ascertain the effects of each single test on IRBD identification. The logistic regression model was statistically significant for UPSIT-40 ($X^2 = 29.563$; $P < .001$), the Sniffin-I ($X^2 = 34.326$; $P < .001$), the Sniffin-Thr ($X^2 = 5.312$; $P = .021$), the Sniffin-D ($X^2 = 19.842$; $P < .001$), and the Sniffin-Total ($X^2 = 23.755$; $P < .001$). The Sniffin-I model explained 70.3% of the variance in IRBD and correctly classified 89.1% of cases; the UPSIT-40 model explained 61.6% of the variance and correctly classified 85.4%; the Sniffin-Total model explained 56.0% of the variance and correctly classified 81.8%; the Sniffin-D model explained 46.8% of the variance and correctly classified 82.6%; finally, the Sniffin-Thr model explained 15.3% and correctly classified 77.3% of the cases (Table 2).

3.5. Optimal cut-off scores and their accuracy measures for the olfactory tests in IRBD

The ROC curve showed that the optimal cut-off score for the UPSIT-40 (AUROC = .902) was ≤ 26 with a sensitivity of 85.7% and a specificity of 85.2%. For the Sniffin-Total (AUROC = .884) the optimal cut-off was ≤ 29 with 89.5% sensitivity and 76.0% specificity. For the Sniffin' subtests the respective optimal cut-off scores, sensitivity, and specificity were: ≤ 11 , 90.5%, and 88.0% for the Sniffin-I (AUROC = .922); ≤ 4 , 73.7%, and 76.0% for the Sniffin-Thr (AUROC = .739); and ≤ 11 , 85.7%, and 76.0% for the Sniffin-D (AUROC = .838) (Fig. 1, Supplementary material 2 and 3). AUROC values comparisons only showed significant differences between UPSIT-40 and Sniffin-Thr ($t = 2.082$; $P = .037$).

3.6. MRI correlates of olfactory test

In IRBD patients with anosmia, the UPSIT-40 test correlated positively with a cluster involving the right medial frontal cortex, right frontal orbital cortex, and left subcallosal cortex (2222 voxels; coordinates of cluster maximum: $X = 12$, $Y = 40$, $Z = -22$) (Fig. 2). No significant correlations were found either for Sniffin' total score or for Sniffin' subtests.

4. Discussion

To our knowledge, this is the first work comparing the UPSIT-40 and the Sniffin' Sticks test in the same sample of IRBD patients. In line with evidence from the literature, IRBD patients showed impaired olfactory identification and discrimination when compared with matched healthy subjects. We found that olfactory identification measures are the best smell predictor of IRBD; in particular, Sniffin' Identification scores had the highest classification accuracy. On the other hand, worse UPSIT-40 performance was related to gray matter reduction in orbitofrontal cortex regions in anosmic IRBD patients, whereas Sniffin' subtests did not show significant structural brain correlates.

We found that IRBD patients had impaired performance in both UPSIT-40 and Sniffin' Sticks total scores, as well as in Sniffin' Identification and Sniffin' Discrimination subtests. Previous studies have reported impaired smell identification in IRBD patients in comparison with HC using the Sniffin' Identification [3,22,23], the UPSIT short version (B-SIT-12) [24,25], the UPSIT-40 [26,27], the Sniffin' Sticks screening 12 test [28], and the Odor Stick Identification Test for Japanese [23]. Smell discrimination impairment has been also reported [3,23], as well as smell threshold increment [3,23,27]. As expected, these results highlight the presence of olfactory impairment in IRBD patients assessed with different smell tests. Otherwise, for clinical purposes it may be interesting to study the ability of tests to identify olfactory deficits in IRBD patients.

Our results showed that smell identification was the olfactory

Table 1

Inter-group comparisons of demographic, clinical, and olfactory measures.

Demographic and clinical characteristics					
	HC (n = 27)		IRBD (n = 21)		T stat/p
Age	66.4(9.9)		71.8(7.9)		69.500/.074
Years of education	12.2(4.3)		11.7(4.9)		262.500/.661
Sex (male/female)	13/14		15/6		2.634/.105
NPI	1.9(2.5)		6.3(5.8)		418.000/ < .001
BDI	5.1(4.7)		6.8(4.9)		298.500/.265
AS	8.9(5.3)		10.8(5.5)		312.000/.155
RBD-I	0.2(0.1)		0.6(0.2)		466.000/ < .001
RBD duration	–		4.62 (3.3)		–
MDS-UPDRS-III	–		2.5 (1.9)		–
Olfactory test					
	adjusted Z	raw score	adjusted Z	raw score	
Sniffin Discrimination	0.1(0.9)	12.3(2.0)	– 1.6(1.6)	8.3(3.2)	82.000/ < .001
Sniffin Identification	0.1(0.8)	13.1(1.6)	– 2.4(1.6)	8.0(2.8)	48.000/ < .001
Sniffin Threshold	– 0.2(0.9)	5.7(2.0)	– 0.9(1.5)	3.8(3.1)	134.000/.014
Sniffin Total	0.0(0.9)	31.1(4.5)	– 2.1(1.6)	20.7(7.0)	65.000/ < .001
UPSIT-40	0.0(0.9)	30.6(4.6)	– 2.1(1.4)	19.7(6.6)	64.000/ < .001

Abbreviations: AS, Starkstein's Apathy Scale; BDI, Beck Depression Inventory II; HC, healthy controls; IRBD, idiopathic form of REM-sleep behavior disorder; NPI, Neuropsychiatric Inventory; RBD-I, Innsbruck REM Sleep Behavior Disorder Inventory; Sniffin, Bughart Sniffin' Sticks extended test; MDS-UPDRS-III, Movement Disorders Society Unified Parkinson's Disease Rating Scale motor section; UPSIT-40, University of Pennsylvania Smell Identification Test. Measures for demographic and clinical data are presented as mean scores (SD). For the olfactory test, measures are presented as mean scores (SD) of the Z scores corrected for age, years of education, and sex; and mean (SD) of the raw scores. Group differences were tested using Mann-Whitney's *U* test or Chi-square test. For the olfactory data group differences in adjusted Z were tested using Mann-Whitney's *U* test. In bold those results that survive Bonferroni correction for multiple comparisons.

Table 2

Logistic regression analyses.

	B	SE	Wald/P value	Exp (B)	Nagelkerke R ²	PAC (HC/IRBD)	X ² /p value
UPSIT-40							
UPSIT-40	-.306	.080	14.630/ < .001	.736	.616	85.4 (88.9/81.0)	29.563/ < .001
Constant	7.636	2.114	13.048/ < .001	2071.755			
Sniffin Identification							
Sniffin-I	-.857	.232	13.688/ < .001	.425	.703	89.1 (92.0/85.7)	34.326/ < .001
Constant	9.202	2.628	12.260/ < .001	9920.754			
Sniffin Threshold							
Sniffin-Thr	-.289	.136	4.544/.033	.749	.153	77.3 (84.0/68.4)	5.312/.021
Constant	1.081	.690	2.453/.117	2.948			
Sniffin Discrimination							
Sniffin-D	-.533	.157	11.526/.001	.587	.468	82.6 (88.0/76.2)	19.842 < .001
Constant	5.479	1.746	9.849/.002	239.535			
Sniffin Total							
Sniffin-Total	-.263	.073	13.120/ < .001	.769	.560	81.8 (88.0/73.7)	23.755/ < .001
Constant	6.708	2.007	11.170/.001	818.587			

Abbreviations: HC, healthy controls; IRBD, idiopathic form of REM-sleep behavior disorder; PAC, percentage accuracy in classification for the whole sample, for HC and for IRBD patients; SE, standard error; Sniffin, Bughart Sniffin' Sticks extended test; Sniffin-D, Sniffin Discrimination; Sniffin-I, Sniffin Identification; Sniffin-Total, Sniffin Total score; Sniffin-Thr, Sniffin Threshold; UPSIT-40, University of Pennsylvania Smell Identification Test. Independent binary logistic regression with stepwise method was computed introducing group membership (HC vs. IRBD) as a dependent variable and the olfactory measures as predictive variables.

domain that better discriminated IRBD patients; specifically, Sniffin' Identification had the greatest accuracy classification for IRBD (89.1%), followed by the UPSIT-40 (85.4%). Although, Sniffin' Identification (AUROC = .922) had slightly higher AUC values than UPSIT-40 (AUROC = .902), these differences did not reach statistical significance. In the same line, Sniffin' Identification was the only olfactory subtest that correlated with the RBD-I scale. A previous longitudinal study found Sniffin' Identification subtest had a diagnostic accuracy of 82.4% in predicting IRBD conversion to a Lewy body disease. Interestingly, in this same study, those subjects who converted had a baseline olfactory performance similar to that described in PD, whereas non-converters had significantly better smell function [3]. Similarly, a 5-year longitudinal study found that, compared with disease-free patients, IRBD subjects who eventually developed PD or DLB had lower scores at baseline in the UPSIT-40 [8]. In the same line, a recent work with a large multicenter cohort of IRBD patients found that the rate of

neurodegenerative phenoconversion was significantly increased with olfactory deficit (hazard ratio = 2.62) [29]. Taken together, these results suggested that olfactory tests may be useful tools for discriminating HC from IRBD. In addition, previous longitudinal studies also showed the relevance of olfactory measures for predicting which IRBD patients are more likely to convert earlier to PD and DLB. Identifying these patients during the prodromal phase will conceivably help to select subjects in the design of future disease-modifying trials.

As far as we know, this is the first study providing diagnostic accuracy data and optimal cut-off scores for both UPSIT-40 and Sniffin' Sticks tests for discriminating IRBD patients from healthy subjects. As suggested by the logistic regression, taking an IRBD optimal cut-off revealed that smell identification tests had the greatest sensitivity and specificity (respectively, 85.7% and 85.2% for the UPSIT-40; and 90.5% and 88.0% for the Sniffin' Identification). In line with our results, data from a previous work with Japanese IRBD patients reflected Sniffin'

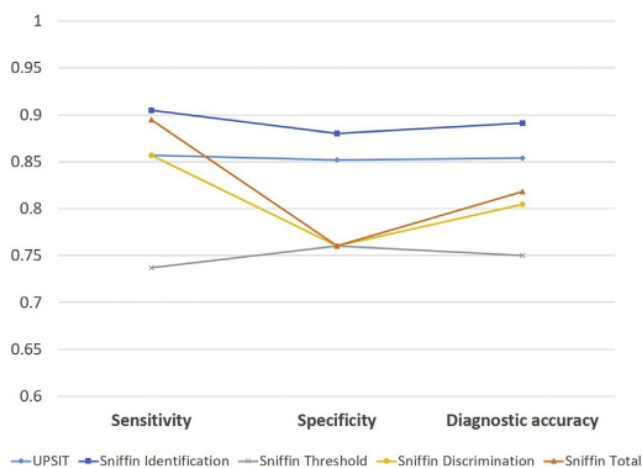


Fig. 1. Diagnostic accuracy measures for UPSIT-40 and Sniffin' Sticks tests.

Identification was the Sniffin' subtest with greatest sensitivity [23]. From a clinical point of view, both UPSIT-40 and Sniffin' identification are useful, but Sniffin' identification is less time consuming.

The voxel-based-morphometry analysis showed that, in anosmic IRBD patients, worse performance in the UPSIT-40 was associated with gray matter reduction in orbitofrontal regions with a right hemisphere predominance. Our results are in line with a previous work in healthy subjects that found a positive correlation between gray matter volume in right orbitofrontal and olfactory performance [30], highlighting the important role of this region in olfactory function. Using cortical thickness analysis, previous studies with IRBD reported lower performance on the UPSIT-40 to be correlated with bilateral orbitofrontal, left precentral [31] and occipital thinning [32]. Another study, assessing brain perfusion in IRBD, showed that performance in the UPSIT 12-item version was related to regional cerebral blood flow reduction in the anterior parahippocampal gyri [33]. Regarding results in PD samples, and in line with our results, a study conducted with early PD patients showed that atrophy in the orbitofrontal cortex was associated with

olfactory dysfunction [34]. On the whole, evidence suggest a potential role of orbitofrontal cortex not only in healthy subjects, but also in IRBD and PD patients. We can speculate that orbitofrontal involvement in anosmic IRBD could be reflecting that patients with worse odor identification may be those that eventually evolve to PD.

The strengths of this work are the inclusion of two smell assessment tools commonly used worldwide, the study of olfactory test structural correlates in IRBD patients, and the exclusion of potential causes of secondary smell loss in our sample. Main limitation is a relatively small sample, especially to detect MRI correlates for the Sniffin' subtests.

In summary, we found that both, UPSIT-40 and Sniffin' Identification, had high accuracy to detect olfactory dysfunction in IRBD patients. Moreover, we found that gray matter reduction in orbitofrontal regions in anosmic IRBD may contribute to the degree of impairment in UPSIT-40 performance.

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Disclosures

The authors declare no conflict of interest.

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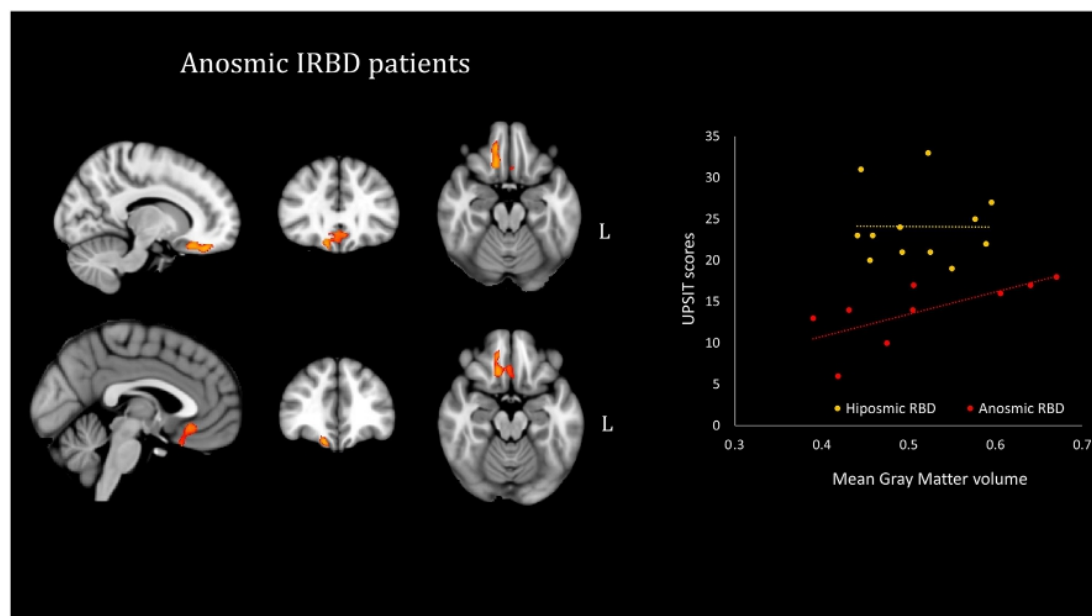


Fig. 2. Correlation between gray matter volume and UPSIT-40 scores in IRBD anosmics. VBM correlation between UPSIT-40 total score and gray matter volume. Significant voxelwise correlation is marked in warm colors. Results are displayed over the sagittal, coronal, and axial sections of the MNI standard brain at $p \leq .05$ FDR-corrected. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.parkreldis.2019.06.013>.

References

- [1] A. Iranzo, The REM sleep circuit and how its impairment leads to REM sleep behavior disorder, *Cell Tissue Res.* 373 (2018) 245–266, <https://doi.org/10.1007/s00441-018-2852-8>.
- [2] A. Iranzo, A. Fernández-Arcos, E. Tolosa, M. Serradell, J.L. Molinuevo, F. Valdeoriola, E. Gelpi, I. Vilaseca, R. Sánchez-Valle, A. Lladó, C. Gaig, J. Santamaría, Neurodegenerative disorder risk in idiopathic REM sleep behavior disorder: study in 174 patients, *PLoS One* 9 (2014), <https://doi.org/10.1371/journal.pone.0089741>.
- [3] P. Mählkecht, A. Iranzo, B. Högl, B. Frauscher, C. Müller, J. Santamaría, E. Tolosa, M. Serradell, T. Mitterling, V. Gschliesser, G. Goebel, F. Brugger, C. Scherfler, W. Poewe, K. Seppi, Olfactory dysfunction predicts early transition to a Lewy body disease in idiopathic RBD, *Neurology* 84 (2015) 654–658, <https://doi.org/10.1212/WNL.0000000000001265>.
- [4] R.B. Postuma, D. Aarsland, P. Barone, D.J. Burn, C.H. Hawkes, W. Oertel, T. Ziemssen, Identifying prodromal Parkinson's disease: pre-Motor disorders in Parkinson's disease, *Mov. Disord.* 27 (2012) 617–626, <https://doi.org/10.1002/mds.24996>.
- [5] A. Haehner, T. Hummel, C. Hummel, U. Sommer, S. Junghanns, H. Reichmann, Olfactory loss may be a first sign of idiopathic Parkinson's disease, *Mov. Disord.* 22 (2007) 839–842, <https://doi.org/10.1002/mds.21413>.
- [6] G.W. Ross, H. Petrovitch, R.D. Abbott, C.M. Tanner, J. Popper, K. Masaki, L. Launer, L.R. White, Association of olfactory dysfunction with risk for future Parkinson's disease, *Ann. Neurol.* 63 (2008) 167–173, <https://doi.org/10.1002/ana.21291>.
- [7] B. Högl, A. Stefani, A. Videnovic, Idiopathic REM sleep behaviour disorder and neurodegeneration - an update, *Nat. Rev. Neurol.* 14 (2018) 40–56, <https://doi.org/10.1038/nrneurol.2017.157>.
- [8] R.B. Postuma, J.-F. Gagnon, M. Vendette, C. Desjardins, J.Y. Montplaisir, Olfaction and color vision identify impending neurodegeneration in rapid eye movement sleep behavior disorder, *Ann. Neurol.* 69 (2011) 811–818, <https://doi.org/10.1002/ana.22282>.
- [9] R.L. Doty, S.M. Bromley, M.B. Stern, Olfactory testing as an aid in the diagnosis of Parkinson's disease: development of optimal discrimination criteria, *Neurodegeneration* 4 (1995) 93–97 <http://www.ncbi.nlm.nih.gov/pubmed/7600189>, Accessed date: 26 June 2017.
- [10] M. Rodríguez-Violante, P. González-Latapi, A. Camacho-Ordoñez, D. Martínez-Ramírez, H. Morales-Briceno, A. Cervantes-Arriaga, Comparing the accuracy of different smell identification tests in Parkinson's disease: relevance of cultural aspects, *Clin. Neurol. Neurosurg.* 123 (2014) 9–14, <https://doi.org/10.1016/j.clineuro.2014.04.030>.
- [11] J. Deeb, M. Shah, N. Muhammed, R. Gunasekera, K. Gannon, L.J. Findley, C.H. Hawkes, A basic smell test is as sensitive as a dopamine transporter scan: comparison of olfaction, taste and DaTSCAN in the diagnosis of Parkinson's disease, *QJM* 103 (2010) 941–952, <https://doi.org/10.1093/qjmed/hcq142>.
- [12] L. Silveira-Moriyama, M. de Jesus Carvalho, R. Katzenschlager, A. Petrie, R. Ranvaud, E.R. Barbosa, A.J. Lees, The use of smell identification tests in the diagnosis of Parkinson's disease in Brazil, *Mov. Disord.* 23 (2008) 2328–2334, <https://doi.org/10.1002/mds.22241>.
- [13] M. Rodríguez-Violante, A.J. Lees, A. Cervantes-Arriaga, T. Corona, L. Silveira-Moriyama, Use of smell test identification in Parkinson's disease in Mexico: a matched case-control study, *Mov. Disord.* 26 (2011) 173–176, <https://doi.org/10.1002/mds.23354>.
- [14] A. Campabadal, B. Segura, H.C. Baggio, A. Abos, C. Uribe, A.I. García-Díaz, M.J. Martí, F. Valdeoriola, Y. Compta, N. Bargallo, C. Junque, Diagnostic accuracy, item analysis and age effects of the UPSIT Spanish version in Parkinson's disease, *Arch. Clin. Neuropsychol.* (2018) 2–11, <https://doi.org/10.1093/arcin/acy053>.
- [15] M. Lawton, M.T.M. Hu, F. Baig, C. Ruffmann, E. Barron, D.M.A. Swallow, N. Malek, K.A. Grosset, N. Bajaj, R.A. Barker, N. Williams, D.J. Burn, T. Foltynie, H.R. Morris, N.W. Wood, M.T. May, D.G. Grosset, Y. Ben-Shlomo, Equating scores of the university of Pennsylvania smell identification test and Sniffin' Sticks test in patients with Parkinson's disease, *Park. Relat. Disord.* 33 (2016) 96–101, <https://doi.org/10.1016/j.parkreldis.2016.09.023>.
- [16] B.F. Boeve, REM sleep behavior disorder: updated review of the core features, the RBD-neurodegenerative disease association, evolving concepts, controversies, and future directions, *Ann. N. Y. Acad. Sci.* (2010) 15–54, <https://doi.org/10.1111/j.1749-6632.2009.05115.x>.RE.M.
- [17] A. Iranzo, J.L. Molinuevo, J. Santamaría, M. Serradell, M.J. Martí, F. Valdeoriola, E. Tolosa, Rapid-eye-movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study, *Lancet Neurol.* 5 (2006) 572–577, [https://doi.org/10.1016/S1474-4422\(06\)70476-8](https://doi.org/10.1016/S1474-4422(06)70476-8).
- [18] T. Hummel, G. Kobal, H. Gudziol, A. Mackay-Sim, Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects, *Eur. Arch. Oto-Rhino-Laryngol.* 264 (2007) 237–243, <https://doi.org/10.1007/s00405-006-0173-0>.
- [19] R.B. Postuma, D. Berg, M. Stern, W. Poewe, K. Marek, I. Litvan, MDS clinical diagnostic criteria for Parkinson's disease, *Mov. Disord.* 30 (2015) 1591–1599, <https://doi.org/10.1002/mds.26424>.
- [20] C.D. Good, I.S. Johnsrude, J. Ashburner, R.N.A. Henson, K.J. Friston, R.S.J. Frackowiak, A voxel-based morphometric study of ageing in 465 normal adult human brains, *Neuroimage* 14 (2001) 21–36, <https://doi.org/10.1006/nimg.2001.0786>.
- [21] S.M. Smith, M. Jenkinson, M.W. Woolrich, C.F. Beckmann, T.E.J. Behrens, H. Johansen-Berg, P.R. Bannister, M. De Luca, I. Drobnjak, D.E. Flitney, R.K. Niazy, J. Saunders, J. Vickers, Y. Zhang, N. De Stefano, J.M. Brady, P.M. Matthews, Advances in functional and structural MR image analysis and implementation as FSL, *Neuroimage* 23 (Suppl 1) (2004) S208–S219, <https://doi.org/10.1016/j.neuroimage.2004.07.051>.
- [22] T.R. Barber, M. Lawton, M. Rolinski, S. Evetts, F. Baig, C. Ruffmann, A. Gornall, J.C. Klein, C. Lo, G. Dennis, O. Bandmann, T. Quinnell, Z. Zaiwalla, Y. Ben-Shlomo, M.T.M. Hu, Prodromal parkinsonism and neurodegenerative risk stratification in REM sleep behavior disorder, *Sleep* 40 (2017) 11–13, <https://doi.org/10.1093/sleep/zsx071>.
- [23] T. Miyamoto, M. Miyamoto, M. Iwanami, K. Hirata, M. Kobayashi, M. Nakamura, Y. Inoue, Olfactory dysfunction in idiopathic REM sleep behavior disorder, *Sleep Med.* 11 (2010) 458–461, <https://doi.org/10.1016/j.sleep.2009.09.013>.
- [24] M.L. Fantini, R.B. Postuma, J. Montplaisir, L. Ferini-Strambi, Olfactory deficit in idiopathic rapid eye movements sleep behavior disorder, *Brain Res. Bull.* 70 (2006) 386–390, <https://doi.org/10.1016/j.brainresbull.2006.07.008>.
- [25] H.Y. Shin, E.Y. Joo, S.T. Kim, H.-J. Dhong, J.W. Cho, Comparison study of olfactory function and substantia nigra hyperechogenicity in idiopathic REM sleep behavior disorder, Parkinson's disease and normal control, *Neurol. Sci.* 34 (2013) 935–940, <https://doi.org/10.1007/s10072-012-1164-0>.
- [26] C. Aguirre-Mardones, A. Iranzo, D. Vilas, M. Serradell, C. Gaig, J. Santamaría, E. Tolosa, Prevalence and timeline of nonmotor symptoms in idiopathic rapid eye movement sleep behavior disorder, *J. Neurol.* 262 (2015) 1568–1578, <https://doi.org/10.1007/s00415-015-7742-3>.
- [27] A. Iranzo, M. Serradell, I. Vilaseca, F. Valdeoriola, M. Salamero, C. Molina, J. Santamaría, E. Tolosa, Longitudinal assessment of olfactory function in idiopathic REM sleep behavior disorder, *Park. Relat. Disord.* 19 (2013) 600–604, <https://doi.org/10.1016/j.parkreldis.2013.02.009>.
- [28] S.F. Huang, K. Chen, J.J. Wu, F.T. Liu, J. Zhao, W. Lin, S.S. Guo, Y.X. Wang, Y. Wang, S.S. Luo, Y.M. Sun, Z.T. Ding, H. Yu, J. Wang, Odor identification test in idiopathic REM-behavior disorder and Parkinson's disease in China, *PLoS One* 11 (2016) 1–13, <https://doi.org/10.1371/journal.pone.0160199>.
- [29] R.B. Postuma, A. Iranzo, M. Hu, B. Högl, B.F. Boeve, R. Manni, W.H. Oertel, I. Arnulf, L. Ferini-Strambi, M. Puligheddu, E. Antelmi, V. Cochen De Cock, D. Arnaldi, B. Mollenhauer, A. Videnovic, K. Sonka, K.-Y. Jung, D. Kunz, Y. Dauvilliers, F. Provini, S.J. Lewis, J. Buskova, M. Pavlova, A. Heidbreder, J.Y. Montplaisir, J. Santamaría, T.R. Barber, A. Stefani, E.K. StLouis, M. Terzaghi, A. Janzen, S. Leu-Semenescu, G. Plazzi, F. Nobili, F. Sixel-Doering, P. Dusek, F. Bes, P. Cortelli, K. Ehgoetz Martens, J.-F. Gagnon, C. Gaig, M. Zucconi, C. Trenkwalder, Z. Gan-Or, C. Lo, M. Rolinski, P. Mählkecht, E. Holzknecht, A.R. Boeve, L.N. Teigen, G. Toscano, G. Mayer, S. Morbelli, B. Dawson, A. Pelletier, Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behaviour disorder: a multicentre study, *Brain* 142 (2019) 744–759, <https://doi.org/10.1093/brain/awz014>.
- [30] J. Seubert, J. Freiherr, J. Frasnelli, T. Hummel, J.N. Lundström, Orbitofrontal cortex and olfactory bulb volume predict distinct aspects of olfactory performance in healthy subjects, *Cerebr. Cortex* 23 (2013) 2448–2456, <https://doi.org/10.1093/cercor/bhs230>.
- [31] J.B. Pereira, D. Weintraub, L. Chahine, D. Aarsland, O. Hansson, E. Westman, Cortical thinning in patients with REM sleep behavior disorder is associated with clinical progression, *NPJ Parkinson's Dis.* (2019), <https://doi.org/10.1038/s41531-019-0079-3>.
- [32] S. Rahayel, R.B. Postuma, J. Montplaisir, Cortical and subcortical gray matter bases of cognitive deficits in REM sleep behaviour, *Neurology* 15 (2018) 90 <https://doi.org/10.1212/WNL.0000000000005523>.
- [33] M. Vendette, J.-F. Gagnon, J.-P. Soucy, N. Gosselin, R.B. Postuma, M. Tuineag, I. Godin, J. Montplaisir, Brain perfusion and markers of neurodegeneration in rapid eye movement sleep behavior disorder, *Mov. Disord.* 26 (2011) 1717–1724, <https://doi.org/10.1002/mds.23721>.
- [34] X. Wu, C. Yu, F. Fan, K. Zhang, C. Zhu, T. Wu, K. Li, P. Chan, Correlation between progressive changes in piriform cortex and olfactory performance in early Parkinson's disease, *Eur. Neurol.* 66 (2011) 98–105, <https://doi.org/10.1159/000329371>.

Supplementary material

Comparing the accuracy and neuroanatomical correlates of the UPSIT-40 and the Sniffin' Sticks test in REM sleep behavior disorder

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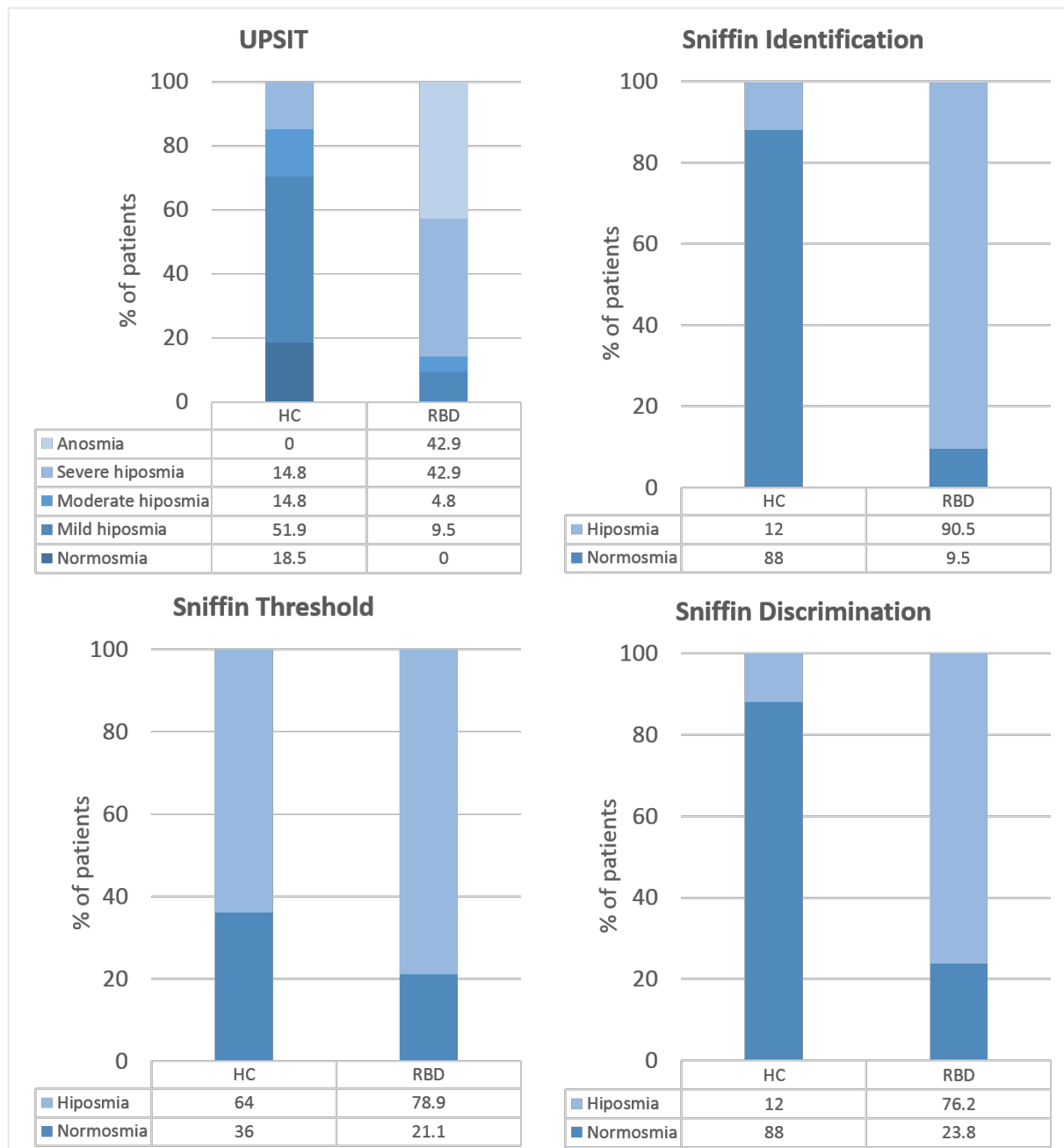
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Supplementary material 1. Percentage of normosmics, hyposmics and anosmics according to olfactory tests



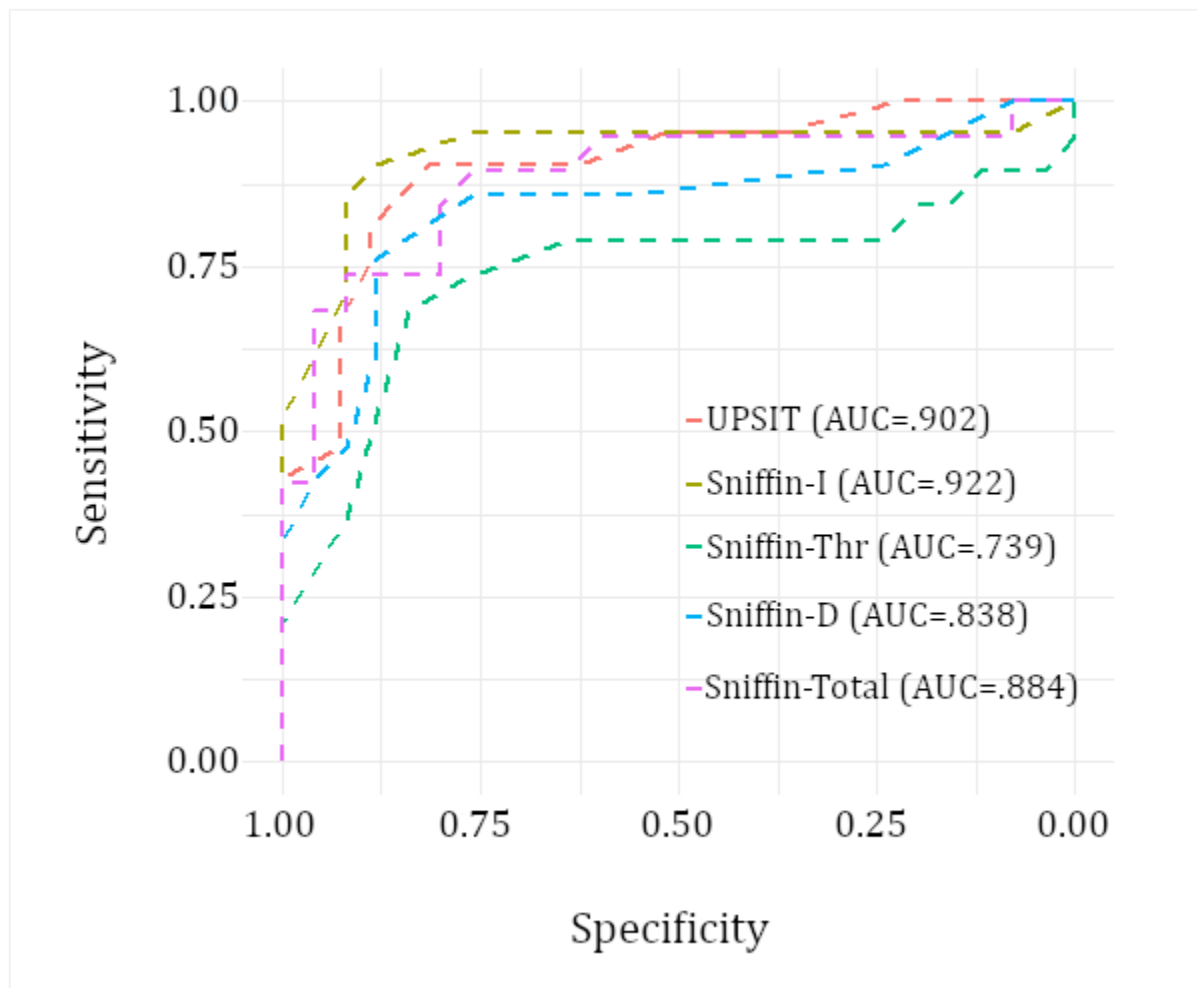
Abbreviations: Sniffin, Bughart Sniffin' Sticks extended test; UPSIT, University of Pennsylvania Smell Identification Test

Supplementary material 2. Diagnostic accuracy measures for UPSIT and Sniffin' tests

Olfactory test	Optimal cut-off	TP	TN	FP	FN	PPV	NPV	Diagnostic accuracy	Sensitivity	Specificity	AUROC	CI	SE/p value
UPSIT	26	18	23	4	3	81.8	88.5	85.4	85.7	85.2	.902	.812-.992	.046/<.001
Sniffin-I	11	19	22	3	2	86.4	91.7	89.1	90.5	88.0	.922	.827-1.0	.048/<.001
Sniffin-Thr	4	14	19	6	5	70.0	79.2	75.0	73.7	76.0	.739	.571-.907	.086/.007
Sniffin-D	11	18	19	6	3	75.0	86.4	80.4	85.7	76.0	.838	.712-.964	.064/<.001
Sniffin-Total	29	17	19	6	2	73.9	90.5	81.8	89.5	76.0	.884	.774-.994	.056/<.001

Abbreviations: AUROC, area under de ROC curve; CI, confidence interval for AUROC; FN, false negative; FP, false positive; NPV, negative predictive value; PPV, Positive predictive value; SE, standard error; Sniffin, Bughart Sniffin' Sticks extended test; Sniffin-D, Sniffin Discrimination; Sniffin-I, Sniffin Identification; Sniffin-Total, Sniffin Total score; Sniffin-Thr, Sniffin Threshold; TN, true negative; TP, true positive; UPSIT, University of Pennsylvania Smell Identification Test

Supplementary material 3. ROC curves for each olfactory measure.



Abbreviations: AUC, Area under the curve; Sniffin, Bughart Sniffin' Sticks extended test; Sniffin-D, Sniffin Discrimination; Sniffin-I, Sniffin Identification; Sniffin-Total, Sniffin Total score; Sniffin-Thr, Sniffin Threshold; UPSIT, University of Pennsylvania Smell Identification Test. AUC values comparisons only showed significant differences between UPSIT and Sniffin-Thr ($t=2.082$; $p=.037$).

Study 5

Campabadal, A., Inguanzo, A., Segura, B., Junque, C., Serradell, M., Abos, A., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Compta, Y., Bargallo, N., Iranzo, A., Cortical Gray Matter progression in Idiopathic REM sleep behavior disorder and its relation to cognitive decline. *Under review*

Cortical Gray Matter progression in Idiopathic REM sleep behavior disorder and its relation to cognitive decline

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Background: Idiopathic Rapid eye movement sleep behavior disorder (IRBD) is recognized as the prodromal stage of the alpha-Synucleinopathies. Although some studies have addressed the characterization of brain structure in IRBD, little is known about its progression. **Objective:** The present work aims at further characterizing cortical gray matter progression throughout IRBD relative to normal aging and investigating how these changes are associated with cognitive decline. **Methods:** Fourteen patients with polysomnography-confirmed IRBD and 18 age-matched healthy controls (HC) underwent neuropsychological, and T1-weighted MRI at baseline and follow-up. We compared the evolution of cortical thickness (CTh) and cognitive performance in IRBD and HC after a mean of 1.6 years. FreeSurfer was used for CTh preprocessing and analyses. The symmetrized percent of change (SPC) of the CTh was correlated with the SPC of neuropsychological performance. **Results:** IRBD and HC differed significantly in the cortical thinning progression in regions encompassing bilateral superior parietal and precuneus, the right cuneus, the left occipital pole and lateral orbitofrontal gyri (FWE corrected, $p < 0.05$). The Visual form discrimination test showed worse progression in the IRBD relative to HC, that was associated with gray matter loss in the right superior parietal and the left precuneus (FWE corrected, $p < 0.05$). **Conclusion:** Progression in IRBD patients is characterized by parieto-occipital and orbitofrontal thinning and visuospatial loss. The cognitive decline in IRBD is associated with degeneration in parietal regions.

Keywords: IRBD, Cortical thickness, Longitudinal data, Visuospatial functions, Parkinson's disease.

Highlights

- Cortical degeneration over time in IRBD patients is larger than in normal aging.
- IRBD patients have progressive parieto-occipital and orbitofrontal thinning.
- Visuospatial decline in IRBD is associated with degeneration in parietal regions.

1. Introduction

There is growing evidence supporting that idiopathic Rapid eye movement (REM) sleep behavior disorder (IRBD) is a prodromal symptom of alpha-Synucleinopathies such as Parkinson's disease (PD) and dementia with Lewy bodies (DLB) as well as multiple system atrophy. A recent multicentric study involving 1280 IRBD patients found that the overall conversion rate to an overt neurodegenerative disease was 6.3% per year, with 73.5% converting after 12-year follow-up (Postuma et al., 2019).

To date, there is a considerable amount of neuroimaging research showing structural (Campabadal et al., 2019b; De Marzi et al., 2016; Ehrminger et al., 2016; Ellmore et al., 2010; Hanyu et al., 2012; Ohlhauser et al., 2019; Park et al., 2018; Pereira et al., 2019; Pyatigorskaya et al., 2017; Rahayel et al., 2015, 2018a, 2018b; Scherfler et al., 2011; Unger et al., 2010) and functional (Byun et al., 2020; Campabadal et al., 2020; Dayan and Browner, 2017; Ellmore et al., 2013; Park et al., 2018; Rolinski et al., 2016) abnormalities in IRBD patients using MRI. Most importantly, owing to all these works, there is strong evidence that brain changes relate to cognitive impairment (Byun et al., 2020; Campabadal et al., 2020; Pereira et al., 2019; Rahayel et al., 2018b; Vendette et al., 2012) and olfactory loss (Campabadal et al., 2019a; Pereira et al., 2019; Rahayel et al., 2018b; Vendette et al., 2011). Nevertheless, few researchers have investigated how the disease progression affects brain structure and function, and how these changes are associated with cognitive

decline. Previous longitudinal investigations have focused on metabolic prognostic biomarkers in IRBD. In this sense, reduced DAT binding in the striatum (Iranzo et al., 2017), increased hippocampal perfusion (Dang-Vu et al., 2012) abnormalities in PD related pattern expression (Holtbernd et al., 2014; Yoon et al., 2019), and hyperechogenicity of the substantia nigra (Iranzo et al., 2010) were found to be able to predict phenocconversion in IRBD patients. As for studies focused on structural progression, a recent work reported that IRBD patients that eventually convert to Lewy body disease had widespread thinning in the left superior frontal, right precentral and lateral occipital gyri compared to non-converters (Pereira et al., 2019). This study found that converters had widespread cortical loss in frontal, precentral and occipital regions compared to HC (Pereira et al., 2019). However, the lack of MRI follow-up for the control group precludes to discard the aging effects on brain changes.

Within this framework, the objectives of the current work were (1) to address differential progressive cortical degeneration between IRBD and normal aging, (2) to study the changes over time in cognitive functions in IRBD and their relationship with progressive cortical thinning. We hypothesized that both healthy individuals and IRBD patients would show an aging-related progressive decline, with larger degeneration in IRBD given that this parasomnia is considered part of the prodromal phase of the Synucleinopathies PD and DLB.

2. Material and methods

2.1. Participants

The study sample included 14 patients with IRBD and 18 healthy subjects who underwent neuropsychological and MRI evaluation at two different time-points; at baseline and after 1.6 ± 0.3 years for patients and 1.6 ± 0.2 years for controls of follow-up ($t=.807$; $p=.426$). All participants are part of a longitudinal cohort recruited between 2015 and 2017 (Campabadal et al., 2019b). IRBD patients were recruited from our sleep disorders center and none of them developed parkinsonism or reported cognitive complaints during the follow-up. Inclusion criteria for IRBD patient, at baseline and at the time of second evaluation were: [1] no cognitive or motor complaints at the time of diagnosis [2] a history of dream-enacting behaviors, video-polysomnographic demonstration of REM sleep without atonia; [3] absence of other neurological diseases (Boeve, 2010; Iranzo et al., 2006). Healthy subjects did not show cognitive, motor, or sleep complaints and were recruited from the Institut de l'Envel·liment (Barcelona, Spain).

Exclusion criteria were: [1] Presence of psychiatric and/or neurologic comorbidity; [2] low global IQ score estimated by the Vocabulary subtest of the Wechsler Adult Intelligence Scale, 3rd edition (scalar score ≤ 7 points); [3] MMSE score < 25 at baseline, [4] claustrophobia; [5] MRI movement artifacts; and [6] evidence in HC of sleep disorders or mild cognitive impairment.

The study was approved by the Ethics Committee of the University of Barcelona (IRB00003099) and Hospital Clinic (HCB/2014/0224). All subjects provided written informed consent to participate after full explanation of the procedures involved.

2.2. Neuropsychological assessment

Participants were evaluated with the same neuropsychological battery at both time points. Attention and working memory were

assessed with the Trail Making Test (TMT, parts A and B) (in seconds), Digit Span Forward and Backward, the Stroop Color-word Test, and the Symbol Digits Modalities Test (SDMT)-Oral version. Executive functions were evaluated with phonetic (words beginning with the letter “p” in 1 min) and semantic (animals in 1 min) fluencies. Language was assessed by the total number of correct responses in the short version of the Boston Naming Test (BNT). In the memory domain, we assessed total learning recall (sum of correct responses from trial I to trial V) (RAVLT). Visuospatial and visuoperceptual (VS/VP) functions were assessed with Benton's Judgement of Line Orientation (BJLO), Visual Form Discrimination (VFD) and Facial Recognition (FRT) tests (Lezak, 2012).

2.3. MRI acquisition and Cortical thickness preprocessing

MRI data were acquired with a 3T scanner (MAGNETOM Trio, Siemens, Germany) at both times. The scanning protocol included high-resolution 3-dimensional T1-weighted images acquired in the sagittal plane (TR=2300 ms, TE=2.98 ms, TI=900 ms, 240 slices, FOV=256 mm, 1-mm isotropic voxel) and an axial FLAIR sequence (TR = 9000 ms, TE = 96 ms).

Cross-sectional preprocessing was performed using the automated FreeSurfer stream (version 5.1; available at: <http://surfer.nmr.harvard.edu>). After processing each subject cross-sectionally, in order to perform the longitudinal analyses of the data, within-subject templates (Reuter and Fischl, 2011) and the corresponding longitudinal files were created for each time point and subject. Briefly, a template volume for each subject, using information from all of their time points, and an average image were created using robust, inverse, consistent registration (Reuter et al., 2010). All time points were constructed through unbiased mean images and later aligned. After registration and creation of the templates,

images from all time points were mapped to the template location and averaged and processed with the default cross-sectional stream. The symmetrized percent change (SPC) was used for longitudinal analyses of cortical thickness. Cortical thickness SPC is the rate in mm/year with respect to the average thickness $[(\text{Thickness at time point 1} - \text{Thickness at time point 2}) / \text{Interval between assessments}] / [0.5 * (\text{Thickness at time point 1} + \text{Thickness at time point 2})]$. In aging or disease, SPC is expected to be negative in most regions (<https://surfer.nmr.mgh.harvard.edu/fswiki/LongitudinalTwoStageModel>).

2.4. Olfactory assessment

Olfactory identification was assessed using the Spanish version of the University of Pennsylvania Smell Identification Test (UPSIT) (Doty et al., 1995). Specific exclusion criteria for the olfaction test were: [1] history of nasal bone fracture, [2] diagnosis of rhinitis or nasal polyps, and [3] upper respiratory tract infections in the two weeks prior to testing.

2.5. Motor assessment

Presence of motor symptoms was evaluated using the International Parkinson and Movement Disorders Society Unified Parkinson's Disease Rating Scale motor section (MDS-UPDRS-III).

2.6. Statistical analyses

Group differences between HC and IRBD at baseline were analyzed using two-sample test and Pearson's chi-squared test. For longitudinal neuropsychological and olfactory variables, repeated measures general linear model was used to assess group differences over time in quantitative variables with sex as a covariate.

Comparisons between groups in the cortical thickness SPC were performed using a vertex-by-vertex general linear model. Time effects in both groups and group-by-time interaction effects were computed, SPC was included as a dependent factor and group as an independent factor. Regression models included SPC as an independent factor and cognitive scores as dependent factors. Sex was introduced as a covariate in all analyses. In order to avoid clusters appearing significant purely by chance (i.e., false positives), Monte Carlo null-Z simulation with 10,000 iterations was applied to cortical thickness maps to provide clusterwise correction for multiple comparisons. Simulation was applied for negative time effects (time 2 < time 1) in each group, on all other models, simulation was applied for absolute results. Results were thresholded at a corrected p value of 0.05.

3. Results

3.1. Neuropsychological changes

Sociodemographic and clinical data of participants at baseline are summarized in Table 1. No significant differences between groups were found in age, education or the interval between assessments. Groups differed significantly in sex ($X^2=5.039$; $P=0.025$), as men were more common in the IRBD group, and for this reason this variable was entered as a covariate in all analyses.

The longitudinal evolution of cognitive and olfactory performance is summarized in Table 2. Significant group differences between patients and HC were found in several measures, namely MMSE, VFD, JLO, semantic fluency, RAVLT, Stroop measures, SDMT, TMTA, TMTB and BNT (see Table 2, for detailed statistical data). Significant group-by-time interaction was seen in VFD test with worse progression in the IRBD group relative to HC ($F=12.76$; $p<0.001$) (Table 2).

Table 1. Demographic and clinical data of the participants at baseline

	IRBD (n=14)	HC (n=18)	Test stat/p
Age, years	70.1 (6.9)	68.3 (7.5)	-0.699/0.49
Education, years	10.1 (5.1)	10.9 (4.2)	0.503/0.62
Sex (male/female)	11/3	7/11	5.039/0.03
Disease duration	3.2 (3.4)	-	-
MDS-UPDRS-III	2.4 (2.1)	-	-

Abbreviations: HC, healthy controls; IRBD, idiopathic rapid eye movement sleep behavior disorder; MDS-UPDRS-III, Movement Disorder Society Unified Parkinson's Disease Rating Scale motor section. Measures are presented as mean (standard deviation). Group differences between HC and IRBD were analyzed using two-sample test. Differences in categorical variables were analyzed with Pearson's chi-squared test. Measures are presented as mean (standard deviation) for continuous variables.

Only this test evidenced the clinically relevant cognitive decline in IRBD. Specifically, 69.2% of the IRBD patients presented a worsening greater than 1.5 standard deviation (SD) in VFD performance in comparison to baseline scores, while non-healthy individual had such a loss in this test ($X^2= 6.036$; $P=0.014$). Olfactory identification was impaired in IRBD relative to HC ($F= 29.4$; $P<0.001$), but we did not find decline across time.

3.2. Cortical thickness evolution

Cortical thickness analysis revealed that both groups had significant progressive GM decline over time (Fig. 1A and Table 3 show the time effect for each group). In IRBD, cortical thinning was found in both hemispheres involving mainly the parietal cortices, but also frontal and occipital regions. Specifically, cortical GM loss was found in the superior and inferior parietal lobes, cuneus, precuneus, precentral and postcentral gyri, the left lateral occipital and orbitofrontal cortices and the right rostral middle frontal gyrus (FWE corrected, $p\text{-value}<0.05$). In controls, changes were seen in the left pars triangularis, supramarginal gyrus and precuneus. As illustrated in Fig. 1B and Table 3, significant group-by-time interactions were found

between groups; in comparison with HC, IRBD patients exhibited significantly greater progressive cortical thinning encompassing the bilateral superior parietal and precuneus, the right cuneus, the left occipital pole and lateral orbitofrontal gyri (FWE corrected, $p\text{-value}<0.05$).

3.3. Correlates of VFD changes

To assess the clinical relevance of VFD decline in IRBD patients, we investigated its structural correlates. Whole-brain analyses showed significant correlations between changes in VFD over time and cortical thinning in the right superior parietal and the left precuneus (FWE corrected, $p\text{-value}<0.05$) (Fig. 2 and Table 3).

4. Discussion

In the present study, we aimed to investigate the progression of cortical thinning in IRBD relative to healthy individuals, and how these changes relate to cognitive decline. As far as we know, this is the first longitudinal work reporting gray matter loss and progressive worsening in visual discrimination performance in IRBD compared to normal aging. It is worth noting, that structural and cognitive changes are identified even in a short interval of less than two years.

Table 2. Neuropsychological decline over time in IRBD and HC

	IRBD (n=13)		HC (n=17)		F (Group)	F (Time)	F (Group by time)
	Baseline	Follow-up	Baseline	Follow-up			
MMSE	27.9 (1.7)	28.2 (1.4)	29.4 (1.0)	29.4 (0.6)	10.9/0.003	0.6/0.44	0.3/0.62
VFD	29.3 (2.8)	26.5 (2.3)	29.6 (2.5)	29.5 (2.8)	5.2/0.03	3.9/0.06	12.8/<0.001
BJLO	22.4 (4.9)	22.5 (4.5)	24.1 (3.9)	24.6 (3.8)	4.9/0.04	0.1/0.82	0.1/0.88
FRT Short	21.4 (2.4)	22.1 (3.7)	22.9 (1.9)	23.3 (2.3)	2.3/0.14	0.7/0.42	0.1/0.79
Phonemic fluency	12.9 (5.7)	12.5 (5.1)	16.1 (4.3)	16.3 (4.1)	3.9/0.06	0.3/0.61	0.5/0.49
Semantic fluency	15.0 (5.1)	16.2 (4.9)	19.1 (3.2)	19.1 (2.6)	7.2/0.01	0.8/0.38	0.4/0.54
RAVLT	39.8(7.2)	41.1 (9.3)	48.7 (7.7)	47.2 (8.2)	6.6/0.02	0.3/0.58	0.6/0.44
Direct Digits	5.3 (1.8)	4.9 (1.1)	5.4 (1.2)	5.3 (0.3)	0.6/0.45	1.2/0.28	0.3/0.64
Indirect Digits	4.2 (0.9)	3.4 (1.1)	4.1 (1.2)	3.9 (1.2)	01.0/0.34	1.0/0.33	3.4/0.08
Stroop W	84.6 (17.6)	81.5 (17.8)	96.3 (14.7)	93.4 (15.7)	8.9/0.01	0.1/0.92	0.6/0.45
Stroop C	54.7 (12.0)	56.2 (13.3)	65.6 (9.5)	60.8 (7.4)	5.2/0.03	0.1/0.81	1.9/0.18
Stroop WC	30.0 (11.3)	29.8 (11.9)	36.5 (9.9)	35.1 (9.6)	4.3/0.05	0.8/0.39	0.4/0.53
SDMT	35.5 (12.6)	35.5 (13.1)	48.1 (9.6)	48.3 (9.8)	11.0/0.003	0.1/0.77	0.1/0.80
TMTA	52.6 (14.1)	54.2 (16.8)	39.4 (17.9)	43.1 (19.3)	15.5/<0.001	2.9/0.09	2.2/0.15
TMTB	161.3 (76.6)	202.2 (102.5)	94.8 (43.2)	126.7 (64.7)	13.8/0.001	4.7/0.04	0.7/0.40
BNT	13.2 (0.9)	13.8 (0.4)	14.1 (0.8)	13.8 (1.2)	4.7/0.04	0.4/0.56	4.7/0.04
UPSIT	17.7 (6.2)	19.2 (6.6)	30.1 (5.6)	30.1 (2.9)	29.4/<0.001	1.2/0.29	0.4/0.53

Abbreviations: BJLO, Benton's Judgment of Line Orientation test; BNT, Boston Naming Test; FRT Short, Facial Recognition test short form; HC, healthy controls; IRBD, idiopathic rapid eye movement sleep behavior disorder; MMSE, Mini-mental state examination; RAVLT, Rey's Auditory Verbal Learning Test sum of correct responses from trial I to trial V; SDMT, Symbol Digits Modalities Test; Stroop W, Stroop Word; Stroop C, Stroop Color; Stroop WC, Stroop Word-Color; TMTA, Trail Making Test part A; TMTB, Trail Making Test part B; UPSIT, The University of Pennsylvania Smell Identification Test; VFD, Visual Form Discrimination. Repeated measures general linear model was used to assess group differences over time with sex as a covariate. Measures are presented as mean (standard deviation).

We found significant group effect in several neuropsychological tests. Similar to previous studies we identify deficits in verbal learning (Gagnon et al., 2009; Massicotte-Marquez et al., 2008), semantic fluency (Gagnon et al., 2009; Massicotte-Marquez et al., 2008), mental processing speed (Pereira et al., 2019), attention and executive functions (Delazer et al., 2012; Ferini-Strambi et al., 2004; Gagnon et al., 2009; Massicotte-Marquez et al., 2008; Terzaghi et al., 2013a). We also observed impaired VS/VP functions, similarly to the findings in PD and DLB (Garcia-Diaz et al., 2018a; Mori et al., 2000; Pereira et al., 2009; Ramírez-Ruiz et al., 2006; Segura et al., 2014; Tiraboschi et al., 2006). As for the cognitive

decline over IRBD, there is still considerable uncertainty. Previous longitudinal studies including follow-up data for IRBD and healthy individuals, did not report an interaction between group and time, so the aging effect could not be ruled out (Fantini et al., 2011; Terzaghi et al., 2013b; Youn et al., 2016). In the current work, after a short period of time, we found cognitive decline in IRBD relative to normal aging. Particularly, we identified VFD progressive worsening, again in agreement with VS/VP findings in PD and DLB (Garcia-Diaz et al., 2018a; Mori et al., 2000; Pereira et al., 2009; Ramírez-Ruiz et al., 2006; Segura et al., 2014; Tiraboschi et al., 2006).

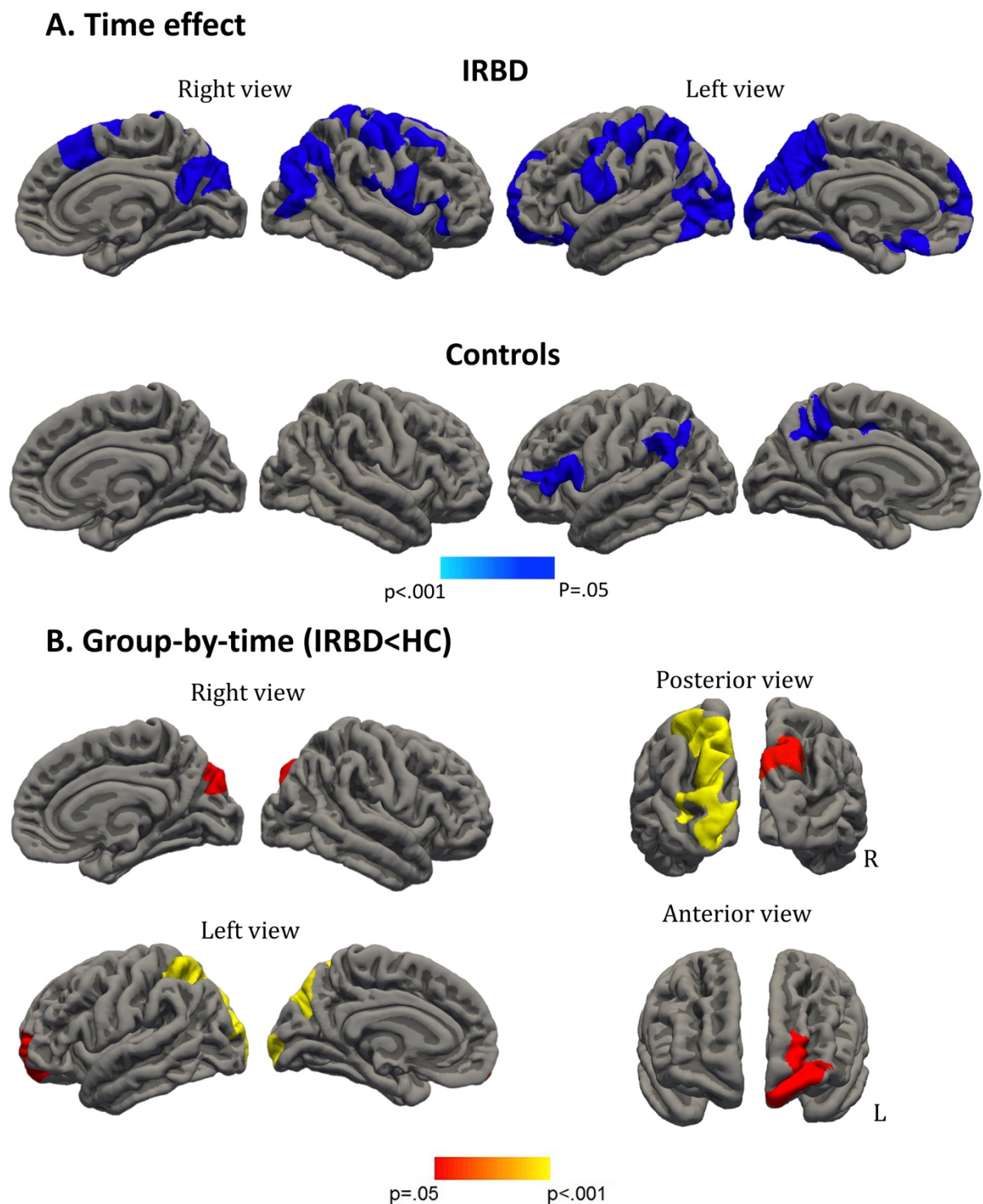


Figure 1. Vertex-wise symmetrized percent change (SPC) in cortical thickness. **A.** Time effect for IRBD and HC group, cold colors represent negative SPC (GM loss across time); **B.** Group-by-time interaction, warm colors depict those regions were IRBD present reduced SPC in comparison with HC over the course of the study. Results were obtained using Monte Carlo simulation with 10,000 iterations applied to cortical thickness maps to provide cluster-wise correction for multiple comparisons (FWE corrected, p-value <0.05). Simulation was applied for negative time effects (time 2 < time 1), whereas, on group-by-time contrast, simulation was applied for absolute results. Sex was used as a covariate. Abbreviations: HC, healthy controls; IRBD, idiopathic REM sleep behavior disorder; L, left; R, right.

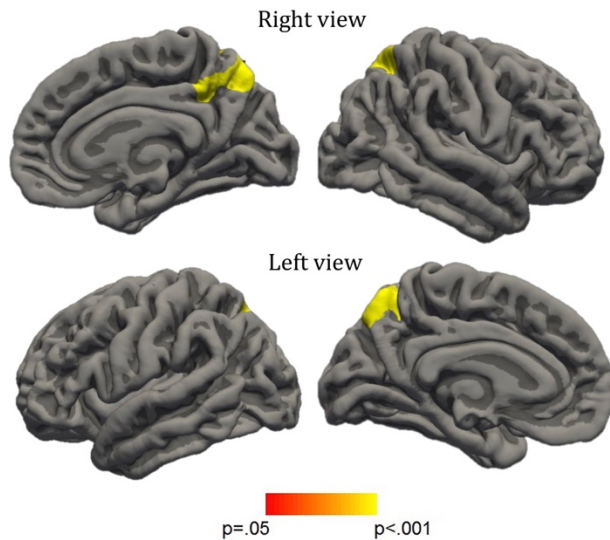


Figure 2. Correlates of VFD changes. Vertex-wise symmetrized percent change (SPC) in cortical thickness correlations with SPC Visual form Discrimination (VFD) scores in IRBD group. Sex was used as a covariate. The scale bar shows P values (FWE corrected, p-value <0.05).

MRI data evidenced aging-related structural changes in both groups with common thinning in the left precuneus and inferior parietal regions but with a larger widespread frontal and parieto-occipital progression in IRBD. Cortical thickness is a sensitive measure to detect time effect in normal aging and PD (Uribe et al., 2019), and likewise, it seems to be useful to track gray matter loss in IRBD patients even in a short interval of less than two years. Indeed, when we compared both groups' cortical thinning pattern across time, we confirm the larger degeneration in IRBD relative to healthy individuals bilaterally in the superior parietal and precuneus, the right cuneus, the left occipital pole and a cluster involving the left lateral orbitofrontal and frontopolar cortices. As stated in the introduction, there is no previous research investigating brain changes in IRBD over time compared to normal aging. It is interesting noting that already in our cross-sectional

study superior parietal thinning was identified in these patients (Campabadal et al., 2019b), with our current results, we highlight the involvement of parietal regions in the progression of IRBD. This predominantly posterior cortical pattern is similar to previous findings in PD and DLB (Delli Pizzi et al., 2014; Ramírez-Ruiz et al., 2007; Segura et al., 2014; Uribe et al., 2016).

Cortical degeneration was also observed in left lateral orbitofrontal and frontopolar cortex. These regions have an important role in complex cognitive processes, namely planning, introspection and problem solving. In PD, orbitofrontal cortex is associated with decision-making and recognition of emotions (Ibarretxe-Bilbao et al., 2009). Despite we found executive dysfunction compared to HC, no decline was found across time, which might be explained by the fact that the current neuropsychological assessment did not evaluate specifically orbitofrontal functions. On the other hand, orbitofrontal regions have also been associated with olfactory dysfunction in IRBD (Campabadal et al., 2019a; Pereira et al., 2019) and PD (Ibarretxe-Bilbao et al., 2010). Nonetheless, despite finding olfactory identification deficits in IRBD relative to healthy controls, our results did not show decline over the disease course. In the same line, after a 4-year follow-up, olfactory decline in IRBD (Iranzo et al., 2013) and PD patients (Campabadal et al., 2017) was similar to that observed in healthy elder controls. Iranzo et al. (2013) hypothesized that the absence of longitudinal changes in IRBD might be explained by a floor effect in olfactory loss reached at early stages of the disease, suggesting that the smell function is already compromised in IRBD (Iranzo, et al., 2013).

Table 3. Differences between HC and IRBD patients in cortical thickness

Cluster size (mm ²)	MNI305 space			Clusterwise p value	Cluster anatomical annotation
	X	Y	Z		
IRBD effect Time					
LH Clusters					
1 17560.6	-17.5	-87.9	19.9	<0.001	Superior parietal
2 5351.7	-10.7	55.5	-18.7	<0.001	Lateral orbitofrontal
3 2248.5	-41.5	-0.3	28.7	0.001	Precentral
RH Clusters					
1 1606.7	23.1	60.7	2.2	0.021	Rostral middle frontal
2 10258.7	7.1	25.7	56.2	<0.001	Superior frontal
3 7201.8	41.6	-63.6	45.7	<0.001	Inferior parietal
HC effect Time					
LH Clusters					
1 1803.3	-47.7	27.9	3.3	0.009	Pars triangularis
2 1615.2	-57.7	-50.2	30.5	0.019	Supramarginal
3 1684.3	-15.0	-44.5	46.5	0.014	Precuneus
Group by time (HC>IRBD)					
LH Clusters					
1 5609.7	-17.5	-87.9	19.9	<0.001	Superior parietal
2 1043.3	-13.4	55.8	-16.7	0.035	Lateral orbitofrontal
RH Clusters					
1 1259.1	20.4	-79.6	43.1	0.012	Superior parietal
Correlation with decline in VFD in IRBD					
LH Clusters					
1 921.2	-8	-72.6	48.7	0.044	Precuneus
RH Clusters					
1 1870.46	21.1	-59.3	58.9	<0.001	Superior parietal

Abbreviations: HC, healthy controls; IRBD, idiopathic rapid eye movement sleep behavior disorder; LH, left hemisphere; RH, right hemisphere; VFD, Visual Form Discrimination. All analyses are covariates by sex. Results after FWE correction with Monte Carlo simulation and threshold at $p \leq 0.05$

Our study provides new evidence for neuroanatomical substrate of progressive worsening in IRBD cognition. In particular, we established that visual discrimination changes over time are related to progressive reductions in bilateral superior parietal cortical regions. As far as we are aware, the neuroanatomical basis of cognitive dysfunction in IRBD has only been studied cross-sectionally (Pereira et al., 2019; Rahayel et al., 2018b). Specifically, structural mechanisms underpinning VS/VP impairment in IRBD are related with cortical thinning in posterior regions (Pereira et al., 2019; Rahayel et al., 2018b), but also anterior temporal (Pereira et al., 2019; Rahayel et al., 2018b) frontal, insular cortices and with right hippocampal regions (Rahayel et al., 2018b). The involvement of frontal regions in Rahayel

et al.'s work, might be explained by the use of copy drawing and block design to compute VS domain, tasks that also demand motor and executive functions. It is worth noting that when assessing specifically VS/VP functions in PD, structural substrates encompass cortical thinning in lateral temporoparietal regions (Garcia-Diaz et al., 2018a, 2014). Moreover, as PD progresses, VS/VP impairment is accompanied by bilateral posterior degeneration (Garcia-Diaz et al., 2018b). Although further longitudinal data with larger samples are needed to confirm our results, the decline in VS/VP performance might help to identify those patients presenting progressive neurodegeneration in posterior cortices. The strengths of our study are that all participants were evaluated by the same

neuropsychologist at both time points, using the same neuropsychological battery and MRI scanning protocol, thus avoiding the variability of multicentric data. Moreover, we used a case control-design to identify different gray matter degeneration in IRBD in comparison to normal aging. However, the reduced sample size limits the generalization of the results. On the other hand, the short time between evaluations may have limited the longitudinal findings of our work. A longer interval between time points would allow detecting a decline in other functions. Prospective studies will help to identify structural and cognitive markers of phenoconversion, with a particular focus on those able to identify whether a patient will eventually convert to PD or DLB.

5. Conclusion

The current study gives evidence of progressive cortical degeneration in IRBD mainly encompassing parieto-occipital and orbitofrontal regions. Additionally, we identified visual discrimination decline over the course of the disease, and we established its structural substrates, highlighting the involvement of bilateral parietal cortices in IRBD's evolution. Overall, these findings widen our knowledge of how IRBD's progression affects brain structure and cognition mainly involving posterior and orbitofrontal regions, similarly to previous findings in PD and DLB.

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Author Contributions

CJ contributed in the design of the study. AA, AC and CU contributed to the data collection. AC, AI, BS and CJ contributed to the analysis of the data and AA, AC, AI, BS, CG, CJ, CU, JS, MS and YC contributed to the interpretation of the data. AC, AI, BS and CJ contributed to the draft of the article. All the authors revised the manuscript critically for important intellectual content and approved the final version of the manuscript.

Declaration of Competing Interest

The authors report no conflicts of interest relevant to this study.

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References

- Boeve, B.F., 2010. REM Sleep Behavior Disorder: Updated Review of the Core Features, the RBD-Neurodegenerative Disease Association, Evolving Concepts, Controversies, and Future Directions. *Ann. N. Y. Acad. Sci.* 15–54. <https://doi.org/10.1111/j.1749-6632.2009.05115.x>.REM
- Byun, J.-I., Kim, H.-W., Kang, H., Cha, K.S., Sunwoo, J.-S., Shin, J.-W., Moon, J., Lee, S.-T., Jung, K.-H., Chu, K., Kim, M.-H., Shin, W.C., Lee,

- D.S., Schenck, C.H., Lee, S.K., Jung, K.-Y., 2020. Altered resting-state thalamo-occipital functional connectivity is associated with cognition in isolated rapid eye movement sleep behavior disorder. *Sleep Med.* 69, 198–203. <https://doi.org/10.1016/j.sleep.2020.01.010>
- Campabadal, A., Abos, A., Segura, B., Serradell, M., Uribe, C., Baggio, H.C., Gaig, C., Santamaria, J., Compta, Y., Bargallo, N., Junque, C., Iranzo, A., 2020. Disruption of posterior brain functional connectivity and its relation to cognitive impairment in idiopathic REM sleep behavior disorder. *NeuroImage Clin.* 25, 102138. <https://doi.org/https://doi.org/10.1016/j.nicl.2019.102138>
- Campabadal, A., Segura, B., Junque, C., Serradell, M., Abos, A., Uribe, C., Baggio, H., Gaig, C., Santamaria, J., Bargallo, N., Iranzo, A., 2019. Comparing the accuracy and neuroanatomical correlates of the UPSIT-40 and the Sniffin' Sticks test in REM sleep behavior disorder. *Park. Relat. Disord.* 65, 197–202. <https://doi.org/10.1016/j.parkreldis.2019.06.013>
- Campabadal, A., Segura, B., Junque, C., Serradell, M., Abos, A., Uribe, C., Baggio, H., Gaig, C., Santamaria, J., Compta, Y., Bargallo, N., Iranzo, A., 2019. Cortical Gray Matter and Hippocampal Atrophy in Idiopathic Rapid Eye Movement Sleep Behavior Disorder. *Front. Neurol.* 10, 1–9. <https://doi.org/10.3389/fneur.2019.00312>
- Campabadal, A., Uribe, C., Segura, B., Baggio, H.C., Abos, A., Garcia-Diaz, A.I., Marti, M.J., Valldeoriola, F., Compta, Y., Bargallo, N., Junque, C., 2017. Brain correlates of progressive olfactory loss in Parkinson's disease. *Parkinsonism Relat. Disord.* 41, 44–50. <https://doi.org/10.1016/j.parkreldis.2017.05.005>
- Dang-Vu, T.T., Gagnon, J.F., Vendette, M., Soucy, J.P., Postuma, R.B., Montplaisir, J., 2012. Hippocampal perfusion predicts impending neurodegeneration in REM sleep behavior disorder. *Neurology* 79, 2302–2306. <https://doi.org/10.1212/WNL.0b013e318278b658>
- Dayan, E., Browner, N., 2017. Alterations in striato-thalamo-pallidal intrinsic functional connectivity as a prodrome of Parkinson's disease. *NeuroImage Clin.* 16, 313–318. <https://doi.org/10.1016/j.nicl.2017.08.003>
- De Marzi, R., Seppi, K., Högl, B., Müller, C., Scherfler, C., Stefani, A., Iranzo, A., Tolosa, E., Santamaria, J., Gizewski, E., Schocke, M., Skalla, E., Kremser, C., Poewe, W., 2016. Loss of dorsolateral nigral hyperintensity on 3.0 tesla susceptibility-weighted imaging in idiopathic rapid eye movement sleep behavior disorder. *Ann. Neurol.* 79, 1026–1030. <https://doi.org/10.1002/ana.24646>
- Delazer, M., Högl, B., Zamarian, L., Wenter, J., Ehrmann, L., Gschliesser, V., Brandauer, E., Poewe, W., Frauscher, B., 2012. Decision Making and Executive Functions in REM Sleep Behavior Disorder 35, 667–673. <https://doi.org/http://dx.doi.org/10.5665/sleep.1828>
- Delli Pizzi, S., Franciotti, R., Tartaro, A., Caulo, M., Thomas, A., Onofri, M., Bonanni, L., 2014. Structural alteration of the dorsal visual network in DLB patients with visual hallucinations: a cortical thickness MRI study. *PLoS One* 9, e86624. <https://doi.org/10.1371/journal.pone.0086624>
- Doty, R.L., Bromley, S.M., Stern, M.B., 1995. Olfactory testing as an aid in the diagnosis of Parkinson's disease: development of optimal discrimination criteria. *Neurodegeneration* 4, 93–7.
- Ehrminger, M., Latimier, A., Pyatigorskaya, N., Garcia-Lorenzo, D., Leu-Semenescu, S.,

- Vidailhet, M., Lehericy, S., Arnulf, I., 2016. The coeruleus/subcoeruleus complex in idiopathic rapid eye movement sleep behaviour disorder. *Brain* 139, 1180–1188. <https://doi.org/10.1093/brain/aww006>
- Ellmore, T.M., Castriotta, R.J., Hendley, K.L., Aalbers, B.M., Furr-Stimming, E., Hood, A.J., Suescun, J., Beurlot, M.R., Hendley, R.T., Schiess, M.C., 2013. Altered nigrostriatal and nigrocortical functional connectivity in rapid eye movement sleep behavior disorder. *Sleep* 36, 1885–1892. <https://doi.org/10.5665/sleep.3222>
- Ellmore, T.M., Hood, A.J., Castriotta, R.J., Stimming, E.F., Bick, R.J., Schiess, M.C., 2010. Reduced volume of the putamen in REM sleep behavior disorder patients. *Park. Relat. Disord.* 16, 645–649. <https://doi.org/10.1016/j.parkreldis.2010.08.014>
- Fantini, M.L., Farini, E., Ortelli, P., Zucconi, M., Manconi, M., Cappa, S., Ferini-Strambi, L., 2011. Longitudinal study of cognitive function in idiopathic REM sleep behavior disorder. *Sleep* 34, 619–625. <https://doi.org/10.1093/sleep/34.5.619>
- Ferini-Strambi, L., Di Gioia, M.R., Castronovo, V., Oldani, A., Zucconi, M., Cappa, S.F., 2004. Neuropsychological assessment in idiopathic REM sleep behavior disorder (RBD): does the idiopathic form of RBD really exist? *Neurology* 62, 41–5.
- Gagnon, J.-F., Vendette, M., Postuma, R.B., Desjardins, C., Jessica Massicotte-Marquez, Panisset, M., Montplaisir, J., 2009. Mild Cognitive Impairment in Rapid Eye Movement Sleep Behavior Disorder and Parkinson's Disease. *Ann. Neurol.* 66, 39–47. <https://doi.org/https://doi.org/10.1002/ana.21680>
- Garcia-Diaz, A.I., Segura, B., Baggio, H.C., Marti, M.J., Valldeoriola, F., Compta, Y., Bargallo, N., Uribe, C., Campabadal, A., Abos, A., Junque, C., 2018a. Structural Brain Correlations of Visuospatial and Visuo-perceptual Tests in Parkinson's Disease. *J. Int. Neuropsychol. Soc.* 24. <https://doi.org/10.1017/S1355617717000583>
- Garcia-Diaz, A.I., Segura, B., Baggio, H.C., Marti, M.J., Valldeoriola, F., Compta, Y., Vendrell, P., Bargallo, N., Tolosa, E., Junque, C., 2014. Structural MRI correlates of the MMSE and pentagon copying test in Parkinson's disease. *Park. Relat. Disord.* 20, 1405–1410. <https://doi.org/10.1016/j.parkreldis.2014.10.014>
- Garcia-Diaz, A.I., Segura, B., Baggio, H.C., Uribe, C., Campabadal, A., Abos, A., Marti, M.J., Valldeoriola, F., Compta, Y., Bargallo, N., Junque, C., 2018b. Cortical thinning correlates of changes in visuospatial and visuo-perceptual performance in Parkinson's disease: A 4-year follow-up. *Park. Relat. Disord.* 46. <https://doi.org/10.1016/j.parkreldis.2017.11.003>
- Hanyu, H., Inoue, Y., Sakurai, H., Kanetaka, H., Nakamura, M., Miyamoto, T., Sasai, T., Iwamoto, T., 2012. Voxel-based magnetic resonance imaging study of structural brain changes in patients with idiopathic REM sleep behavior disorder. *Parkinsonism Relat. Disord.* 18, 136–9. <https://doi.org/10.1016/j.parkreldis.2011.08.023>
- Holtbernd, F., Gagnon, J.-F., Postuma, R.B., Ma, Y., Tang, C.C., Feigin, A., Dhawan, V., Vendette, M., Soucy, J.-P., Eidelberg, D., Montplaisir, J., 2014. Abnormal metabolic network activity in REM sleep behavior disorder. *Neurology* 82, 620–627. <https://doi.org/10.1212/WNL.0000000000000130>

- Ibarretxe-Bilbao, N., Junque, C., Marti, M.J., Valldeoriola, F., Vendrell, P., Bargallo, N., Zarei, M., Tolosa, E., 2010. Olfactory impairment in Parkinson's disease and white matter abnormalities in central olfactory areas: A voxel-based diffusion tensor imaging study. *Mov. Disord.* 25, 1888–1894. <https://doi.org/10.1002/mds.23208>
- Ibarretxe-Bilbao, N., Junque, C., Tolosa, E., Marti, M.-J., Valldeoriola, F., Bargallo, N., Zarei, M., 2009. Neuroanatomical correlates of impaired decision-making and facial emotion recognition in early Parkinson's disease. *Eur. J. Neurosci.* 30, 1162–71. <https://doi.org/10.1111/j.1460-9568.2009.06892.x>
- Iranzo, A., Lomeña, F., Stockner, H., Valldeoriola, F., Vilaseca, I., Salamero, M., Molinuevo, J.L., Serradell, M., Duch, J., Pavía, J., Gallego, J., Seppi, K., Högl, B., Tolosa, E., Poewe, W., Santamaria, J., 2010. Decreased striatal dopamine transporter uptake and substantia nigra hyperechogenicity as risk markers of synucleinopathy in patients with idiopathic rapid-eye-movement sleep behaviour disorder: A prospective study. *Lancet Neurol.* 9, 1070–1077. [https://doi.org/10.1016/S1474-4422\(10\)70216-7](https://doi.org/10.1016/S1474-4422(10)70216-7)
- Iranzo, A., Molinuevo, J.L., Santamaría, J., Serradell, M., Martí, M.J., Valldeoriola, F., Tolosa, E., 2006. Rapid-eye-movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study. *Lancet Neurol.* 5, 572–577. [https://doi.org/10.1016/S1474-4422\(06\)70476-8](https://doi.org/10.1016/S1474-4422(06)70476-8)
- Iranzo, A., Santamaría, J., Valldeoriola, F., Serradell, M., Salamero, M., Gaig, C., Niñerola-Baizán, A., Sánchez-Valle, R., Lladó, A., De Marzi, R., Stefani, A., Seppi, K., Pavia, J., Högl, B., Poewe, W., Tolosa, E., Lomeña, F., 2017. Dopamine transporter imaging deficit predicts early transition to synucleinopathy in idiopathic rapid eye movement sleep behavior disorder. *Ann. Neurol.* 82, 419–428. <https://doi.org/10.1002/ana.25026>
- Iranzo, A., Serradell, M., Vilaseca, I., Valldeoriola, F., Salamero, M., Molina, C., Santamaria, J., Tolosa, E., 2013. Longitudinal assessment of olfactory function in idiopathic REM sleep behavior disorder. *Park. Relat. Disord.* 19, 600–604. <https://doi.org/10.1016/j.parkreldis.2013.02.009>
- Lezak, M.D., 2012. Neuropsychological assessment. Oxford University Press.
- Massicotte-Marquez, J., Décar, A., Gagnon, J.-F., Vendette, M., Mathieu, A., Postuma, R.B., Carrier, J., Montplaisir, J., Petersen, R.C., 2008. Executive dysfunction and memory impairment in idiopathic REM sleep behavior disorder. *Neurology* 70, 1250–7. <https://doi.org/10.1212/01.wnl.0000286943.79593.a6>
- Mori, E., Shimomura, T., Fujimori, M., Hirano, N., Imamura, T., Hashimoto, M., Tanimukai, S., Kazui, H., Hanihara, T., 2000. Visuo-perceptual Impairment in Dementia With Lewy Bodies 57, 489–493.
- Ohlhauser, L., Smart, C.M., Gawryluk, J.R., 2019. Tract-Based Spatial Statistics Reveal Lower White Matter Integrity Specific to Idiopathic Rapid Eye Movement Sleep Behavior Disorder as a Proxy for Prodromal Parkinson's Disease. *J. Parkinsons. Dis.* 9, 723–731. <https://doi.org/10.3233/JPD-191688>
- Park, K.M., Lee, H.J., Lee, B.I., Kim, S.E., 2018. Alterations of the brain network in idiopathic rapid eye movement sleep behavior disorder: structural connectivity analysis. *Sleep Breath.* 23, 587–593. <https://doi.org/10.1007/s11325-018-1737-0>

- Pereira, J.B., Junqué, C., Martí, M.J., Ramirez-Ruiz, B., Bargalló, N., Tolosa, E., 2009. Neuroanatomical substrate of visuospatial and visuoperceptual impairment in Parkinson's disease. *Mov. Disord.* 24, 1193–1199. <https://doi.org/10.1002/mds.22560>
- Pereira, J.B., Weintraub, D., Chahine, L., Aarsland, D., Hansson, O., Westman, E., 2019. Cortical thinning in patients with REM sleep behavior disorder is associated with clinical progression. *npj Park. Dis.* 3, 5:7. <https://doi.org/10.1038/s41531-019-0079-3>
- Postuma, R.B., Iranzo, A., Hu, M., Högl, B., Boeve, B.F., Manni, R., Oertel, W.H., Arnulf, I., Ferini-Strambi, L., Puligheddu, M., Antelmi, E., Cohen De Cock, V., Arnaldi, D., Mollenhauer, B., Videnovic, A., Sonka, K., Jung, K.Y., Kunz, D., Dauvilliers, Y., Provini, F., Lewis, S.J., Buskova, J., Pavlova, M., Heidbreder, A., Montplaisir, J.Y., Santamaria, J., Barber, T.R., Stefani, A., Louis, S.E.K., Terzaghi, M., Janzen, A., Leu-Semenescu, S., Plazzi, G., Nobili, F., Sixel-Doering, F., Dusek, P., Bes, F., Cortelli, P., Ehgoetz Martens, K., Gagnon, J.F., Gaig, C., Zucconi, M., Trenkwalder, C., Gan-Or, Z., Lo, C., Rolinski, M., Mhlknecht, P., Holzknecht, E., Boeve, A.R., Teigen, L.N., Toscano, G., Mayer, G., Morbelli, S., Dawson, B., Pelletier, A., 2019. Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behaviour disorder: A multicentre study. *Brain* 142, 744–759. <https://doi.org/10.1093/brain/awz030>
- Pyatigorskaya, N., Gaurav, R., Arnaldi, D., Leu-Semenescu, S., Yahia-Cherif, L., Valabregue, R., Vidailhet, M., Arnulf, I., Lehericy, S., 2017. Magnetic Resonance Imaging Biomarkers to Assess Substantia Nigra Damage in Idiopathic Rapid Eye Movement Sleep Behavior Disorder. *Sleep* 40. <https://doi.org/10.1093/sleep/zsx149>
- Rahayel, S., Montplaisir, J., Monchi, O., Bedetti, C., Postuma, R.B., Brambati, S., Carrier, J., Joubert, S., Latreille, V., Jubault, T., Gagnon, J.F., 2015. Patterns of cortical thinning in idiopathic rapid eye movement sleep behavior disorder. *Mov. Disord.* 30, 680–687. <https://doi.org/10.1002/mds.25820>
- Rahayel, S., Postuma, R.B., Montplaisir, J., Bedetti, C., Brambati, S., Carrier, J., Monchi, O., Bourgouin, P.A., Gaubert, M., Gagnon, J.F., 2018a. Abnormal Gray Matter Shape, Thickness, and Volume in the Motor Cortico-Subcortical Loop in Idiopathic Rapid Eye Movement Sleep Behavior Disorder: Association with Clinical and Motor Features. *Cereb. Cortex* 28, 658–671. <https://doi.org/10.1093/cercor/bhx137>
- Rahayel, S., Postuma, R.B., Montplaisir, J., Génier Marchand, D., Escudier, F., Gaubert, M., Bourgouin, P.-A., Carrier, J., Monchi, O., Joubert, S., Blanc, F., Gagnon, J.-F., 2018b. Cortical and subcortical gray matter bases of cognitive deficits in REM sleep behavior disorder. *Neurology* 90, e1759–e1770. [https://doi.org/10.1016/S0306-4522\(99\)00316-4](https://doi.org/10.1016/S0306-4522(99)00316-4)
- Ramírez-Ruiz, B., Junqué, C., Martí, M.-J., Valldeoriola, F., Tolosa, E., 2006. Neuropsychological deficits in Parkinson's disease patients with visual hallucinations. *Mov. Disord.* 21, 1483–7. <https://doi.org/10.1002/mds.20965>
- Ramírez-Ruiz, B., Martí, M.J., Tolosa, E., Giménez, M., Bargalló, N., Valldeoriola, F., Junqué, C., 2007. Cerebral atrophy in Parkinson's disease patients with visual hallucinations. *Eur. J. Neurol.* 14, 750–756. <https://doi.org/10.1111/j.1468-1331.2007.01768.x>
- Reuter, M., Fischl, B., 2011. Avoiding asymmetry-induced bias in longitudinal image processing. *Neuroimage* 57, 19–21. <https://doi.org/10.1016/j.neuroimage.2011.02.076>

- Reuter, M., Rosas, H.D., Fischl, B., 2010. Highly accurate inverse consistent registration: A robust approach. *Neuroimage* 53, 1181–1196. <https://doi.org/10.1016/j.neuroimage.2010.07.020>
- Rolinski, M., Griffanti, L., Piccini, P., Roussakis, A.A., Szewczyk-krolkowski, K., Menke, R.A., Quinnell, T., Zaiwalla, Z., Klein, J.C., Mackay, C.E., Hu, M.T.M., 2016. Basal ganglia dysfunction in idiopathic REM sleep behaviour disorder parallels that in early Parkinson's disease. *Brain* 2224–2234. <https://doi.org/10.1093/aww131>
- Scherfler, C., Frauscher, B., Schocke, M., Iranzo, A., Gschliesser, V., Seppi, K., Santamaria, J., Tolosa, E., Högl, B., Poewe, W., 2011. White and gray matter abnormalities in idiopathic rapid eye movement sleep behavior disorder: A diffusion-tensor imaging and voxel-based morphometry study. *Ann. Neurol.* 69, 400–407. <https://doi.org/10.1002/ana.22245>
- Segura, B., Baggio, H.C., Marti, M.J., Valldeoriola, F., Compta, Y., Garcia-Diaz, A.I., Vendrell, P., Bargallo, N., Tolosa, E., Junque, C., 2014. Cortical thinning associated with mild cognitive impairment in Parkinson's disease. *Mov. Disord.* 29, 1495–1503. <https://doi.org/10.1002/mds.25982>
- Terzaghi, M., Zucchella, C., Rustioni, V., Sinforiani, E., Manni, R., 2013a. Cognitive Performances and Mild Cognitive Impairment in Idiopathic Rapid Eye Movement Sleep Behavior Disorder: Results of a Longitudinal Follow-Up Study. *Sleep* 36, 1527–1532. <https://doi.org/10.5665/sleep.3050>
- Terzaghi, M., Zucchella, C., Rustioni, V., Sinforiani, E., Manni, R., 2013b. Cognitive Performances and Mild Cognitive Impairment in Idiopathic Rapid Eye Movement Sleep Behavior Disorder: Results of a Longitudinal Follow-Up Study. *Sleep* 36, 1527–1532. <https://doi.org/10.5665/sleep.3050>
- Tiraboschi, P., Salmon, D.P., Hansen, L.A., Hofstetter, R.C., Thal, L.J., Corey-Bloom, J., 2006. What best differentiates Lewy body from Alzheimer's disease in early-stage dementia? *Brain* 129, 729–735. <https://doi.org/10.1093/brain/awh725>
- Unger, M.M., Belke, M., Menzler, K., Heverhagen, J.T., Keil, B., Stiasny-Kolster, K., Rosenow, F., Diederich, N.J., Mayer, G., Möller, J.C., Oertel, W.H., Knake, S., 2010. Diffusion tensor imaging in idiopathic REM sleep behavior disorder reveals microstructural changes in the brainstem, substantia nigra, olfactory region, and other brain regions. *Sleep* 33, 767–773. <https://doi.org/10.1093/sleep/33.6.767>
- Uribe, C., Segura, B., Baggio, H.C., Abos, A., Garcia-Diaz, A.I., Campabadal, A., Marti, M.J., Valldeoriola, F., Compta, Y., Bargallo, N., Junque, C., 2019. Progression of Parkinson's disease patients' subtypes based on cortical thinning: 4-year follow-up. *Parkinsonism Relat. Disord.* 64, 286–292. <https://doi.org/10.1016/j.parkreldis.2019.05.012>
- Uribe, C., Segura, B., Baggio, H.C., Abos, A., Marti, M.J., Valldeoriola, F., Compta, Y., Bargallo, N., Junque, C., 2016. Patterns of cortical thinning in nondemented Parkinson's disease patients. *Mov. Disord.* 31, 699–708. <https://doi.org/10.1002/mds.26590>
- Vendette, M., Gagnon, J.-F., Soucy, J.-P., Gosselin, N., Postuma, R.B., Tuineag, M., Godin, I., Montplaisir, J., 2011. Brain perfusion and markers of neurodegeneration in rapid eye movement sleep behavior disorder. *Mov. Disord.* 26, 1717–1724. <https://doi.org/10.1002/mds.23721>

Vendette, M., Montplaisir, J., Gosselin, N., Soucy, J.-P., Postuma, R.B., Dang-Vu, T.T., Gagnon, J.-F., 2012. Brain perfusion anomalies in rapid eye movement sleep behavior disorder with mild cognitive impairment. *Mov. Disord.* 27, 1255–1261. <https://doi.org/10.1002/mds.25034>

Yoon, E.J., Lee, J.Y., Nam, H., Kim, H.J., Jeon, B., Jeong, J.M., Kim, Y.K., 2019. A new metabolic network correlated with olfactory and

executive dysfunctions in idiopathic rapid eye movement sleep behavior disorder. *J. Clin. Neurol.* 15, 175–183. <https://doi.org/10.3988/jcn.2019.15.2.175>

Youn, S., Kim, T., Yoon, I.Y., Jeong, J., Kim, H.Y., Han, J.W., Kim, J.M., Kim, K.W., 2016. Progression of cognitive impairments in idiopathic REM sleep behaviour disorder. *J. Neurol. Neurosurg. Psychiatry* 87, 890–896. <https://doi.org/10.1136/jnnp-2015-311437>

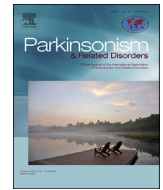
Study 6

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Brain correlates of progressive olfactory loss in Parkinson's disease



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ABSTRACT

Background: Olfactory dysfunction is present in a large proportion of patients with Parkinson's disease (PD) upon diagnosis. However, its progression over time has been poorly investigated. The few available longitudinal studies lack control groups or MRI data.

Objective: To investigate the olfactory changes and their structural correlates in non-demented PD over a four-year follow-up.

Methods: We assessed olfactory function in a sample of 25 PD patients and 24 normal controls of similar age using the University of Pennsylvania Smell Identification test (UPSIT). Structural magnetic resonance imaging data, obtained with a 3-T Siemens Trio scanner, were analyzed using FreeSurfer software.

Results: Analysis of variance showed significant group ($F = 53.882$; $P < 0.001$) and time ($F = 6.203$; $P = 0.016$) effects, but the group-by-time interaction was not statistically significant. UPSIT performance declined ≥ 1.5 standard deviations in 5 controls and 7 patients. Change in UPSIT scores of patients correlated positively with volume change in the left putamen, right thalamus, and right caudate nucleus.

Conclusion: Olfactory loss over time in PD and controls is similar, but we have observed significant correlation between this loss and basal ganglia volumes only in patients.

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1. Introduction

Olfactory dysfunction is very frequent in PD. This impairment can already be observed in *de novo* patients, and is unrelated to dopaminergic treatment [1]. In a multicenter study, the prevalence of olfactory deficits was estimated to be 96.7% when compared with young subjects, and 74.5% when adjusted for age [2]. However, the degree of olfactory impairment in PD can range from normal to

severe hyposmia. It has been suggested that normosmic patients could be a unique clinical phenotype with fewer motor deficits and a more benign disease course [3]. In contrast, severe olfactory dysfunctions have been associated with worse cognitive performance, more cognitive decline over time, and higher risk of progression to mild cognitive impairment (MCI) and to dementia [4–6].

Olfactory impairment is observed in *de novo* patients and can therefore occur regardless of dopaminergic treatment [7,8]. Olfactory dysfunction has also been found to be a preclinical or premotor manifestation in PD [1,9]. In a study of asymptomatic relatives of PD patients, it has been found that hyposmia is associated with a 12.5% risk of developing PD within a five-year period [10]. Similarly, patients diagnosed with idiopathic anosmia are at a higher risk of developing PD [11]. Moreover, hyposmia is observed in patients with idiopathic REM sleep behavior disorder, a prodromal PD symptom [12].

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The progression of olfactory deficits in PD is unclear. Cross-sectional studies are inconsistent, with some suggesting that olfactory loss does not progress over the course of PD since it does not correlate with disease duration [1,13,14]. Nonetheless, correlations with disease severity and symptom duration was also reported [15]. The lack of worsening in olfaction is consistent with Braak staging describing the olfactory bulb as a region affected in the first stages of the disease [16]. However, the progression of degenerative changes in other regions involved in olfaction might contribute to increase the degree of olfactory impairment. Results from post-mortem studies revealed pathological changes (Lewy body formation) in the olfactory bulb but also in other brain regions such as the anterior olfactory nucleus, the piriform cortex, the amygdaloid complex, the entorhinal cortex, and the hippocampal formation [1]. In agreement with the involvement of several regions explaining olfactory deficits in PD, MRI studies have reported correlations of olfactory deficits with olfactory bulb volume [17–19], but also with piriform and orbitofrontal cortical volumes [20,21].

To the best of our knowledge, only three longitudinal studies of olfactory deficits in PD have been published [22–24]. These studies did not include a control group, which makes it difficult to distinguish disease-related decline from the effects of aging on olfactory function [25]. Moreover, there are no previous longitudinal studies including MRI data. In the current work, we investigated the olfactory changes in a sample of patients compared with a control group of similar age. We also performed correlation analyses between olfactory loss and measures of brain atrophy.

2. Method

2.1. Participants

The study sample included 25 PD patients and 24 healthy controls (HC) who underwent olfactory and MRI evaluation twice. These subjects are part of a cohort of 98 PD patients and 33 HC recruited between October 2010, and March 2012. In the present study, we only included subjects who underwent olfactory and MRI evaluation at both time points. In the 2010 cohort, the UPSIT was only administered to a small subsample. The mean ($\pm$ standard deviation) follow-up interval was 44.9 ± 5.7 months for patients and 45.9 ± 3.5 months for controls ($F = 0.732$; $P = 0.468$). Inclusion criteria for patients at time 1 were: (1) the fulfillment of UK PD Society Brain Bank diagnostic criteria for PD; (2) no surgical treatment with deep brain stimulation. Exclusion criteria were: (1) dementia according to Movement Disorders Society criteria; (2) Hoehn and Yahr (H&Y) score > 3 ; (3) significant psychiatric, neurological, or systemic comorbidity; (4) low global intelligence quotient estimated by the Vocabulary subtest of the Wechsler Adult Intelligence Scale, 3rd edition (scaled score ≤ 7); (5) Mini Mental State Examination (MMSE) score < 25 ; (6) presence of claustrophobia; (7) significant pathological MRI findings other than mild white matter hyperintensities in the FLAIR sequence; (8) MRI artefacts.

All PD patients were taking antiparkinsonian medication consisting of different combinations of L-DOPA, COMT inhibitors, MAO inhibitors, dopamine agonists, and amantadine. In order to standardize doses, levodopa equivalent daily dose (LEDD) was calculated as suggested by Tomlinson et al. [26]. All assessments were done in the *on* state. Motor disease severity was evaluated using H&Y staging and the Unified Parkinson's Disease Rating Scale motor section (UPDRS-III).

The study was approved by the Ethics Committee of the University of Barcelona (IRB00003099). All subjects provided written informed consent to participate after full explanation of the procedures involved.

2.2. Olfactory and clinical assessment

Odor identification was assessed using the Spanish version of the University of Pennsylvania Smell Identification Test [27]. The UPSIT is a standardized multiple-choice scratch-and-sniff test consisting of four test booklets with 10 items each. Subjects scratched the impregnated area and were asked to select from one of four possible answers for each item. If the patient could not identify an odor, and the examiner observed that the area was not sufficiently scratched, the examiner either scratched the area or suggested that the patient do so until an odor was selected. Subjects were also asked for their smoking history and whether they were aware of having any smell dysfunction. Following normative data presented in the UPSIT manual, which includes adjustment for age and sex, scores greater than 33 in males and 34 in females were considered to reflect normosmia, and scores lower or equal to 18 reflected anosmia. Olfactory decline was established if the total UPSIT score was at least 1.5 standard deviation (based on the distribution of scores in the HC group at baseline) lower at time 2 than at time 1.

Participants were assessed with a neuropsychological battery recommended by the Movement Disorder Society task force to evaluate cognitive functions in PD [28]. Attention and working memory were assessed with the Trail Making Test (TMT) (in seconds), part A (TMT A) and part B (TMT B), Digit Span Forward and Backward, and the Stroop Color-word Test and Symbol Digits Modalities Tests (SDMT); executive functions were evaluated with phonemic (words beginning with the letter “p” in 1 min) and semantic (animals in 1 min) fluencies; language was assessed by the total number of correct responses in the short version of the Boston Naming Test; and memory through total learning recall (sum of correct responses from trial I to trial V) and delayed recall (total recall after 20 min) using Rey's Auditory Verbal Learning Test (RAVLT). Visuospatial and visuoperceptual functions were assessed with Benton's Judgement of Line Orientation (JLO) and Visual Form Discrimination (VFD) tests.

As in a previous study [29], the presence of MCI was established if the z-score for a given test was at least 1.5 lower than the expected score in at least two tests in one domain, or in at least one test per domain in at least two domains. Furthermore, the presence of dementia was determined if MMSE score was below 25, or if there was cognitive impairment in more than one domain and impaired instrumental activities of daily living (IADL), as recommended by Dubois et al. [30].

The Beck Depression Inventory II, Starkstein's Apathy Scale, and the Neuropsychiatric Inventory were administered to all subjects to explore the presence of psychiatric symptoms.

2.3. MRI analyses

2.3.1. Preprocessing of longitudinal imaging data

MRI data were acquired with a 3T scanner (MAGNETOM Trio, Siemens, Germany) at both times. The scanning protocol included high-resolution 3-dimensional T1-weighted images acquired in the sagittal plane (TR = 2300 ms, TE = 2.98 ms, TI = 900 ms, 240 slices, FOV = 256 mm; 1 mm isotropic voxel) and an axial FLAIR sequence (TR = 9000 ms, TE = 96 ms).

Cross-sectional preprocessing of both times was performed using the automated FreeSurfer stream (version 5.1; available at: <http://surfer.nmr.harvard.edu>) as previously described in Segura et al. [29]. Detailed information about the longitudinal FreeSurfer stream is described in Ibarretxe-Bilbao et al. [31].

Longitudinal cortical thickness comparisons were performed using the longitudinal two-stage model. In this analysis, we computed the symmetrized percent of change (SPC) of cortical

thickness. Cortical thickness SPC is the rate in mm/year ($(thickness2 - thickness1) / (time2 - time1)$) with respect to the average thickness ($0.5 * (thickness1 + thickness2)$). In aging or disease, SPC is expected to be negative in most regions (<https://surfer.nmr.mgh.harvard.edu/fswiki/LongitudinalTwoStageModel>). We also obtained striatal (putamen and caudate) volumes via whole-brain segmentation.

Comparisons between groups were performed using a vertex-by-vertex general linear model. One-sample t-tests were performed to test time effects in both groups (if the SPC was different from zero). To test group-by-time interaction effects, SPC was included as a dependent factor and group as an independent factor (Supplementary Table 1).

In addition, supplementary cross-sectional intergroup cortical thickness comparisons were performed using a vertex-by-vertex general linear model with FreeSurfer at time 1 and time 2 (Supplementary Table 1).

All results were corrected for multiple comparisons using pre-cached cluster-wise Monte Carlo simulation with 10,000 iterations. Simulation was applied for negative time effects (time 2 < time 1) in each group, whereas, on all other models, simulation was applied for absolute results. Reported cortical regions reached one-tailed and two-tailed corrected significance level of $p < 0.05$ respectively.

The SPC of striatal volumes was computed and introduced in independent t-tests in order to compare it between groups. Significant results were corrected by general cognitive status (MMSE), age and gender. The SPC of UPSIT scores was also calculated. Pearson's correlation test was used to study the relationship between SPC of striatal volumes and SPC of UPSIT scores, and control of the false-discovery rate to 5% (FDR) was used to correct for multiple testing [32].

2.4. Statistical analyses of demographic, clinical, and olfactory data

Statistical analyses of demographic, clinical, and olfactory data were performed using IBM SPSS Statistics 22.0.0 (2013; Armonk, NY: IBM Corp). Group differences in demographic and clinical variables between HC and PD patients were analyzed with independent t-tests for quantitative measures or with Pearson's chi-squared test for categorical measures. Mann-Whitney's *U* test was used when comparing PD with olfactory decline and PD non-

decliners, due to small subsamples. In order to study the relationship between clinical and olfactory data, Pearson's correlation coefficient test was used. Group-by-time interaction effects in clinical variables such as UPSIT, neuropsychological tests, and neuropsychiatric symptoms between PD and HC were assessed through a repeated-measures general linear model.

3. Results

3.1. Demographic and clinical characteristics

There were no differences between groups in age ($t = 1.468$; $P = 0.149$), years of education ($t = 1.235$; $P = 0.223$), estimated IQ scores ($t = 1.045$; $P = 0.301$), or sex ($\chi^2 = 0.506$; $P = 0.477$). Regarding psychiatric symptoms, there were no significant interactions between group and time in Beck Depression Inventory II ($F = 1.777$; $P = 0.190$), Starkstein's Apathy Scale ($F = 0.090$; $P = 0.766$), or in Cummings' Neuropsychiatric Inventory scores ($F = 0.992$; $P = 0.325$) (Table 1).

Significant interactions between group and time were found in Stroop Color ($F = 5.445$; $P = 0.024$), Stroop Word-Color ($F = 5.121$; $P = 0.029$), SDMT ($F = 8.107$; $P = 0.007$), and the short version of the BNT ($F = 5.077$; $P = 0.029$). At follow-up, PD-MCI criteria were fulfilled by 12 (25%) patients, from whom 6 were cognitively preserved at baseline. Two (4.1%) PD-MCI patients had converted to PD dementia at follow-up.

3.2. Olfactory decline over time

Table 2 shows the UPSIT performance at baseline and after four years of follow-up. The distribution of the degree of impairment in PD patients in the first assessment was 36% anosmics, 60% hyposmics, and 4% normosmic. Repeated-measures analysis showed a significant effect of group ($F = 53.882$; $P < 0.001$) and time ($F = 6.203$; $P = 0.016$), but the group-by-time interaction did not achieve statistical significance ($F = 0.277$; $P = 0.601$).

In patients, UPSIT scores correlated significantly with age at time 1 ($r = -0.469$; $P = 0.018$), and with years of disease duration ($r = -0.434$; $P = 0.030$) and LEDD ($r = -0.463$; $P = 0.020$) at time 2. Qualitatively taking the cut-off of 1.5 standard deviation decline from the baseline scores, 5 controls and 7 patients displayed significant olfactory function loss over time. Patients with olfactory

Table 1
Demographic and clinical characteristics of PD patients and HC at both times.

	PD (n = 25)	HC (n = 24)	Test stats/P-value
Age at baseline, yrs., mean (SD)	61.4 (10.0)	65.3 (8.9)	1.468/0.149 ^a
Education, yrs., mean (SD)	12.9 (4.8)	11.2 (4.4)	1.235/0.223 ^a
Vocabulary subtest, mean (SD)	49.0 (6.8)	46.8 (7.7)	1.045/0.301 ^a
Sex, male, n (%)	14 (56.0)	11 (45.8)	0.506/0.477 ^b
Disease duration, yrs., mean (SD)			
Time 1	4.9 (3.5)	NA	NA
Time 2	8.7 (3.4)	NA	NA
Age of onset, yrs., mean (SD)	56.4 (10.3)	NA	NA
UPDRS part III, mean (SD)			
Time 1	14.04 (8.7)	NA	NA
Time 2	16.25 (7.7)	NA	NA
Hoehn & Yahr stage, n 1/1.5/2/2.5/3/4			
Time 1	9/1/12/2/1/0	NA	NA
Time 2	4/0/11/0/10/0	NA	NA
LEDD, mg, mean (SD)			
Time 1	607.8 (433.4)	NA	NA
Time 2	659.2 (371.4)	NA	NA

Abbreviations: HC: healthy controls; LEDD: L-dopa equivalent daily dose; NA: not applicable; PD: Parkinson's disease; UPDRS part III: Unified Parkinson's Disease Rating Scale motor section. Data are presented as mean (SD) or n (%).

^a Independent t-tests were used for continuous variables when comparing HC with all PD patients.

^b Pearson's chi-squared tests were used for categorical variables.

Table 2
Olfactory results in PD and HC at both times.

	PD (n = 25)	HC (n = 24)	Test stats/P-value
<i>University of Pennsylvania Smell Identification Test</i>			
Time 1, mean (SD)	20.6 (7.5)	31.3 (3.1)	6.481/<0.001 ^a
Time 2, mean (SD)	18.7 (6.4)	30.0 (4.7)	7.036/<0.001 ^a
<i>Olfactory status according to UPSIT, n (%)</i>			
Time 1 Anosmics	9 (36.0)	0 (0.0)	
Time 2 Anosmics	12 (48.0)	0 (0.0)	

Abbreviations: HC, healthy controls; PD, Parkinson's disease; UPSIT, *University of Pennsylvania Smell Identification Test*. Data are presented as mean (SD) or n (%).
^a Independent t-tests were used for continuous variables when comparing HC with all PD patients.

decline did not differ from non-decliners in any clinical or demographic variable.

3.3. Cortical thickness and volumetric changes

Comparison of whole-brain cortical thickness maps showed that both groups had significant progressive cortical thinning in several

regions of the posterior cortex. In PD patients, significant cortical thinning was found in the left isthmus of the cingulate gyrus and in the right inferior parietal lobule. In controls, changes over time were seen in the left lingual gyrus and insula, and in the right caudal middle frontal gyrus and lateral occipital regions (Fig. 1). No significant group-by-time interactions were seen between controls and patients.

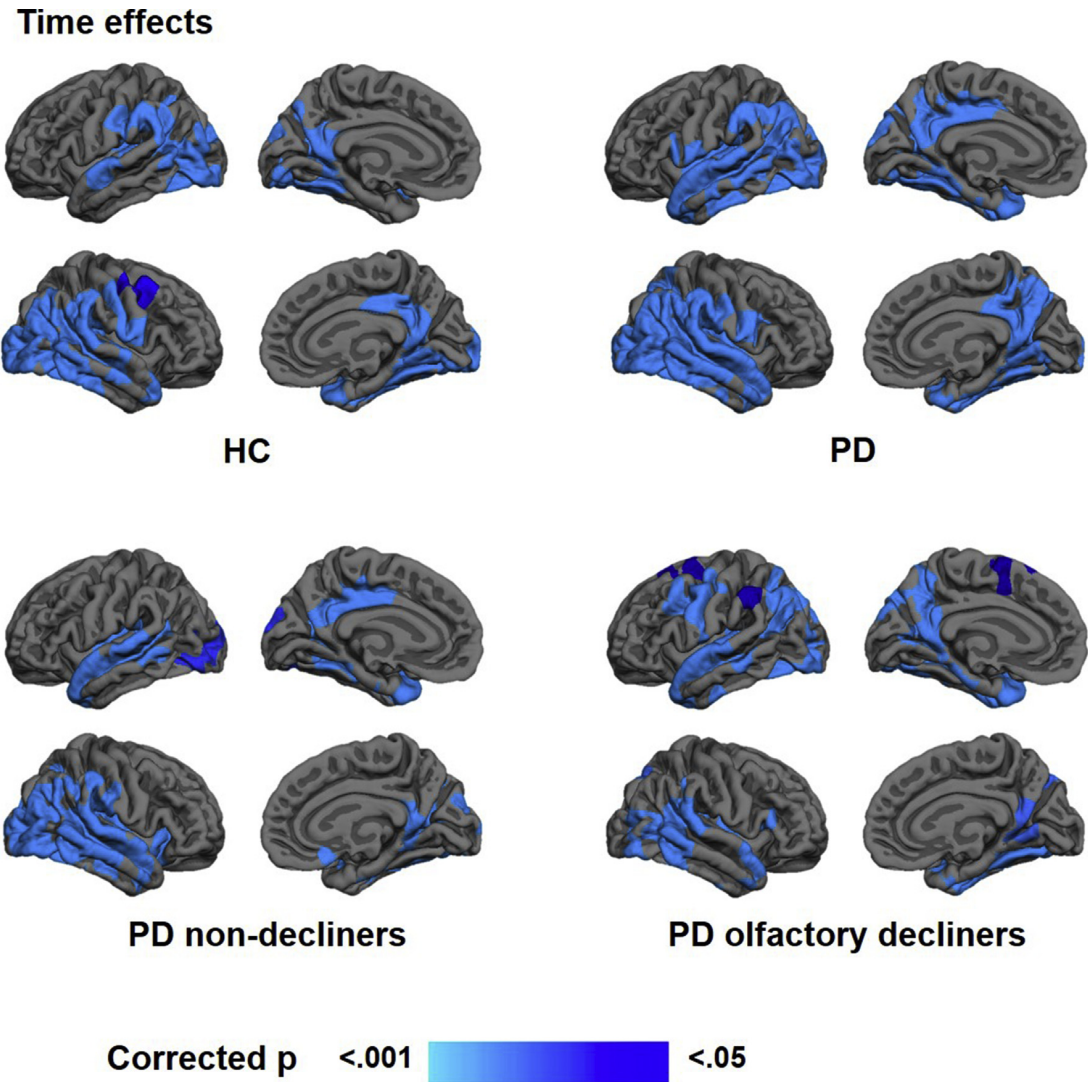


Fig. 1. Abbreviations: HC: healthy controls; PD: Parkinson's disease. Results were obtained using Monte Carlo simulation with 10,000 iterations applied to cortical thickness maps to provide cluster-wise correction for multiple comparisons. Simulation was applied for negative time effects (time 2 < time 1) in each group. Clusters with corrected p < .05 are shown.

The analysis of volumetric changes in the regions of interest of the olfactory system showed that PD patients had greater percentage of volume loss in the right putamen in comparison with HC ($t = 2.676$; $P = 0.010$). Differences remain significant after controlling for general cognitive status, age, and gender ($F = 6.504$; $P = 0.014$).

Correlations between the percentage of change in UPSIT scores and basal ganglia volumes, corrected for multiple comparisons using FDR, showed statistical significance for PD patients in the right thalamus ($P = 0.044$), right caudate ($P = 0.049$), and left putamen ($P = 0.044$) (Fig. 2).

Regarding changes in subcortical volumes over time, PD with olfactory decline had greater right caudate loss in comparison with PD without olfactory decline ($U = 22.000$; $P = 0.012$).

4. Discussion

Our results demonstrate that olfactory impairments progress over time in PD patients, as we detected significant worsening after four years of follow-up. However, the progression of smell impairment was similar to that identified in the control group. These results seem to suggest that aging per se could be responsible for the observed changes in olfactory ability. An alternative explanation could be that atrophy in different brain structures might produce similar clinical changes.

In our study, the prevalence of olfactory deficits as well as the mean scores observed in patients and controls are similar to previous reports using UPSIT with larger samples [1]. Our sample could thus be considered representative of olfactory dysfunctions in PD. We found that the distribution of the degree of impairment in PD patients in the first assessment was 36% anosmics, 60% hyposmics, and 4% normosmic. Regarding the decline in olfactory performance, our results agree with those from a previous longitudinal study performed in a sample of 19 PD patients studied twice with an interval of five years using the “sniffin’ sticks”; in that study, however, aging effects could not be excluded because the authors did not include a control group in the design [24]. In the present study, we found a significant effect of time during the four-year follow-up in both patients and controls. The scores obtained in the UPSIT diminished a mean of two points in PD patients, and 1.3 points in HC. Taking the cut-off point of 1.5 standard deviation

below the baseline score as indicative of decline, we observed olfactory decline in 7 patients, but also in 5 controls. It is well known that aging per se is associated with olfactory decline; McKinnon et al. (2010), using data collected from 732 subjects, estimated that the decrease in UPSIT scores is 3.2 points for every 10 years of age [33]. Loss of olfactory identification ability is seen from the 5th decade onwards, but is most remarkable between the 7th and 8th decades of life [25]. The correlations between UPSIT scores and disease duration that we and other researchers have observed could reflect both aging effects and the progression of the degenerative process.

In the current four-year longitudinal study, we found evidence of progressive cortical thickness reduction, but this atrophy was similar for patients and controls, and was unrelated to the olfactory loss. From a neuropathological point of view, the severity of abnormal protein aggregates (including alpha-synuclein, hyperphosphorylated tau protein, and neurofilament protein) in the olfactory bulb and tract increases significantly in neurodegenerative diseases with increasing neuritic Braak stages and McKeith criteria for Lewy body disease [34]. Odor impairment in PD may be due to defective adult neurogenesis. In animal models, dopaminergic deafferentation may result in impaired precursor cell proliferation in the subventricular zone, which provides new olfactory bulb neurons. It also results in an increased number of dopaminergic neurons in the glomerular cell layer of the olfactory bulb. In PD, the survival of newly generated neurons in the bulb is decreased [35]. Moreover, it has been experimentally demonstrated that dopamine modulates adult neurogenesis in vivo and in vitro and that adult neurogenesis is impaired in individuals with PD [36].

It is thus clear that there is a neuropathological basis for progressive odor impairment. Regarding its anatomical correlates, olfactory impairment in PD has been associated with atrophy of the olfactory bulb [17–19], piriform cortex [21,37], and orbitofrontal cortex [21]. The gray matter correlates of olfactory deficits differ according to the disease stage; in early PD patients, significant correlations are seen in the right piriform cortex, while in moderate and advanced patients the right amygdala is implicated [37]. According to Braak stages [16], the orbitofrontal cortex, a tertiary olfactory structure, is affected relatively early, and prior to posterior parietal regions. Changes in orbitofrontal cortex are therefore expected in early disease stages. Lack of change over the study time in

Correlation between the percentage of change of UPSIT and structural measures in PD patients

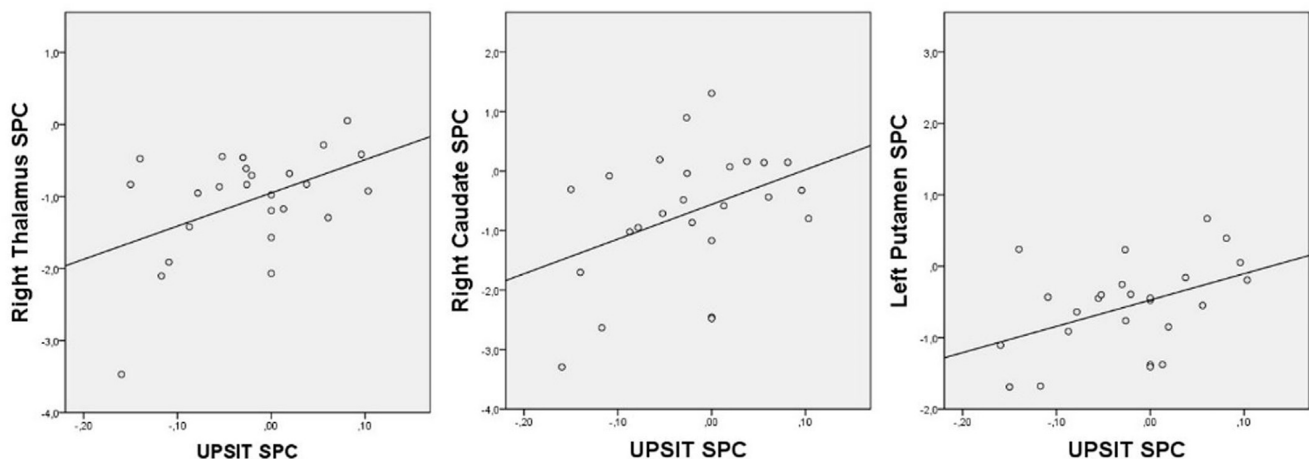


Fig. 2. Significant correlation between the percentages of change in UPSIT scores and subcortical volumes. SPC: symmetrized percentage change.

these structures might be explained by the topographical progression of Lewy-type lesions proposed by the Braak staging. It is possible that further longitudinal studies in *de novo* samples could help identify the relationship between orbital frontal atrophy and loss of odor identification.

In contrast to the lack of cortical correlates of smell loss in PD, we observed significant correlations between the percentage of change in UPSIT scores and the percentage of volume loss in some striatal regions related to the olfactory system. In normal subjects, PET studies showed activation in the thalamus and right caudate during olfactory perception [38]. In fMRI studies, activation was reported in the left putamen [39,40] and caudate [40]. In PD, odor perception activates the bilateral putamen [39], left caudate, and left putamen [40]. Thus, although the striatal nuclei are not classically considered as olfactory structures, they appear to be part of circuitry involved in olfactory perception. Moreover, olfactory performance in PD have been reported to be correlated with gray matter density in the caudate and putamen [41]. However, an alternative interpretation of our results is that striatal atrophy may reflect the rapid disease progression in PD with severe olfactory impairment. In a cross-sectional study, it has been observed that abnormal odor identification was associated with more advanced stages of PD and increased severity of motor symptoms [42]. Similarly, it has been reported that regional mean diffusivity values of the substantia nigra and the olfactory tract correlated significantly with putaminal dopaminergic dysfunction [43].

In conclusion, PD patients and healthy controls matched by age showed a significant similar progressive olfactory loss and a progressive reduction of cortical thickness mainly involving posterior regions, but olfactory loss was associated with basal ganglia volume reductions only in PD.

Disclosures

Authors CU, BS, HCB, AA, AGD, AC, MJM, FV, NB and CJ report no disclosure. YC has received funding, research support and/or honoraria in the last 5 years from Union Chimique Belge (UCB pharma), Lundbeck, Medtronic, Abbvie, Novartis, GSK, Boehringer, Pfizer, Merz, Piramal Imaging and Esteve.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.parkreldis.2017.05.005>.

References

- [1] R.L. Doty, Olfactory dysfunction in parkinson disease, *Nat. Publ. Gr* 8 (2012) 329–339, <http://dx.doi.org/10.1038/nrneurol.2012.80>.
- [2] A. Haehner, S. Boesveldt, H.W. Berendse, A. Mackay-Sim, J. Fleischmann, P.A. Silburn, A.N. Johnston, G.D. Mellick, B. Herting, H. Reichmann, T. Hummel, Prevalence of smell loss in Parkinson's disease - a multicenter study, *Park. Relat. Disord.* 15 (2009) 490–494, <http://dx.doi.org/10.1016/j.parkreldis.2008.12.005>.
- [3] D.H. Lee, J.S. Oh, J.H. Ham, J.J. Lee, I. Lee, P.H. Lee, J.S. Kim, Y.H. Sohn, Is normosmic Parkinson disease a unique clinical phenotype? *Neurology* 85 (2015) 1270–1275, <http://dx.doi.org/10.1212/WNL.0000000000001999>.
- [4] R. Postuma, J.F. Gagnon, Cognition and olfaction in Parkinson's disease, *Brain* 133 (2010) 1–2, <http://dx.doi.org/10.1093/brain/awq225>.
- [5] M.E. Fullard, B. Tran, S.X. Xie, J.B. Toledo, C. Scordia, C. Linder, R. Purri, D. Weintraub, J.E. Duda, L.M. Chahine, J.F. Morley, Olfactory impairment predicts cognitive decline in early Parkinson's disease, *Park. Relat. Disord.* 25 (2016) 45–51, <http://dx.doi.org/10.1016/j.parkreldis.2016.02.013>.
- [6] T. Baba, A. Kikuchi, K. Hirayama, Y. Nishio, Y. Hosokai, S. Kanno, T. Hasegawa, N. Sugeno, M. Konno, K. Suzuki, S. Takahashi, H. Fukuda, M. Aoki, Y. Itoyama, E. Mori, A. Takeda, Severe olfactory dysfunction is a prodromal symptom of dementia associated with Parkinson's disease: a 3 year longitudinal study, *Brain* 135 (2012) 161–169, <http://dx.doi.org/10.1093/brain/awr321>.
- [7] R.L. Doty, M.B. Stern, C. Pfeiffer, S.M. Gollomp, H.I. Hurtig, Bilateral olfactory dysfunction in early stage treated and untreated idiopathic Parkinson's disease, *J. Neurol. Neurosurg. Psychiatry* 55 (1992) 138–142, <http://dx.doi.org/10.1136/jnnp.55.2.138>.
- [8] G. Tissingh, H.W. Berendse, P. Bergmans, R. DeWaard, B. Drukarch, J.C. Stoof, E.C. Wolters, Loss of olfaction in *de novo* and treated Parkinson's disease: possible implications for early diagnosis, *Mov. Disord.* 16 (2001) 41–46, [http://dx.doi.org/10.1002/1531-8257\(200101\)16:1<41::AID-MDS1017>3.0.CO;2-M](http://dx.doi.org/10.1002/1531-8257(200101)16:1<41::AID-MDS1017>3.0.CO;2-M).
- [9] H.W. Berendse, D.S. Roos, P. Rajmakers, R.L. Doty, Motor and non-motor correlates of olfactory dysfunction in Parkinson's disease, *J. Neurol. Sci.* 310 (2011) 21–24, <http://dx.doi.org/10.1016/j.jns.2011.06.020>.
- [10] H.W. Berendse, M.M. Ponsen, Diagnosing premotor Parkinson's disease using a two-step approach combining olfactory testing and DAT SPECT imaging, *Park. Relat. Disord.* 15 (2009) S26–S30, [http://dx.doi.org/10.1016/S1353-8020\(09\)70774-6](http://dx.doi.org/10.1016/S1353-8020(09)70774-6).
- [11] A. Haehner, T. Hummel, C. Hummel, U. Sommer, S. Junghanns, H. Reichmann, Olfactory loss may be a first sign of idiopathic Parkinson's disease, *Mov. Disord.* 22 (2007) 839–842, <http://dx.doi.org/10.1002/mds.21413>.
- [12] A. Iranzo, M. Serradell, I. Vilaseca, F. Valdeoriola, M. Salameiro, C. Molina, J. Santamaria, E. Tolosa, Longitudinal assessment of olfactory function in idiopathic REM sleep behavior disorder, *Park. Relat. Disord.* 19 (2013) 600–604, <http://dx.doi.org/10.1016/j.parkreldis.2013.02.009>.
- [13] C.H. Hawkes, B.C. Shephard, S.E. Daniel, Olfactory dysfunction in Parkinson's disease, *J. Neurol. Neurosurg. Psychiatry* 62 (1997) 436–446, <http://dx.doi.org/10.1038/nrneurol.2012.80>.
- [14] A. Haehner, T. Hummel, H. Reichmann, A clinical approach towards smell loss in Parkinson's disease, *J. Park. Dis.* 4 (2014) 189–195, <http://dx.doi.org/10.3233/JPD-130278>.
- [15] J. Deeb, M. Shah, N. Muhammed, R. Gunasekera, K. Gannon, L.J. Findley, C.H. Hawkes, A basic smell test is as sensitive as a dopamine transporter scan: comparison of olfaction, taste and DaTSCAN in the diagnosis of Parkinson's disease, *Qjm* 103 (2010) 941–952, <http://dx.doi.org/10.1093/qjmed/hcq142>.
- [16] H. Braak, K. Del Tredici, U. Rub, R.A.I. de Vos, E.N.H. Jansen Steur, E. Braak, Staging of brain pathology related to sporadic Parkinson's disease, *Neurobiol. Aging* 24 (2003) 197–211.
- [17] J. Wang, H. You, J.F. Liu, D.F. Ni, Z.X. Zhang, J. Guan, Association of olfactory bulb volume and olfactory sulcus depth with olfactory function in patients with Parkinson disease, *Am. J. Neuroradiol.* 32 (2011) 677–681, <http://dx.doi.org/10.3174/ajnr.A2350>.
- [18] S. Chen, H. Tan, Z. Wu, C. Sun, J. He, X. Li, M. Shao, Imaging of olfactory bulb and gray matter volumes in brain areas associated with olfactory function in patients with Parkinson's disease and multiple system atrophy, *Eur. J. Radiol.* 83 (2014) 564–570, <http://dx.doi.org/10.1016/j.ejrad.2013.11.024>.
- [19] R. Sengoku, S. Matsushima, K. Bono, K. Sakuta, M. Yamazaki, S. Miyagawa, T. Komatsu, H. Mitsumura, Y. Kono, T. Kamiyama, K. Ito, S. Mochio, Y. Iguchi, Olfactory function combined with morphology distinguishes Parkinson's disease, *Park. Relat. Disord.* 21 (2015) 771–777, <http://dx.doi.org/10.1016/j.parkreldis.2015.05.001>.
- [20] X. Wu, C. Yu, F. Fan, K. Zhang, C. Zhu, T. Wu, K. Li, P. Chan, Correlation between progressive changes in piriform cortex and olfactory performance in early Parkinson's disease, *Eur. Neurol.* 66 (2011) 98–105, <http://dx.doi.org/10.1159/000329371>.
- [21] E.-Y. Lee, P.J. Eslinger, G. Du, L. Kong, M.M. Lewis, X. Huang, Olfactory-related cortical atrophy is associated with olfactory dysfunction in Parkinson's disease, *Mov. Disord.* 29 (2014) 1205–1208, <http://dx.doi.org/10.1002/mds.25829>.
- [22] A. Müller, H. Reichmann, A. Livermore, T. Hummel, Olfactory function in idiopathic Parkinson's disease (IPD): results from cross-sectional studies in IPD patients and long-term follow-up of *de-novo* IPD patients, *J. Neural Transm.* 190 (2002) 805–811.
- [23] B. Herting, S. Schulze, H. Reichmann, A. Haehner, T. Hummel, A longitudinal study of olfactory function in patients with idiopathic Parkinson's disease, *J. Neurol.* 255 (2008) 367–370, <http://dx.doi.org/10.1007/s00415-008-0665-5>.
- [24] T. Meusel, B. Westermann, P. Fuhr, T. Hummel, A. Welge-Lüssen, The course of olfactory deficits in patients with Parkinson's disease-A study based on psychophysical and electrophysiological measures, *Neurosci. Lett.* 486 (2010) 166–170, <http://dx.doi.org/10.1016/j.neulet.2010.09.044>.
- [25] A. Sorokowska, V.A. Schriever, V. Gudziol, C. Hummel, A. Hähner, E. Iannilli, C. Sinding, M. Aziz, H.S. Seo, S. Negoias, T. Hummel, Changes of olfactory abilities in relation to age: odor identification in more than 1400 people aged 4 to 80 years, *Eur. Arch. Oto-Rhino-Laryngology* 272 (2015) 1937–1944, <http://dx.doi.org/10.1007/s00405-014-3263-4>.

- [26] C.L. Tomlinson, R. Stowe, S. Patel, C. Rick, R. Gray, C.E. Clarke, Systematic review of levodopa dose equivalency reporting in Parkinson's disease, *Mov. Disord.* 25 (2010) 2649–2653, <http://dx.doi.org/10.1002/mds.23429>.
- [27] R.L. Doty, *The Smell Identification Test Administration Manual*, Senonics, Haddon Heights, third ed., 1995.
- [28] I. Litvan, D. Aarsland, C.H. Adler, J.G. Goldman, J. Kulisevsky, B. Mollenhauer, M.C. Rodriguez-Oroz, A.I. Troster, D. Weintraub, MDS Task Force on mild cognitive impairment in Parkinson's disease: critical review of PD-MCI, *Mov. Disord.* 26 (2011) 1814–1824, <http://dx.doi.org/10.1002/mds.23823>.
- [29] B. Segura, H.C. Baggio, M.J. Marti, F. Valldeoriola, Y. Compta, A.I. Garcia-Diaz, P. Vendrell, N. Bargallo, E. Tolosa, C. Junque, Cortical thinning associated with mild cognitive impairment in Parkinson's disease, *Mov. Disord.* 29 (2014) 1495–1503, <http://dx.doi.org/10.1002/mds.25982>.
- [30] B. Dubois, D. Burn, C. Goetz, D. Aarsland, R.G. Brown, G.A. Broe, D. Dickson, C. Duyckaerts, J. Cummings, S. Gauthier, A. Korczyn, A. Lees, R. Levy, I. Litvan, Y. Mizuno, I.G. McKeith, C.W. Olanow, W. Poewe, Diagnostic Procedures for Parkinson's Disease Dementia: Recommendations from the Movement Disorder Society Task Force, vol. 22, 2007, pp. 2314–2324, <http://dx.doi.org/10.1002/mds.21844>.
- [31] N. Ibarretxe-Bilbao, C. Junque, B. Segura, H.C. Baggio, M.J. Marti, F. Valldeoriola, N. Bargallo, E. Tolosa, Progression of cortical thinning in early Parkinson's disease, *Mov. Disord.* 27 (2012) 1746–1753, <http://dx.doi.org/10.1002/mds.25240>.
- [32] R.S. Society, Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing Author (s): Yoav Benjamini and Yosef Hochberg Source: Journal of the Royal Statistical Society. Series B (Methodological), Vol. 57, No. 1 Published by: Wiley for the Royal Statistical Society Stable URL: <http://www.jstor.org/stable/2346101>, 57 (2017) 289–300.
- [33] J. McKinnon, V. Evidente, E. Driver-Dunckley, A. Premkumar, J. Hentz, H. Shill, M. Sabbagh, J. Caviness, D. Connor, C. Adler, Olfaction in the elderly: a cross-sectional analysis comparing Parkinson's disease with controls and other disorders, *Int. J. Neurosci.* 120 (2010) 36–39, <http://dx.doi.org/10.3109/00207450903428954>.
- [34] J. Attens, L. Walker, K.A. Jellinger, Olfactory bulb involvement in neurodegenerative diseases, *Acta Neuropathol.* 127 (2014) 459–475, <http://dx.doi.org/10.1007/s00401-014-1261-7>.
- [35] F. Marxreiter, M. Regensburger, J. Winkler, Adult neurogenesis in Parkinson's disease, *Cell. Mol. Life Sci.* 70 (2013) 459–473, <http://dx.doi.org/10.1007/s00018-012-1062-x>.
- [36] G.U. Hoglinger, P. Rizk, M.P. Muriel, C. Duyckaerts, W.H. Oertel, I. Caille, E.C. Hirsch, Dopamine depletion impairs precursor cell proliferation in Parkinson disease, *Nat. Neurosci.* 7 (2004) 726–735, <http://dx.doi.org/10.1038/nn1265>.
- [37] E. Wattendorf, A. Welge-Lüssen, K. Fiedler, D. Bilecen, M. Wolfensberger, P. Fuhr, T. Hummel, B. Westermann, Olfactory impairment predicts brain atrophy in Parkinson's disease, *J. Neurosci.* 29 (2009) 15410–15413, <http://dx.doi.org/10.1523/JNEUROSCI.1909-09.2009>.
- [38] I. Savic, B. Gulyas, M. Larsson, P. Roland, Olfactory functions are mediated by parallel and hierarchical processing, *Neuron* 26 (2000) 735–745, [http://dx.doi.org/10.1016/S0896-6273\(00\)81209-X](http://dx.doi.org/10.1016/S0896-6273(00)81209-X).
- [39] C. Moessnang, G. Frank, U. Bogdahn, J. Winkler, M.W. Greenlee, J. Klucken, Altered activation patterns within the olfactory network in Parkinson's disease, *Cereb. Cortex* 21 (2011) 1246–1253, <http://dx.doi.org/10.1093/cercor/bhq202>.
- [40] B. Westermann, E. Wattendorf, U. Schwerdtfeger, A. Husner, P. Fuhr, O. Gratzl, T. Hummel, D. Bilecen, A. Welge-Lüssen, A. Welge-Lussen, Functional imaging of the cerebral olfactory system in patients with Parkinson's disease, *J. Neurol. Neurosurg. Psychiatry* 79 (2008) 19–24, <http://dx.doi.org/10.1136/jnnp.2006.113860>.
- [41] J.E. Lee, K.H. Cho, J.H. Ham, S.K. Song, Y.H. Sohn, P.H. Lee, Olfactory performance acts as a cognitive reserve in non-demented patients with Parkinson's disease, *Park. Relat. Disord.* 20 (2014) 186–191, <http://dx.doi.org/10.1016/j.parkreldis.2013.10.024>.
- [42] S. Cavaco, A. Goncalves, A. Mendes, N. Vila-Cha, I. Moreira, J. Fernandes, J. Damasio, A. Teixeira-Pinto, A. Bastos Lima, Abnormal olfaction in Parkinson's disease is related to faster disease progression, *Behav. Neurol.* 2015 (2015) 976589, <http://dx.doi.org/10.1155/2015/976589>.
- [43] C. Scherfler, R. Esterhammer, M. Nocker, P. Mahlknecht, H. Stockner, B. Warwitz, S. Spielberger, B. Pinter, E. Donnemiller, C. Decristoforo, I. Virgolini, M. Schocke, W. Poewe, K. Seppi, Correlation of dopaminergic terminal dysfunction and microstructural abnormalities of the basal ganglia and the olfactory tract in Parkinson's disease, *Brain* 136 (2013) 3028–3037, <http://dx.doi.org/10.1093/brain/awt234>.

Supplementary material

Brain correlates of progressive olfactory loss in Parkinson's disease.

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Supplementary Table 1 Cortical thickness data – longitudinal analysis

Cortical area	Cluster size (mm ²)	Stats	P-value	MNI coordinates (x,y,z) ¹
<i>Cross-sectional intergroup at baseline</i>				
Healthy controls vs PD				
Left rostral middle frontal gyrus	1,623.44	4.305	.014	-37 44 9
Left cuneus	2,189.75	2.551	.001	-6 -75 26
PD non-decliners vs PD decliners				
No significant differences at baseline				
<i>Cross-sectional intergroup comparison at time 2</i>				
Healthy controls vs PD				
No significant differences at baseline				
PD non-decliners vs PD decliners				
No significant differences at time 2				
<i>Time effects</i>				
Healthy controls				
Left insula	2,924.46	-5.872	<.001	-34 -26 20
Left lingual gyrus	15,510.76	-4.015	<.001	-12 87 -13
Right lateral occipital cortex	25,992.79	-5.491	<.001	37 -76 7
Right caudal middle frontal gyrus	1,847.14	-3.424	.002	40 6 48
PD patients				
Left isthmus of cingulate gyrus	29,571.77	-5.836	<.001	-10 -52 27
Right inferior parietal lobule	33,174.32	-6.217	<.001	49 -44 17
PD decliners				
Left inferior parietal lobule	11,123.29	-4.563	<.001	-45 -69 22
Left superior parietal lobule	1,313.45	-3.378	.018	-33 -45 38
Left superior temporal gyrus	6,549.03	-3.399	<.001	-58 -17 2
Left superior frontal gyrus	1,246.60	-3.073	.024	-9 6 49
Left caudal middle frontal gyrus	3,530.03	-2.927	<.001	-36 4 34
Right inferior temporal gyrus	9,899.71	-4.910	<.001	55 -58 -10
Right pars opercularis	3,070.32	-2.711	<.001	42 19 9
Right isthmus of cingulate gyrus	2,087.46	-2.663	<.001	14 -44 -1
PD non-decliners				
Left posterior cingulate gyrus	3,146.51	-5.834	<.001	-4 -23 37
Left postcentral gyrus	9,562.30	-3.909	<.001	-40 -16 21
Left lateral occipital cortex	2,410.78	-3.018	<.001	-32 -81 -13
Right lateral occipital cortex	20,992.62	-6.181	<.001	31 -90 -2
Right isthmus of cingulate gyrus	2,519.62	-5.904	<.001	9 -42 7
<i>Group-by-time interaction effects</i>				
Healthy controls vs PD patients				
No significant interactions				

¹MNI305 space. PD, Parkinson's disease. Results were obtained using Monte Carlo simulation with 10,000 iterations applied to cortical thickness maps to provide clusterwise correction for multiple comparisons. Clusters with corrected p<0.05 are reported.

CHAPTER 5

General discussion

In the present Doctoral Thesis, we aimed at characterizing brain and cognitive changes associated with IRBD as well as investigating the assessment and evolution of olfactory dysfunction in prodromal stages of Synucleinopathies and PD. To this end, six studies were performed to address specific questions. In this final section, I will discuss the most significant results.

Cognitive impairment in IRBD

In *studies 1* and *2*, we aimed at identifying neuropsychological impairment in IRBD and its neuroanatomical substrates. To achieve these objectives, we compared the cognitive performance between IRBD patients and healthy individuals, afterward, we studied its structural and functional correlates. Furthermore, in *study 5*, we investigated whether some functions show decline throughout the disease.

In keeping with previous neuropsychological findings on IRBD, in *studies 1, 2, and 5*, patients differed from controls in several cognitive domains, namely attention and executive functions (Delazer et al., 2012; Gagnon et al., 2009; Massicotte-Marquez et al., 2008; Ferini-Strambi et al., 2004; Terzaghi et al., 2013), mental processing speed (Pereira et al., 2019), verbal memory (Fantini et al., 2011; Gagnon et al., 2009; Her et al., 2019; Li et al., 2018; Massicotte-Marquez et al., 2008; Shin et al., 2019; Terzaghi et al., 2013, 2008) and semantic fluency (Gagnon et al., 2009; Massicotte-Marquez et al., 2008). Moreover, for the first time, we also identified facial recognition and VS impairment. Our results showed large effect size for semantic fluency, RAVLT total learning, SDMT, TMTA, TMTB and FRT tests. Medium effect size was obtained for RAVLT recall and recognition, Stroop Color and BNT. As was reported earlier, executive functions, attention (Marchand et al., 2017; Terzaghi et al., 2019; Youn et al., 2016), semantic fluency (Marchand et al., 2017), and verbal memory (Terzaghi et al., 2019) are significantly impaired in IRBD converters compared to non-converters. Interestingly, Marchand et al., (2017) found that performance in TMT, verbal fluency and Stroop Color Word test, are the best predictors of DLB conversion in IRBD patients. These findings suggest that in our sample the

Cognitive impairment in IRBD

Our results in *studies 1, 2, and 5* showed impairment in verbal memory, semantic fluency, mental processing speed and executive functions in IRBD relative to healthy individuals in line with findings in DLB and PD.

prevalence of future converters is probably high, thus PD- and DLB-like cognitive characteristics are already present.

Visuospatial and Visuo-perceptual impairment in IRBD

Study 1 is the first investigation testing facial recognition in IRBD patients. We found significantly lower scores in IRBD in comparison to healthy individuals with a large effect size. Facial recognition is a VP function that has its neural basis located in occipitotemporal regions, namely in the fusiform gyrus (Haxby et al., 2002). Interestingly, in *study 1* IRBD patients also presented cortical thinning in this region. Previous literature in PD, identified facial recognition impairment (Pereira et al., 2009a), specifically in PD with hallucinations (Ramírez-Ruiz et al., 2006), as well as atrophy in the fusiform gyrus, both in PD (Garcia-Diaz et al., 2018b, 2018a) and in PD with hallucinations (Ramírez-Ruiz et al., 2007). These results suggest that this posterior-based dysfunction could be a cognitive biomarker of PD pathology in IRBD.

In *study 5*, we found differences between IRBD and HC in JLO, a test that measures VS function in isolation. This finding is also remarkable, considering that the specific and independent assessment of VS functions has been overlooked in the literature, with only two previous works assessing this function (Iranzo et al., 2006; Pereira et al., 2019). The main limitation in Iranzo, et al.'s work, is that they lacked a control group. In the other study, they found a clear tendency to significance in VS impairment in IRBD relative to HC (Pereira et al., 2019). In a similar way, our results are in line with previously reported VS/VP dysfunction in DLB (Levy and Chelune, 2007; Tiraboschi et al., 2006) and PD (Garcia-Diaz et al., 2018a; Pereira et al., 2009a; Ramírez-Ruiz et al., 2006).

Facial recognition and Visuospatial impairment in IRBD

Studies 1 and 5, identified for the first time, impairment in facial recognition and in visuospatial functions in agreement with previously reported in overt Synucleinopathies, such as VS/VP dysfunctions in PD and DLB.

Neuroanatomical basis of cognitive dysfunction in IRBD

Contrary to our hypotheses and previous literature (Pereira et al., 2019; Rahayel et al., 2018b), in *study 1* we did not find significant correlations between neuropsychological impairment and MRI measures of brain atrophy such as cortical thickness. However, there

was coherence between the structural changes detected by MRI and neuropsychological impairment. For instance, as mentioned above, IRBD subjects had facial recognition impairment and cortical atrophy in the fusiform gyrus, a region that is known to be critical for such function (Haxby et al., 2002). In the same way, patients showed memory impairment and hippocampal atrophy, a core structure for learning and memory. Particularly, we found larger atrophy in the right posterior hippocampus, a region that is related to spatial memory (Ezzati et al., 2016). The lack of association between hippocampal volume reduction and memory impairment in our work might be improved in the future by using specific visuospatial memory tests.

On the other hand, *study 2* evidenced the potential role of altered brain functional connectivity in IRBD's cognitive impairment. We found a positive correlation between mental processing speed and temporoparietal functional connectivity in the IRBD group. It is interesting to note that the mental processing speed domain in *study 2* was measured using the Stroop Color and SDMT-oral version, tests that are known to have a strong posterior cortical component (Forn et al., 2009; Garcia-Diaz et al., 2018a, 2018b; Lezak, 2012). Specifically, in agreement with our results, SDMT is associated with cortical atrophy in temporoparietal regions in PD patients (Garcia-Diaz et al., 2018a). Presumably, the relationship among these tests and the loss of connectivity between right superior parietal and left inferior temporal regions could be reflecting integration abnormalities between the right dorsal-stream, required for VS processing, and regions of the left ventral-stream, that may be involved in visuo-verbal decodification required in both tasks. Such findings might be underlying the specific vulnerability of long-distance connections in IRBD patients. Further research is needed to elucidate possible changes in the organization of white matter tracts in IRBD.

An alternative explanation might be that the correlation between mental speed processing and the loss of connectivity between right superior parietal and left inferior temporal regions is reflecting a failure of coherence between the dorsal and ventral attention networks. This hypothesis is even more interesting if we consider that neither of the two networks controls attentional processes in isolation and that flexible interaction between both systems is responsible for the dynamic control of attention in relation to top-down goals and bottom-up sensory stimulation (Vossel et al., 2014). The lack of co-activation between these two networks might explain the common presence of

fluctuating cognition in DLB patients, which mechanisms might be already ongoing in IRBD patients. Furthermore, the presence of hallucinations in PD is associated with disruptions in inter-network connectivity between ventral and dorsal attention networks (Shine et al., 2014). Minor hallucinations appeared as a frequent early non-motor symptom that may even predate the onset of parkinsonism (Pagonabarraga et al 2016). In this sense, a more thorough clinical evaluation in future studies with IRBD patients, might help to elucidate the role of attentional control networks in minor hallucinations.

Mental speed processing impairment is related to the temporoparietal connectivity disruption

In *study 2*, we identified a positive correlation between mental speed processing and the reduction of connectivity between right superior parietal and left inferior temporal regions. This finding suggests a loss of coherence between the dorsal and ventral attention networks.

Finally, it is worth noting that among all cognitive domains, only mental speed processing significantly correlated with brain functional connectivity. It may be assumed that SDMT and, Stroop Color test require not only mental speed processing, but also the integration of attention, VS, and VP functions. So, this may be the reason why they are more sensitive when it comes to reflecting brain dysfunctions in IRBD patients.

Impairment in SDMT and Stroop Color tests reflect brain dysfunctions in IRBD

Only mental speed processing domain was associated with disrupted functional connectivity (*study 2*). It may be assumed that SDMT and Stroop Color test require not only mental speed processing, but also integration of attention and VS/VP functions. This may be the reason why they are better when it comes to reflecting brain dysfunctions in IRBD patients.

VS/VP decline in IRBD is related to progressive atrophy in posterior cortices

In *study 5*, we found cognitive decline in IRBD relative to normal aging after less than two years. Namely, we identified VFD progressive worsening, in agreement with VS/VP findings on progressive cognitive impairment in PD and DLB (Garcia-Diaz et al., 2018a; Mori et al., 2000; Pereira et al., 2009a; Ramírez-Ruiz et al., 2006; Segura et al., 2014;

Tiraboschi et al., 2006). Although previous longitudinal studies including follow-up data for IRBD and healthy individuals are already published, these works did not report an interaction between group and time, (Fantini et al., 2011; Terzaghi et al., 2013; Youn et al., 2016) so the aging effect could not be ruled out. More importantly, we identified that visual discrimination changes over time are related to progressive reductions in bilateral superior parietal cortical regions. As far as we are aware, the neuroanatomical basis of cognitive dysfunction in IRBD has been tackled only from cross-sectional approaches (Pereira et al., 2019; Rahayel et al., 2018b). Specifically, structural mechanisms of VS/VP dysfunction are related to atrophy in the posterior cortex (Pereira et al., 2019; Rahayel et al., 2018b), but also anterior temporal (Pereira et al., 2019; Rahayel et al., 2018b), frontal, insular cortices, and right hippocampal regions (Rahayel et al., 2018b). The fact that in Rahayel et al.'s work, VS/VP substrates include frontal areas, might be explained by the use of copy drawing and block design, tasks that also demand motor and executive functions. Interestingly, when assessing precisely VS/VP functions without motor component in PD, neural correlates include cortical thinning in lateral temporoparietal regions (Garcia-Diaz et al., 2018a, 2014). Moreover, as PD progresses, VS/VP impairment is accompanied by bilateral posterior degeneration (Garcia-Diaz et al., 2018b).

Overall, results in *studies 1, 2, and 5* confirmed previous findings on the impairment in verbal memory, semantic fluency, mental processing speed, and executive functions and highlighted the importance to assess VS/VP in IRBD, since they are impaired in early stages and significantly progress over time. Although further longitudinal data with larger samples are needed to confirm our results in *study 5*, the decline in VS/VP performance might help to identify those patients evolving to an overt PD or DLB.

VS/VP decline in IRBD and progressive atrophy in posterior cortices

Our results in *study 5* show cognitive decline in IRBD relative to normal aging even in a short period. Specifically, we found visual discrimination worsening over the course of the disease, and we identified its structural substrates, highlighting the involvement of bilateral parietal cortices in IRBD's evolution. The decline in VS/VP performance might help to identify those patients that will eventually develop PD or DLB.

Characterization of GM changes and functional connectivity in IRBD

As stated before, one of the main goals of the present Doctoral Thesis was to characterize structural and functional brain substrates underlying Idiopathic REM sleep behavior disorder. This objective was pursued in *studies 1, 2, and 5* through different neuroimaging techniques, both with cross-sectional and longitudinal data.

Cortical degeneration in IRBD

In *study 1*, we identified cortical thinning in IRBD relative to HC in left superior parietal, postcentral, and fusiform regions, as well as in right superior frontal and lateral occipital cortices. These results agree with those obtained by Rahayel et al. (Rahayel et al., 2018a, 2014) regarding the involvement of dorsolateral prefrontal and occipital-medial regions. Besides, the superior parietal thinning concurs well with cortical atrophy in non-demented PD patients (Segura et al., 2014; Uribe et al., 2016). Interestingly, similar to our results, fusiform gyrus and parietal lobe thinning were observed in cross-sectional (Blanc et al., 2015; Watson et al., 2015) and longitudinal DLB studies (Mak et al., 2015).

In *study 5*, we found cortical degeneration in IRBD, even in a short interval of less than two years. MRI data evidenced aging-related structural changes in IRBD and controls with common thinning in the left precuneus and inferior parietal regions but with a larger widespread frontal and parieto-occipital progression in IRBD. Indeed, group-by-time interaction confirms the larger degeneration in IRBD relative to healthy individuals bilaterally in the superior parietal and precuneus, the right cuneus, the left occipital pole, and a cluster involving the left lateral orbitofrontal and frontopolar cortices. As stated in the introduction, there is no previous research investigating brain changes in IRBD over time compared to normal aging. It is interesting noting that already in our cross-sectional *study 1*, superior parietal thinning was identified in these patients. With *study 5*, we

Cortical degeneration in IRBD

Study 1 identified cortical thinning in IRBD relative to HC in the left superior parietal, postcentral, and fusiform regions, as well as in right superior frontal and lateral occipital cortices. After a short interval, *study 5* evidenced larger degeneration in IRBD relative to healthy individuals bilaterally in the superior parietal and precuneus, the right cuneus, the left occipital pole, and a cluster involving the left lateral orbitofrontal and frontopolar cortices. This finding is in agreement with cortical atrophy present in PD and DLB.

demonstrated the involvement of parietal regions in the progression of IRBD's brain degeneration. This predominantly posterior cortical pattern is similar to previous findings in PD and DLB (Delli Pizzi et al., 2014; Ramírez-Ruiz et al., 2007; Segura et al., 2014; Uribe et al., 2016), and further supports the idea of IRBD as a potential harbinger for the development of a Synucleinopathy.

Early implication of the temporal lobe in prodromal PD

In *study 1*, the deep gray matter analysis showed a reduction in the right hippocampus in IRBD patients, which was confirmed by different techniques. This finding is in line with previous works reporting the existence of medial temporal lobe abnormalities in such patients using other neuroimaging approaches. For instance, studies using whole-brain VBM approach reported increased hippocampal GM density (Scherfler et al., 2011), but also GM reduction in related structures such as the parahippocampal gyrus (Hanyu et al., 2012). On the other hand, brain perfusion studies have found a heightened activity of the hippocampus in IRBD patients (Dang-Vu et al., 2012; Ge et al., 2015; Mazza et al., 2006; Vendette et al., 2012; Wu et al., 2014). Some of these works have found this effect specifically in the right hippocampus (Mazza et al., 2006; Vendette et al., 2012). Furthermore, hippocampal perfusion in IRBD has been reported as a predictor of PD or DLB evolution (Dang-Vu et al., 2012). Additionally, we underwent an exploratory analysis to investigate specific changes in hippocampal subfields (*study 1*). The results revealed greater atrophy in the IRBD group in the right CA1 and CA4 subfields, right molecular layer, and right granule cell layer of the dentate gyrus. In agreement with our findings, DLB studies identified reductions in the CA1 (Chow et al., 2012; Sabattoli et al., 2008), CA4-DG (Delli Pizzi et al., 2016), subiculum, and presubiculum (Sabattoli et al., 2008). By contrast, a previous work reported the preservation of hippocampal subfields in DLB patients (Mak et al., 2017). Neuropathological studies in DLB evidenced greater Lewy pathology in the CA2 (Harding et al., 2002a), but also in the entorhinal cortex, CA1, CA3, CA4, and the subiculum (Adamowicz et al., 2017).

Early implication of the hippocampus and neocortex in prodromal PD

Study 1 identified atrophy of the right posterior hippocampus in IRBD patients and of diverse neocortical regions. The fact that these structures are already affected in prodromal PD might explain why some patients present cognitive impairment even before the motor onset.

Taken as a whole, *study 1* offers compelling evidence for the early implication of the hippocampal formation in IRBD, with particular involvement of the right posterior hippocampus. The fact that the hippocampus is already affected in prodromal PD might explain why some IRBD patients present memory impairment even before the motor onset.

Disruption of posterior brain functional connectivity

Previous works focused on rs-fMRI data in IRBD, found altered basal ganglia functional connectivity in these patients when using previously selected regions (Dayan and Browner, 2017; Ellmore et al., 2013; Rolinski et al., 2016a). Interestingly, *study 2* demonstrates that when whole-brain analysis is conducted using a complex-network approach, reduced cortico-cortical connectivity is also present. Particularly, the whole-brain analysis showed decreased posterior cortico-cortical functional connectivity strength in IRBD patients compared with healthy individuals. In summary, our results revealed disrupted connectivity involving associative regions of the temporal and parietal lobes that are known to support complex cognitive functions. To better characterize this posterior-predominant pattern, we underwent independent analyses for anterior and posterior networks. Whereas the results evidenced a significant posterior connectivity reduction in IRBD patients, no results were found in the anterior network. These findings indicate that the presence of IRBD might be associated with altered posterior connectivity. Both PD and DLB (Peraza et al., 2016, 2014) patients manifest connectivity changes in motor regions, however, disrupted function in posterior areas is more commonly reported in DLB (Borroni et al., 2015; Peraza et al., 2015, 2014; Sourty et al., 2016), hence suggesting that changes in such regions might be characteristic of this disease (Hohenfeld et al., 2018). The early disruption of posterior cortico-cortical connectivity seen in *study 2*, has further strengthened the hypothesis that DLB-like mechanisms are already ongoing in IRBD patients. Longitudinal studies on large datasets might confirm whether this posterior pattern is a reliable marker of phenoconversion and if it is a good predictor of the eventual diagnosis.

Disruption of posterior functional connectivity

Study 2 showed reduced posterior cortico-cortical functional connectivity strength in IRBD patients compared with healthy individuals, similar to that found in Synucleinopathies.

It is worth noting that functional disruption of the parietal cortex was also confirmed by the graph analysis that identified decreased centrality for a node located in the left superior parietal lobule. This finding is in complete agreement with previous results in DLB (Peraza et al., 2015) and further support the role of the parietal lobule in IRBD. In brain network analysis, the betweenness centrality of an individual node measures the impact and importance of a region on the flow of information across the brain network (Bullmore and Sporns, 2009; Rubinov and Sporns, 2010). Thus, this result might reflect that the parietal cortex is losing its known role in integrating sensory and cognitive information, which might explain the cognitive impairment present in some patients. Indeed, we found an association between abnormalities in this structure with neuropsychological dysfunction in *studies 2* and *5*. Interestingly, the functional disruption of the parietal cortex is consistent with the cortical degeneration of this region seen in structural *studies 1* and *5*. Neuropathological explanations for the involvement of such structure in IRBD remain speculative at this point but could include disease-specific regional preferences of Lewy body pathology that have been evidenced previously (Hansen et al., 2019).

The parietal cortex in IRBD

Our work highlights the involvement of the parietal cortex in IRBD. Particularly, *studies 1, 2, and 5* demonstrated cortical degeneration and disrupted functional connectivity in this brain region. Remarkably, such abnormalities are underlying cognitive impairment in IRBD patients, specially VS/VP and mental speed processing.

It should be pointed out that our results manifest brain connectivity reduction with left-hemispheric predominance in IRBD (*study 2*). In a recent work, right-handed IRBD patients exhibit a left-hemispheric predominance of subclinical nigrostriatal dysfunction, suggesting that the neurodegenerative process begins asymmetrically at prodromal stages (Iranzo et al., 2020). This finding is also reported in prior studies showing left asymmetries in motor symptoms and nigrostriatal function in right-handed PD subjects (Barrett et al., 2011; Scherfler et al., 2012; Uitti et al., 2005). Interestingly, in keeping with these works in our IRBD sample, eighteen out of twenty patients were right-handed (90%). Based on this, it could be hypothesized that in line with the nigrostriatal deficit, functional brain connectivity changes have an asymmetric vulnerability at prodromal stages that might be related to hemispheric dominance for motor functions.

Olfactory dysfunction: from prodromal stages to PD

The study of olfactory dysfunction was addressed through 4 works. In *studies 3* and *4*, we sought to assess the sensitivity and specificity of olfactory tests in IRBD and PD. In *study 4*, we analyzed MRI data to examine structural substrates of different smell tools in IRBD. Finally, in *studies 5* and *6*, we aimed at exploring the olfactory decline and its neural correlates in IRBD and PD.

In agreement with previous literature, *studies 3* and *4* showed impaired olfactory function both in IRBD and PD. The distribution of the degree of olfactory impairment in IRBD patients at baseline (*study 4*) according to the UPSIT normative data was 43% anosmic and 57% hyposmic, whereas, for PD (*study 6* baseline data), 36% patients were anosmic, 60% hyposmic, and 4% normosmic. In our work, we did not compute a statistical comparison between IRBD and PD patients. Even so, it is worth noting that the mean UPSIT score for PD and IRBD at baseline was similar (20.6 ± 7.5 and 19.7 ± 6.6 , respectively), as well as the percentage of patients with severe hyposmia or anosmia that, according to the normative data, was around 80% for both samples. Moreover, in *study 6* some PD patients remain normosmic, whereas all IRBD patients in *study 4* present olfactory dysfunction even before developing overt parkinsonism. Previous works suggest that normosmic PD patients could be a unique clinical phenotype with fewer motor deficits and a more benign disease course (Lee et al., 2015). In contrast, severe olfactory dysfunction in PD is associated with worse prognosis. (Baba et al., 2012; Damholdt et al., 2011; Fullard et al., 2016; J.-W. Park et al., 2018; Postuma and Gagnon, 2010). Likewise, on combining our results, we might deduce that patients with IRBD and severe olfactory impairment are those that will eventually convert to an overt Synucleinopathy, which will presumably present a more severe disease course. This hypothesis is supported by those studies that report worse prognosis of patients with RBD and hyposmia. Altogether, these results further strengthened the hypothesis that there are different clinical subtypes of PD.

Studies 5 and *6* showed similar smell loss over time in both conditions to that found in normal aging. In agreement with our findings, longitudinal works in IRBD (Iranzo et al., 2013; Postuma et al., 2011) showed that olfaction changes only slightly with time and that the decline is similar to that observed in healthy elder controls. In the same line with

earlier studies (Herting et al., 2008; Meusel et al., 2010; Müller et al., 2002b), in our work, only some PD patients showed smell decline, which again points towards the existence of different PD subtypes according to the degree of olfactory impairment. These previous works lack a control group; thus, the aging effect could not be ruled out. In our study, the fact that the interaction between group and time is not significant indicates that the decline in PD might be explained by aging per se. Nevertheless, the correlation between smell progression and striatal degeneration and the fact that PD with olfactory decline showed greater right caudate atrophy (*study 6*), supports the idea that olfactory loss over time worsens with disease severity.

Olfactory dysfunction in IRBD

Studies 3 and 4 confirmed significant olfactory identification deficits in PD and IRBD. Longitudinal *studies 5 and 6* did not show a significant decline over time in comparison to normal aging in such function. In our work, only some PD patients showed smell decline, which points towards the existence of different PD subtypes according to the degree of olfactory impairment.

Neuroanatomical substrates of olfactory dysfunction in IRBD and PD

MRI data in *study 4* showed that in anosmic IRBD, worse olfactory identification was related to GM reduction in orbitofrontal regions. This result is in line with a recent work in IRBD (Pereira et al., 2019) and previous literature in PD samples (Ibarretxe-Bilbao et al., 2010; Wu et al., 2011). This finding reinforces the implication of the orbitofrontal cortex in the early stages of Synucleinopathies, and thus olfactory dysfunction cannot be attributed only to the olfactory bulb. It can be conceivably hypothesized that the orbitofrontal involvement in anosmic IRBD is reflecting that patients with worse odor identification maybe those that eventually evolve to a Synucleinopathy.

Subsequently, in *studies 5 and 6*, we investigated for the first time the neural mechanisms underlying olfactory progression throughout IRBD and PD. Despite the presence of cortical degeneration in both groups over time, cortical atrophy was not significantly correlated with the smell loss in these patients. In contrast, as mentioned before, we observed significant associations between the percentage of change in the UPSIT and the volume loss in the striatal nuclei in PD. Recently, olfactory performance has been also related to striatal uptake in IRBD patients (Dušek et al., 2019; Lee et al., 2019). The

involvement of the striatum during olfactory perception has been demonstrated previously in functional neuroimaging studies (J. E. Lee et al., 2014; Moessnang et al., 2011; Savic et al., 2000; Westermann et al., 2008). Thus, although the striatum is not classically considered an olfactory structure, it appears to be part of the circuitry involved in smell perception. An alternative explanation is that the striatal atrophy might be reflecting the rapid progression in PD with severe hyposmia. Large longitudinal datasets, including patients at different stages, are needed to elucidate the implication of different substrates on the putative worsening of olfactory function in PD.

Diagnostic accuracy and neural correlates of olfactory tests

As explained in the introduction, odor identification tools, such as the UPSIT and the Sniffin' Sticks tests, are used in both research and clinical settings to assess olfactory dysfunction in Synucleinopathies. In *studies 3* and *4*, we sought to investigate the diagnostic accuracy of such tests in PD and IRBD.

In *study 3*, we report for the first time the age-related discrimination criteria and diagnostic measures for the UPSIT Spanish version for PD. We revealed that the normative cut-off score for anosmia (≤ 18) proposed by the UPSIT manual has high specificity (100%) but low sensitivity (54.6%) for PD. We demonstrated that age should be taken into account, so when optimal cut-off scores are considered according to age ranges, both sensitivity and specificity are above 80%. Age contribution to olfactory deficits is well known both in healthy subjects (Mckinnon et al., 2010; Sorokowska et al., 2015) and in PD patients (Doty et al., 1995). This is demonstrated by the fact that in the original English version discriminatory UPSIT scores are presented for different age groups (Doty et al., 1995). Unfortunately, we were unable to investigate the optimal cut-off according to different age ranges in IRBD due to the sample size in *study 4*. If we keep in mind that the onset of motor features in PD takes place around the age of 60, there is a need for developing more accurate procedures for detecting olfactory impairment in young PD patients and the prodromal stages. Finally, in agreement with previous works with Italian (Picillo et al., 2014), Brazilian (Silveira-Moriyama et al., 2008), Mexican (Rodríguez-Violante et al., 2014b) and Japanese (Kondo H, Matsuda T, Hashiba M, 1998) population, the item analysis of the UPSIT Spanish version demonstrated low percentage of success in control subjects for some items. As suggested previously, this finding might be an

indicator of culturally unsuitable items (Picillo et al., 2014), evidencing the need to provide and to promote the use of country-specific normative data. Therefore, our *study 3* gives evidence of those items that need to be reviewed in order to adapt them culturally so their sensitivity and specificity in Spanish PD may be improved.

In *study 4*, we aimed at comparing the sensitivity and specificity of the UPSIT and the Sniffin' Sticks tests, as well as its neural correlates. As expected, we found lower scores in smell identification and discrimination in IRBD, when compared with matched healthy individuals (Aguirre-Mardones et al., 2015; Barber et al., 2017; Dušek et al., 2019; Fantini et al., 2006; Huang et al., 2016; Iranzo et al., 2013; Mahlknecht et al., 2015; Miyamoto et al., 2011, 2010; Pereira et al., 2019; Shin et al., 2019, 2013; Stiasny-Kolster et al., 2005). Moreover, we confirmed that olfactory tests are useful tools for discriminating HC from IRBD (Huang et al., 2016; Miyamoto et al., 2010). Being the smell identification the olfactory measure that better discriminates patients from controls. Namely, Sniffin' Identification had the highest classification accuracy (89.1%), sensitivity (90.5%) and specificity (88.0%), followed by the UPSIT (85.4%, 85.7% and 85% respectively). As mentioned before, lower performance in the UPSIT was associated with atrophy in orbitofrontal regions in those IRBD patients with anosmia, whereas no Sniffin' subtests correlated with structural data. However, only Sniffin' Identification correlated with RBD severity. From a clinical point of view, both the UPSIT and the Sniffin Identification have proved to be useful and demonstrated high accuracy to detect olfactory impairment in IRBD. Whereas the UPSIT seems to be more sensitive when it comes to identifying structural atrophy in olfactory regions for basic research, the Sniffin' Identification is less time consuming and relates to symptoms' severity, thus could be more useful for clinical practice.

UPSIT vs Sniffin Identification

Both tests have proved to be accurate to detect olfactory impairment in IRBD. The UPSIT but not the Sniffin, correlated with associative olfactory regions, whereas the Sniffin' Identification is less time consuming and its performance is related to RBD symptom's severity (*study 4*).

Final remarks

The present Doctoral Thesis has identified new cognitive deficits in IRBD patients and has shed some light on its progression throughout the disease. In particular, our work has shown the importance of VS/VP functions as a measure able to identify cognitive changes across time in IRBD, and its potential in identifying those patients with progressive neurodegeneration in posterior cortices. Besides, we have exhaustively described the characteristics of severe and early olfactory dysfunction in IRBD and described its neuroanatomical correlates. The neuroimaging data from this Thesis points towards an early implication of the temporal-occipital-parietal cortices in IRBD. Additionally, our longitudinal work has shown that IRBD patients have brain degeneration of the occipitoparietal and orbitofrontal cortex over time, in line with the degenerative changes reported in PD and DLB. Abnormalities in such regions are supported by the association between impaired cognition and both functional and structural brain changes.

Cortical atrophy and disrupted cortico-cortical functional connectivity highlight that cortical degeneration and functional abnormalities already exist in patients with IRBD before they are diagnosed with DLB, PD, or MSA. Thus, suggesting that in prodromal PD, the alpha-synuclein pathology is already involving these structures earlier than would be expected according to the Braak degeneration model proposed from neuropathological data. Cortical atrophy and dysfunction may explain why some patients present neuropsychological impairment before the motor onset. We hope that our research will serve as a basis for further multicentric studies that confirm our findings and to identify the optimal neuropsychological and neuroimage markers for diagnostic, prognostic, and efficacy of future neuroprotective therapies.

CHAPTER 6

Conclusions

Through the combined analysis of the studies included in this Doctoral Thesis, we can conclude that:

- 1 IRBD patients show facial recognition and visuospatial impairment in agreement with the cognitive profile characteristic of PD and DLB. Moreover, we observed cortical thinning in the fusiform gyrus and the parietal cortex, regions that are known to be critical for such functions.
- 2 Structural data points towards an early atrophy of medial temporal regions and posterior cortices in IRBD. Progression over time in IRBD is characterized by occipitoparietal and orbitofrontal degeneration, in line with GM changes reported in PD and DLB.
- 3 IRBD patients show decreased posterior cortico-cortical functional connectivity, including associative regions of the temporal and parietal lobes that are known to support complex cognitive functions. Mental processing speed impairment reflects disrupted temporo-parietal connectivity.
- 4 The olfactory tests have a high accuracy distinguishing IRBD and PD from controls, especially when age is taken into account. Olfactory identification is the measure that better discriminates IRBD from healthy individuals with an accuracy above 85%. Both the Sniffin' Identification and the UPSIT tests have proved to be accurate to detect olfactory impairment in IRBD. The UPSIT performance is associated with the degree of orbitofrontal atrophy, whereas the Sniffin' Identification is less time consuming and relates to symptom severity.
- 5 IRBD and PD patients show similar smell impairment severity. In anosmic IRBD, structural imaging reinforces the implication of the orbitofrontal cortex. Thus, olfactory dysfunction cannot be attributed only to olfactory bulb degeneration.
- 6 Longitudinal data evidenced that olfaction changes only slightly with time in some patients and that the decline is similar to that observed in healthy aging. However, the fact that smell progression is associated with striatal degeneration in PD supports the hypothesis that this function worsens with disease course.
- 7 The results highlight the relevance of VS/VP functions as a measure able to identify cognitive changes across time in IRBD, and its potential in identifying those patients with progressive neurodegeneration in posterior cortices.

In summary, in the present Doctoral Thesis, we have found strong evidence that IRBD is characterized by differential structural and functional connectivity changes, mainly involving temporo-parieto-occipital regions, consistent with previous reports on Synucleinopathies. The combination of different MRI approaches indicates an early neocortical degeneration in prodromal PD that is accompanied by neuropsychological impairment. IRBD and PD patients show severe smell impairment that changes only slightly with time similar to that observed in healthy aging.

ABSTRACT

Background

The present Doctoral Thesis is focused on Rapid-eye-movement (REM) sleep behavior disorder (RBD) and olfactory dysfunction as biomarkers of Synucleinopathies, such as Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). RBD is a parasomnia characterized by loss of atonia during REM sleep that provokes dream-enacting behaviors. Clinically isolated RBD (IRBD) is considered a prodromal stage of alpha-Synucleinopathies since almost 75% of patients after 12 years of the disease develop a PD or atypical parkinsonism. Specifically, in a multicentric study, 56.5% of IRBD patients converted to parkinsonism as the first manifestation, while 43.5% developed dementia first. Subtle motor symptoms, hyposmia, and cognitive impairment are the best predictors of phenoconversion in IRBD.

In this context, there is a growing interest in characterizing the cognitive changes associated with IRBD. Studies published so far conclude that between 15-50% of these patients have mild cognitive impairment. Despite well-documented evidence of neuropsychological deficits in IRBD, additional research is needed to assess visuospatial and visuoperceptive functions (VS/VP) in isolation, since impairment in these functions is characteristic of PD and DLB neuropsychological profile. As for the cognitive decline throughout the disease, there is still considerable uncertainty. Longitudinal studies, including data for IRBD and healthy individuals, have not properly investigated the effect of aging on progressive cognitive decline.

In the past decade, the characterization of brain structure and function in IRBD has become an emergent field of study. Most neuroimaging research has focused on identifying metabolic changes. Owing to these studies, there is strong evidence of a dopaminergic deficit in the striatum, as well as cortical and subcortical metabolic abnormalities with a PD-like pattern. Works on structural neuroimaging in IRBD identified white matter and gray matter (GM) abnormalities in regions that regulate REM sleep, and that are known to have neurodegenerative pathology in early PD. Functional imaging, highlights disrupted functional connectivity within the basal ganglia and also in cortico-striatal networks in IRBD. However, no previous works addressed the study of functional changes in the whole-brain connectivity, without focusing on specific circuits.

As for longitudinal changes, few studies have investigated how disease progression affects brain structure and function, and how these changes may explain the cognitive decline. Previous works have focused on prognostic metabolic biomarkers and there is only one study on the progression of structural degeneration. Unfortunately, the lack of longitudinal MRI for the control group in this work precludes to discard the aging effects on brain changes.

There is a large body of scientific literature demonstrating the relation between Synucleinopathies and smell dysfunction. The high prevalence of hyposmia and anosmia in de novo PD is in accordance with the involvement of the olfactory bulb in the early stages of the disease, as proposed by the Braak scheme. On the other hand, the evidence of olfactory dysfunction in premotor stages is supported by the presence of hyposmia in IRBD patients, even 20 years before the motor onset. The importance of olfactory dysfunction in PD and IRBD is such that hyposmia can differentiate patients from healthy individuals of similar age. Furthermore, olfactory testing may be useful in distinguishing among motor neurodegenerative disorders, as well as in predicting conversion from IRBD to parkinsonism. The progression of olfactory deficits throughout the disease is still unclear. Previous evidence suggests that olfaction changes only slightly over time across the PD course, but since these works did not include a control group, it is difficult to distinguish disease-related decline from the effects of aging on olfactory function. Improving the accuracy of smell clinical testing in IRBD could help identify patients at a higher risk of conversion, and thus define target populations for disease-prevention trials.

Objectives and Hypotheses

The main objectives of this Thesis are:

- 1 To characterize structural and functional brain substrates underlying Idiopathic REM sleep behavior disorder as well as to relate these findings to cognitive performance,
- 2 To study olfactory dysfunction as a preclinical and clinical biomarker of alpha-Synucleinopathies, and its progression throughout the disease,
- 3 To investigate progressive brain degeneration throughout IRBD, and to investigate how these changes relate to cognitive decline.

The main hypotheses are:

- 1 IRBD will be characterized by specific changes in brain structure and functional connectivity that will be associated with olfactory and cognitive impairment,
- 2 IRBD patients will show structural brain changes over time and a cognitive decline superior to that seen in normal aging,
- 3 Smell dysfunction will be identified in IRBD and PD, and it is expected to progress slightly with the disease course.

Material and Methods

This Doctoral Thesis is presented as a compendium of six studies that were carried out to achieve the above-mentioned objectives.

Studies comprised samples of patients with IRBD, PD, and healthy controls (HC). Namely, samples 1, 2, and 4 consist of twenty-one patients with IRBD and twenty-seven healthy participants. Sample 3 included 97 non-demented PD patients and 65 healthy subjects. In *study 5*, the sample consists of 14 IRBD and 18 healthy subjects, and the study sample 6 included 25 PD patients and 24 HC. In *studies 5* and *6*, subjects were evaluated twice (interval between evaluations of 1.6 and 3.8 years, respectively).

A comprehensive battery of neuropsychological tests was administered for *studies 1, 2*, and *5*. Olfaction was assessed in *studies 3, 4, 5*, and *6*. In *studies 3* and *4*, we calculated diagnostic accuracy measures and the optimal cut-off score for each olfactory test and condition (PD vs HC and IRBD vs HC, respectively). In *study 3*, we conducted an item analysis of the University of Pennsylvania Smell Identification Test (UPSIT).

In *studies 1, 2, 4, 5*, and *6* participants underwent an MRI scan acquired in a 3-Tesla Siemens scanner (Magnetom Trio Tim scanner syngo). T1-weighted images were used in *studies 1, 2, 4, 5*, and *6* (participants were scanned twice in *studies 5* and *6*). In *studies 1, 4, 5*, and *6*, FSL and FreeSurfer were used to compute structural analyses. Specifically, in *study 1* cortical thickness, hippocampal shape analysis, subcortical, and hippocampal subfield segmentation approaches were applied. In *study 4*, voxel-based morphometry was used to study the relationship between smell impairment and GM volume in the olfactory system. In *studies 5* and *6*, the longitudinal analysis of cortical thickness was used to study the GM progression in IRBD and PD. Finally, in *study 6*, the percentage of change

in the striatal nuclei was calculated. In *study 2* MRI resting-state functional images were acquired. We used threshold-free network-based statistics and graph analysis to characterized whole-brain functional connectivity in IRBD.

Results

In *studies 1, 2, and 5*, IRBD patients differed from controls in several cognitive domains, namely attention and executive functions, mental processing speed, verbal memory, and semantic fluency. Besides, *studies 1 and 5* identified for first-time impairment in facial recognition and visuospatial functions. Contrary to our hypothesis in *study 1*, we did not find significant correlations between cognitive performance and brain atrophy. However, there was coherence between the structural changes detected by MRI and neuropsychological impairment. For instance, IRBD subjects had facial recognition impairment and cortical atrophy in the fusiform gyrus, a region that is known to be critical for such function. In the same way, patients showed memory impairment and hippocampal atrophy, a core structure for learning and memory. On the other hand, *study 2* evidenced the potential role of altered brain functional connectivity in IRBD's cognitive impairment. We found a positive correlation between mental processing speed and temporoparietal functional connectivity in the IRBD group. In *study 5*, we reported visual form discrimination decline in IRBD relative to normal aging after less than two years. More importantly, we identified that visual form discrimination worsening over time was explained by progressive reductions of the cortical thickness in the superior parietal.

Structural imaging in *study 1* identified cortical thinning in IRBD in left superior parietal, postcentral, and fusiform regions, as well as in right superior frontal and lateral occipital cortices. In *study 1*, the deep gray matter analysis showed a reduction in the right posterior hippocampus in IRBD patients, which was confirmed by different techniques. In *study 5*, we found cortical degeneration in IRBD, in a short period. MRI data evidenced aging-related structural changes in IRBD and controls with common thinning in the left precuneus and inferior parietal regions but with a larger widespread frontal and parieto-occipital progression in IRBD. Indeed, group-by-time contrast confirms the larger degeneration in IRBD patients bilaterally in the superior parietal and precuneus, the right cuneus, the left occipital pole, and a cluster involving the left lateral orbitofrontal and frontopolar cortices.

Focusing on the resting-state fMRI data, *study 2* showed that when we analyze the whole-brain, patients with IRBD show decreased posterior cortico-cortical functional connectivity strength. In summary, our results revealed disrupted connectivity involving associative regions of the temporal and parietal lobes that are known to support complex cognitive functions. An additional analysis evidenced a significant posterior connectivity reduction in IRBD patients, in contrast with preserved connectivity in the anterior network. Moreover, the graph analysis identified decreased centrality for a node located in the left superior parietal lobule.

Studies 3 and *4* showed impaired olfactory performance both in IRBD and PD. The distribution of the degree of olfactory impairment in IRBD patients at baseline (*study 4*) according to the UPSIT normative data was 43% anosmic and 57% hyposmic, whereas, for PD (*study 6* baseline data), 36% patients were anosmic, 60% hyposmic, and 4% normosmic. In our work, we did not compute a statistical comparison between IRBD and PD patients. Even so, it is worth noting that the mean UPSIT score for PD and IRBD at baseline was similar (20.6 ± 7.5 and 19.7 ± 6.6 , respectively), as well as the percentage of patients with severe hyposmia or anosmia that, according to the normative data, was around 80% for both samples. MRI data in *study 4* showed that in anosmic IRBD, worse olfactory identification was related to GM reduction in orbitofrontal regions. Whereas, smell decline in PD was associated with striatal atrophy. On the other hand, *studies 5* and *6* allow concluding that both conditions present similar smell loss over time to that found in normal aging.

In *studies 3* and *4*, we sought to investigate the diagnostic accuracy of olfactory tests in PD and IRBD. In *study 3*, we describe that the normative cut-off score for anosmia (≤ 18) proposed by the UPSIT manual has high specificity (100%) but low sensitivity (54.6%) for PD. Results indicate that age should be taken into account, so when optimal cut-off scores are considered according to age ranges, both sensitivity and specificity are above 80%. Besides, the item analysis identified that some items are of great difficulty even for the normal population. Finally, when comparing different smell functions (threshold, discrimination, and identification) in *study 4*, olfactory identification is the measure that better discriminates IRBD patients from healthy individuals with an accuracy above 85%. Both, The Sniffin' Identification and the UPSIT tests have proved to be accurate to detect olfactory impairment in IRBD. The UPSIT seems to be more sensitive when it comes to

identifying GM atrophy in olfactory regions, whereas the Sniffin' Identification performance is related to RBD symptom's severity.

Conclusions

The present Doctoral Thesis has identified new cognitive deficits in IRBD patients and has shed some light on its progression throughout the course of the disease. In particular, our work has shown the importance of VS/VP functions as a measure able to identify cognitive changes across time in IRBD, and its potential in identifying those patients with progressive neurodegeneration in posterior cortices. Besides, we have exhaustively described the characteristics of severe and early olfactory dysfunction in IRBD and described for the first time its neuroanatomical correlates. The neuroimaging data from this Thesis points towards an early implication of the temporal-occipital-parietal cortices in IRBD. Additionally, our longitudinal work has shown that IRBD patients have brain degeneration of the occipitoparietal and orbitofrontal cortex over time, in line with the degenerative changes reported in PD and DLB. Abnormalities in such regions are supported by the association between impaired cognition and both functional and structural brain changes.

Cortical atrophy and disrupted cortico-cortical functional connectivity highlight that cortical degeneration and functional abnormalities already exist in patients with IRBD before they are diagnosed with DLB, PD, or MSA. Thus, suggesting that in prodromal PD, the alpha-synuclein pathology is already involving these structures earlier than would be expected according to the Braak degeneration model proposed from neuropathological data. Cortical atrophy and dysfunction may explain why some patients present neuropsychological impairment before the motor onset. We hope that our research will serve as a basis for further multicentric studies that confirm our findings, and to identify the optimal neuropsychological and neuroimaging markers for diagnostic, prognostic, and efficacy of future neuroprotective therapies.

RESUM EN CATALÀ

Marc Teòric

La present Tesi Doctoral estudia el trastorn del comportament del son REM (RBD, per les seves sigles en anglès) i la disfunció olfactiva com a biomarcadors de les alfa-Sinucleinopaties, com ara la malaltia de Parkinson (PD, per les seves sigles en anglès), la demència per cossos de Lewy (DLB, per les seves sigles en anglès) i l'atròfia multisistèmica (MSA, per les seves sigles en anglès). El RBD és una parasòmia caracteritzada per la pèrdua d'atonía durant el son REM que provoca episodis on els pacients mostren moviments que reflecteixen el contingut dels seus somnis. El RBD que es presenta clínicament aïllat (IRBD, per les seves sigles en anglès), es considera un estadi prodròmic de les Sinucleinopaties, doncs s'ha vist que gairebé el 75% dels pacients després de 12 anys amb diagnòstic d'IRBD acaben sent diagnosticats de malaltia de Parkinson o parkinsonismes atípics. Concretament, en un estudi multicèntric el 56.5% dels pacients amb IRBD varen desenvolupar parkinsonisme com a primera manifestació, mentre que el 43.5% va presentar demència en primer lloc. La simptomatologia motora, la hipòsmia i el deteriorament cognitiu han demostrat ser els millors predictors de conversió en aquests pacients.

En aquest context, la comunitat científica ha demostrat un interès creixent en definir els canvis cognitius associats a l'IRBD. Els estudis publicats fins ara conclouen que entre el 15-50% dels pacients tenen deteriorament cognitiu lleu. Malgrat l'evidència ben documentada sobre la presència d'afectació neuropsicològica en pacients amb IRBD, cal una recerca addicional que valori de forma aïllada i específica les funcions visuoespacials i visuoperceptives (VS/VP), ja que l'alteració d'aquestes funcions és característica del perfil neuropsicològic dels pacients amb PD i DLB. Pel que fa al declivi cognitiu al llarg de la malaltia, encara hi ha una gran incertesa. Els estudis longitudinals que inclouen dades de controls sans i pacients afectats d'IRBD, no han investigat de forma adequada l'efecte de l'envelliment en les pèrdues progressives de funcions cognitives.

En l'última dècada, la caracterització dels canvis cerebrals estructurals i funcionals en l'IRBD ha esdevingut un camp d'estudi emergent i amb progressió exponencial. La majoria de les investigacions en neuroimatge s'han focalitzat en l'estudi dels canvis metabòlics. Gràcies a aquests treballs s'ha palesat un dèficit dopaminèrgic a l'estriat, així com

alteracions metabòliques corticals i subcorticals que sugereixen un patró similar al descrit en la PD. Els estudis centrats en neuroimatge estructural en IRBD, han identificat anomalies en la substància blanca i la substància grisa (SG) en les regions que regulen el son REM, i que mostren patologia neurodegenerativa en fases inicials de la PD. En la mateixa línia, la neuroimatge funcional, posa de relleu l'alteració de la connectivitat en els ganglis basals i també en xarxes cortico-estriatals en els pacients amb IRBD. Malgrat això, no hi ha cap treball previ que hagi abordat l'estudi dels canvis funcionals de tot el cervell, sense focalitzar en circuits concrets. Pel que fa als canvis longitudinals, pocs estudis han investigat com la progressió de la malaltia afecta l'estructura i la funció del cervell, i com aquests canvis poden explicar el declivi cognitiu. Els treballs anteriors s'han centrat en biomarcadors metabòlics de pronòstic i només hi ha un estudi sobre la progressió de degeneració estructural. Malauradament, la manca de seguiment longitudinal de les ressonàncies magnètiques del grup control en aquest treball impedeix descartar els efectes de l'envelliment normal en els canvis cerebrals.

Hi ha una gran quantitat de literatura científica que demostra la relació entre les Sinucleinopaties i la disfunció de l'olfacte. L'alta prevalença d'hipòsmia i anòsmia en PD de recent diagnòstic, dona suport a l'afectació del bulb olfactori en els primers estadis de la malaltia, tal com proposa l'esquema de Braak. D'altra banda, l'evidència de disfunció olfactiva en les etapes premotors està recolzada per la presència d'hipòsmia en pacients amb IRBD, fins a 20 anys abans de l'aparició dels símptomes motors. La identificació de la disfunció olfactiva en la PD i l'IRBD és molt important ja que la presència d'hipòsmia pot diferenciar els pacients de les persones sanes d'edat similar. A més, les proves olfactives poden ser útils per distingir entre trastorns neurodegeneratius motors, així com per predir la conversió d'IRBD a parkinsonismes. La progressió dels dèficits olfactius en el transcurs de la malaltia encara no és prou clara. L'evidència prèvia suggereix que l'olfacte canvia només lleugerament durant el curs de la PD, però, atès que aquests treballs no van incloure un grup control, és difícil distingir la pèrdua relacionada amb la malaltia, d'aquells efectes produïts pel mateix envelliment. Millorar la precisió dels tests d'olfacte podria ajudar a identificar pacients amb un risc més elevat de conversió, i així definir poblacions que puguin ser objectiu per a assajos clínics adreçats a la prevenció de la malaltia.

Objectius i Hipòtesis

Els objectius principals d'aquesta Tesi són:

- 1 Caracteritzar els substrats cerebrals estructurals i funcionals subjacents a l'RBD, així com relacionar aquestes troballes amb el rendiment cognitiu,
- 2 Estudiar la disfunció olfactiva com a biomarcador clínic i preclínic de les alfa-Sinucleinopaties, i la seva progressió al llarg de la malaltia,
- 3 Investigar la degeneració cerebral progressiva al llarg de l'IRBD i com aquests canvis es relacionen amb el declivi cognitiu.

Les principals hipòtesis són:

- 1 L'IRBD es caracteritzarà per canvis específics en l'estructura cerebral i la connectivitat funcional que estarà associada a un deteriorament olfactiu i cognitiu,
- 2 Els pacients amb IRBD mostraran canvis cerebrals estructurals amb el pas del temps i un declivi cognitiu superior a l'observat en l'envelliment normal,
- 3 S'espera identificar reducció de la capacitat olfàctòria en l'IRBD i la PD, així com que progressi lleugerament amb el curs de la malaltia.

Material i mètodes

La present Tesi Doctoral consta de sis estudis realitzats per donar resposta als objectius esmenats anteriorment.

Els estudis inclouen mostres de pacients amb IRBD, PD i controls sans (CS). Concretament, les mostres 1, 2, i 4 consten de vint-i-un pacients amb IRBD i vint-i-set participants sans. A la mostra 3 es van incloure 97 pacients amb PD sense demència i 65 subjectes sans. A l'*estudi 5*, la mostra està formada per 14 IRBD i 18 subjectes sans, i la mostra de l'*estudi 6* va incloure 25 pacients amb PD i 24 controls sans. Als *estudis 5* i *6*, els subjectes es van avaluar dues vegades (interval entre avaluacions d'1.6 i 3.8 anys, respectivament).

Es va administrar una bateria exhaustiva de proves neuropsicològiques per als *estudis 1*, *2*, i *5*. L'olfacció es va valorar en els *estudis 3*, *4*, *5*, i *6*. Als *estudis 3* i *4*, es varen calcular mesures de precisió diagnòstica i el punt de tall òptim per a cada test olfactiu i per cada

condició (PD vs CS i IRBD vs CS, respectivament). A l'estudi 3 es va fer una anàlisi d'ítems del test UPSIT (*University of Pennsylvania Smell Identification Test*).

En els *estudis 1, 2, 4, 5, i 6* els participants van realitzar una exploració de ressonància magnètica (RM) adquirida en un escàner de 3 Tesla Siemens (magnetom Trio Tim scanner). Les seqüències d'adquisició estructurals potenciades en T1 es van utilitzar en els *estudis 1, 2, 4, 5, i 6* (els participants es van escanejar dues vegades en els *estudis 5 i 6*). En els *estudis 1, 4, 5, i 6*, es van utilitzar els *softwares* FSL i FreeSurfer per analitzar les imatges estructurals. Concretament, a l'*estudi 1* es va calcular el gruix cortical, es va realitzar un àlisis morfolòfic de l'hipocamp i es van obtenir les segmentacions subcorticals i de l'hipocamp. A l'*estudi 4*, es va utilitzar la morfometria basada en vòxel per estudiar la relació entre el deteriorament de l'olfacte i el volum de SG en el sistema olfactiu. En els *estudis 5 i 6*, es va dur a terme una anàlisi longitudinal del gruix cortical per estudiar la progressió de la SG en IRBD i PD. A l'*estudi 6* es va calcular el percentatge de canvi dels nuclis de l'estriat. Finalment, a l'*estudi 2* es van obtenir imatges de RM funcional en estat de repòs. Per tal de caracteritzar la connectivitat funcional del cervell sencer dels pacients amb IRBD, s'utilitzaren les tècniques *threshold-free network-based statistics* i anàlisis de graf.

Resultats

En els *estudis 1, 2 i 5*, els pacients amb IRBD es diferencien dels controls en diversos dominis cognitius, com ara, funcions executives i atenció, velocitat de processament de la informació, memòria verbal i fluència semàntica. A més, en els *estudis 1 i 5* es van identificar per primera vegada alteració en el reconeixement de cares i en les funcions visuoespacials. Al contrari del que havíem hipotetitzat en l'*estudi 1*, no s'han trobat correlacions significatives entre el rendiment cognitiu i l'atròfia cerebral. Malgrat això, hi ha coherència entre els canvis estructurals detectats per la RM i l'alteració neuropsicològica. Per exemple, els pacients amb IRBD tenen afectació del reconeixement de cares i atròfia cortical del gir fusiforme, una regió que és crítica per a aquesta funció. De forma similar, els pacients van mostrar alteració de memòria i atròfia de l'hipocamp, estructura bàsica per a l'aprenentatge i la memòria. D'altra banda, l'*estudi 2* va evidenciar el paper de la disrupció de la connectivitat funcional cerebral en el deteriorament cognitiu de l'IRBD. Es va trobar una correlació positiva entre la velocitat de processament de la

informació i la connectivitat funcional temporoparietal en el grup IRBD. A l'*estudi 5*, es va observar un empitjorament de la discriminació de les formes en els pacients amb IRBD en comparació amb l'envelliment normal en un breu període de menys dos anys. Més important encara, es va identificar que l'empitjorament en la capacitat de discriminar formes s'explicava, en part, per la reducció progressiva del gruix cortical de regions parietals superiors.

L'anàlisi de la imatge estructural de l'*estudi 1* va permetre identificar atròfia cortical en l'IRBD al parietal superior, al gir postcentral i al fusiforme esquerres, així com als còrtex frontal superior i lateral occipital drets. A l'*estudi 1*, l'anàlisi de la SG profunda va mostrar una reducció de l'hipocamp posterior dret en pacients amb IRBD, fet que es va confirmar mitjançant diferents tècniques. A l'*estudi 5*, es va trobar una degeneració cortical en el grup d'IRBD, en un breu període de temps. Les dades de ressonància magnètica van evidenciar canvis estructurals relacionats amb l'envelliment en l'IRBD i en controls amb atròfia en tots dos grups al precuneus esquerre i a les regions parietals inferiors, però amb una progressió frontal i parieto-occipital més important en els IRBDs. De fet, la interacció grup per temps confirma la major degeneració dels pacients amb IRBD, en les regions de l'escorça parietal superior i precuneus, en el cuneus dret, el pol occipital esquerra, i en un clúster localitzat en el gir lateral orbitofrontal i el pol frontal esquerres.

Pel que fa a les dades de RM funcional, l'*estudi 2* va demostrar que quan s'analitza el cervell com un tot, els pacients amb IRBD presenten disminució de la força de la connectivitat funcional en nodes cortico-corticals de l'escorça posterior. En resum, els nostres resultats van palesar una connectivitat funcional reduïda que involucrava regions associatives dels lòbuls temporals i parietals que se sap que suporten funcions cognitives complexes. Una anàlisi addicional, va evidenciar una important reducció de la connectivitat posterior en pacients amb IRBD, que contrastava amb una connectivitat preservada a la xarxa anterior. D'altra banda, l'anàlisi de graf va permetre identificar disminució de la centralitat d'un node situat al lòbul parietal superior esquerre.

Els *estudis 3 i 4* van permetre caracteritzar el deteriorament de la capacitat olfactiva tant en IRBD com en PD. La distribució del grau de pèrdua olfactiva en pacients amb IRBD en el primer moment temporal (*estudi 4*) segons les dades normatives de la UPSIT, va ser 43% de pacients anòsmics i 57% hipòsmics, mentre que, per a la PD (*estudi 6* dades

basals), el 36% dels pacients era anòsmic, 60 % hipòsmic i 4% normòsmic. En el nostre treball no es van comparar estadísticament els pacients amb IRBD i PD. Tot i així, cal destacar que la puntuació en l'UPSIT per a PD i IRBD a la línia de base va ser similar (20.6 ± 7.5 i 19.7 ± 6.6 , respectivament), així com el percentatge de pacients amb hipòsmia severa o anòsmia que, segons les dades normatives, era del 80% aproximadament en les dues mostres. Les dades de ressonància magnètica de l'*estudi 4* van demostrar que en els pacients amb IRBD anòsmics, un pitjor rendiment en la capacitat d'identificació olfactiva està relacionat amb la reducció de SG en les regions orbitofrontals. Mentre que, la pèrdua d'olfacte al llarg del temps en els pacients amb PD es va associar amb reducció del volum de l'estriat. D'altra banda, els *estudis 5 i 6* van permetre concloure que la pèrdua d'olor al llarg del temps en ambdues condicions era similar a la que es va trobar associada a l'envelliment normal.

En els *estudis 3 i 4*, es va investigar la validesa i precisió diagnòstica de les proves olfactives en PD i IRBD. A l'*estudi 3*, es va descriure que la puntuació de tall normativa per al criteri d'anòsmia (≤ 18) proposada pel manual de l'UPSIT té alta especificitat (100%) però baixa sensibilitat (54,6%) per a la PD. Els resultats indicaren que cal tenir en compte l'edat, de manera que quan es consideren punts de tall òptims segons els intervals d'edat, tant la sensibilitat com l'especificitat se situen per sobre del 80%. A més, l'anàlisi dels ítems va identificar que hi ha ítems de molta dificultat fins i tot per la població normal. Finalment, quan es comparen diferents funcions de l'olfacte (llindar, discriminació i identificació) a l'*estudi 4*, la identificació és la mesura que discrimina millor els pacients amb IRBD d'individus sans amb una precisió superior al 85%. Tant la prova d'identificació de Sniffin com l'UPSIT han demostrat alta sensibilitat i especificitat en la detecció del deteriorament de l'olfacte en pacients amb IRBD. Mentre que el rendiment en l'UPSIT sembla estar relacionat amb l'atròfia en regions olfactives, les puntuacions del test d'Identificació de l'*Sniffin*, s'associen a la severitat de la simptomatologia d'RBD.

Conclusions

La present Tesi Doctoral ha identificat nous dèficits cognitius en pacients amb IRBD i ha perfilar la seva progressió al llarg de l'evolució de la malaltia. En particular, el nostre treball ha demostrat la importància de les funcions VS/VP com a mesura capaç d'identificar els canvis cognitius a través del temps en l'IRBD i el seu potencial per

identificar aquells pacients amb neurodegeneració progressiva en el còrtex posterior. A més, hem descrit de forma exhaustiva les característiques de la disfunció olfactiva precoç i greu de l'IRBD i hem descrit per primera vegada els seus correlats neuroanatòmics. Les dades de neuroimatge d'aquesta Tesi assenyalen una implicació primerenca del còrtex temporal-occipital-parietal a l'IRBD. De forma addicional, el nostre estudi longitudinal ha demostrat que al llarg del temps els pacients amb IRBD desenvolupen una degeneració cerebral de les regions occipitoparietals i orbitofrontals, que coincideixen amb els canvis degeneratius descrits en PD i DLB. Les anomalies en aquestes regions també es posen de manifest per l'associació entre l'afectació cognitiva i els canvis cerebrals tant estructurals com funcionals.

L'atròfia cortical i la reducció en la connectivitat funcional cortico-cortical posa de manifest que ja hi ha una degeneració cortical i alteracions funcionals en els pacients amb IRBD abans que tinguin un diagnòstic de DLB, PD o MSA. Això suggereix que en les fases prodròmiques de la PD, la alfa-sinucleïna ja està afectant aquestes estructures més aviat del que seria esperable segons el model de degeneració de Braak, proposat a partir de dades neuropatològiques. L'atròfia i disfunció cortical podria explicar per què alguns pacients presenten alteració neuropsicològica abans de l'aparició dels signes motors. Esperem que la nostra recerca serveixi de base per a estudis posteriors multicèntrics que permetin confirmar les nostres troballes i identificar quins són els marcadors neuropsicològics i de neuroimatge òptims a efectes diagnòstics, pronòstics i d'eficàcia de futures teràpies neuroprotectores.

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References

- Aarsland, D., Brønnick, K., Larsen, J.P., Tysnes, O.B., Alves, G., 2009. Cognitive impairment in incident, untreated Parkinson disease: the Norwegian ParkWest study. *Neurology* 72, 1121–6. <https://doi.org/10.1212/01.wnl.0000338632.00552.cb>
- Adamowicz, D.H., Roy, S., Salmon, D.P., Galasko, D.R., Hansen, L.A., Masliah, E., Gage, F.H., 2017. Hippocampal α -Synuclein in Dementia with Lewy Bodies Contributes to Memory Impairment and Is Consistent with Spread of Pathology. *J. Neurosci.* 37, 1675–1684. <https://doi.org/10.1523/JNEUROSCI.3047-16.2016>
- Aguirre-Mardones, C., Iranzo, A., Vilas, D., Serradell, M., Gaig, C., Santamaría, J., Tolosa, E., 2015. Prevalence and timeline of nonmotor symptoms in idiopathic rapid eye movement sleep behavior disorder. *J. Neurol.* 262, 1568–1578. <https://doi.org/10.1007/s00415-015-7742-3>
- Albin, R.L., Koeppe, R.A., Chervin, R.D., Consens, F.B., Wernette, K., Frey, K.A., Aldrich, M.S., 2000. Decreased striatal dopaminergic innervation in REM sleep behavior disorder. *Neurology* 55, 1410 LP – 1412. <https://doi.org/10.1212/WNL.55.9.1410>
- American Psychiatric Association. (2013). *Diagnostic and statistical manual of mental disorders* (5th ed.). Washington, DC
- Anang, J.B.M., Gagnon, J.-F., Bertrand, J.-A., Romenets, S.R., Latreille, V., Panisset, M., Montplaisir, J., Postuma, R.B., 2014. Predictors of dementia in Parkinson disease. *Neurology* 83, 1253 LP – 1260. <https://doi.org/10.1212/WNL.0000000000000842>
- Anderson, M.J., Robinson, J., 2001. Permutation Tests for Linear Models. *Aust New Zeal J Stat Stat* 43, 75–88. <https://doi.org/10.1111/1467-842X.00156>
- Arnaldi, D., De Carli, F., Picco, A., Ferrara, M., Accardo, J., Bossert, I., Famà, F., Girtler, N., Morbelli, S., Sambuceti, G., Nobili, F., 2015. Nigro-caudate dopaminergic deafferentation: A marker of REM sleep behavior disorder? *Neurobiol. Aging* 36, 3300–3305. <https://doi.org/10.1016/j.neurobiolaging.2015.08.025>
- Arnulf, I., 2012. REM sleep behavior disorder: Motor manifestations and pathophysiology. *Mov. Disord.* 27, 677–689. <https://doi.org/10.1002/mds.24957>
- Baba, T., Kikuchi, A., Hirayama, K., Nishio, Y., Hosokai, Y., Kanno, S., Hasegawa, T., Sugeno, N., Konno, M., Suzuki, K., Takahashi, S., Fukuda, H., Aoki, M., Itoyama, Y., Mori, E., Takeda, A., 2012. Severe olfactory dysfunction is a prodromal symptom of dementia associated with Parkinson's disease: a 3 year longitudinal study. *Brain* 135, 161–9. <https://doi.org/10.1093/brain/awr321>
- Bae, Y.J., Kim, J.M., Kim, K.J., Kim, E., Park, H.S., Kang, S.Y., Yoon, I.Y., Lee, J.Y., Jeon, B., Kim, S.E., 2018. Loss of Substantia Nigra Hyperintensity at 3.0-T MR Imaging in Idiopathic REM Sleep Behavior Disorder: Comparison with 123I-FP-CIT SPECT. *Radiology* 287, 285–293.
- Baggio, H.C., Abos, A., Segura, B., Campabadal, A., Garcia-Diaz, A., Uribe, C., Compta, Y., Marti, M.J., Valldeoriola, F., Junque, C., 2018. Statistical inference in brain graphs using threshold-free network-based statistics. *Hum. Brain Mapp.* 39. <https://doi.org/10.1002/hbm.24007>
- Baggio, H.C., Segura, B., Ibarretxe-Bilbao, N., Valldeoriola, F., Marti, M.J., Compta, Y., Tolosa, E., Junque, C., 2012. Structural correlates of facial emotion recognition deficits in Parkinson's disease patients. *Neuropsychologia* 50, 2121–8. <https://doi.org/10.1016/j.neuropsychologia.2012.05.020>
- Barber, T.R., Lawton, M., Rolinski, M., Evetts, S., Baig, F., Ruffmann, C., Gornall, A., Klein, J.C., Lo, C., Dennis, G., Bandmann, O., Quinnell, T., Zaiwalla, Z., Ben-Shlomo, Y., Hu, M.T.M., 2017. Prodromal Parkinsonism and Neurodegenerative Risk Stratification in REM Sleep Behavior Disorder. *Sleep* 40, 11–13. <https://doi.org/10.1093/sleep/zsx071>
- Barrett, M.J., Wylie, S.A., Harrison, M.B., Wooten, G.F., 2011. Handedness and motor symptom asymmetry in Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 82, 1122–1124. <https://doi.org/10.1136/jnnp.2010.209783>

- Bauckneht, M., Chincari, A., De Carli, F., Terzaghi, M., Morbelli, S., Nobili, F., Arnaldi, D., 2018. Presynaptic dopaminergic neuroimaging in REM sleep behavior disorder: A systematic review and meta-analysis. *Sleep Med. Rev.* 41, 266–274. <https://doi.org/10.1016/j.smr.2018.04.001>
- Beach, T.G., Adler, C.H., Lue, L.F., Sue, L.I., Bachalakuri, J., Henry-Watson, J., Sasse, J., Boyer, S., Shirohi, S., Brooks, R., Eschbacher, J., White, C.L., Akiyama, H., Caviness, J., Shill, H.A., Connor, D.J., Sabbagh, M.N., Walker, D.G., 2009a. Unified staging system for Lewy body disorders: Correlation with nigrostriatal degeneration, cognitive impairment and motor dysfunction. *Acta Neuropathol.* 117, 613–634. <https://doi.org/10.1007/s00401-009-0538-8>
- Beach, T.G., White, C.L., Hladik, C.L., Sabbagh, M.N., Connor, D.J., Shill, H.A., Sue, L.I., Sasse, J., Bachalakuri, J., Henry-Watson, J., Akiyama, H., Adler, C.H., 2009b. Olfactory bulb α -synucleinopathy has high specificity and sensitivity for Lewy body disorders. *Acta Neuropathol.* 117, 169–174. <https://doi.org/10.1007/s00401-008-0450-7>
- Beck, A., Steer, R., B.G., 1996. Manual for the Beck Depression Inventory-II. Psychological Corporation, San Antonio, Texas.
- Bedard, M.A., Aghourian, M., Legault-Denis, C., Postuma, R.B., Soucy, J.P., Gagnon, J.F., Pelletier, A., Montplaisir, J., 2019. Brain cholinergic alterations in idiopathic REM sleep behaviour disorder: a PET imaging study with 18 F-FEOBV. *Sleep Med.* 58, 35–41. <https://doi.org/10.1016/j.sleep.2018.12.020>
- Benjamini, Y. Discovering the false discovery rate. *J. R. Stat. Soc. Ser. B (Statistical Methodol.)* 72, 405–416 (2010).
- Benton, A., Sivan, A., Hamsher, K., 1994. Contributions to neuropsychological assessment. A clinical manual, 2nd ed. New York.
- Benton, A., Varney, N., Hamsher, K., 1978. Visuospatial judgment. A clinical test. *Arch. Neurol.* 364–367.
- Berendse, H.W., Ponsen, M.M., 2009. Diagnosing premotor Parkinson's disease using a two-step approach combining olfactory testing and DAT SPECT imaging. *Park. Relat. Disord.* 15, S26–S30. [https://doi.org/10.1016/S1353-8020\(09\)70774-6](https://doi.org/10.1016/S1353-8020(09)70774-6)
- Berendse, H.W., Roos, D.S., Raijmakers, P., Doty, R.L., 2011. Motor and non-motor correlates of olfactory dysfunction in Parkinson's disease. *J. Neurol. Sci.* 310, 21–24. <https://doi.org/10.1016/j.jns.2011.06.020>
- Berg, D., Hochstrasser, H., 2006. Iron metabolism in parkinsonian syndromes. *Mov. Disord.* 21, 1299–1310. <https://doi.org/10.1002/mds.21020>
- Berg, D., Postuma, R.B., Adler, C.H., Bloem, B.R., Chan, P., Dubois, B., Gasser, T., Goetz, C.G., Halliday, G., Joseph, L., Lang, A.E., Liepelt-Scarf, I., Litvan, I., Marek, K., Obeso, J., Oertel, W., Olanow, C.W., Poewe, W., Stern, M., Deuschl, G., 2015. MDS research criteria for prodromal Parkinson's disease. *Mov. Disord.* 30, 1600–1611. <https://doi.org/10.1002/mds.26431>
- Bertrand, J.A., Bedetti, C., Postuma, R.B., Monchi, O., Génier Marchand, D., Jubault, T., Gagnon, J.F., 2012. Color discrimination deficits in Parkinson's disease are related to cognitive impairment and white-matter alterations. *Mov. Disord.* 27, 1781–1788. <https://doi.org/10.1002/mds.25272>
- Bezdicek, O., Nikolai, T., Nepožitek, J., Peřinová, P., Kemlink, D., Dušek, Pavel, Přihodová, I., Dostálová, S., Ibarburu, V., Trnka, J., Kupka, K., Mecková, Z., Keller, J., Vymazal, J., Růžička, E., Šonka, K., Dušek, Petr, 2018. Prospective memory impairment in idiopathic REM sleep behavior disorder. *Clin. Neuropsychol.* 32, 1019–1037. <https://doi.org/10.1080/13854046.2017.1394493>

- Biederman, I., Cooper, E.E., 1991. Priming contour-deleted images: Evidence for intermediate representations in visual object recognition. *Cogn. Psychol.* 23, 393–419. [https://doi.org/10.1016/0010-0285\(91\)90014-F](https://doi.org/10.1016/0010-0285(91)90014-F)
- Bijsterbosch, J., Smith, S., Beckmann, C., 2017. Introduction to Resting State fMRI Functional Connectivity, First. ed. Oxford University Press, Oxford.
- Bjørnarå, K.A., Dietrichs, E., Toft, M., 2013. REM sleep behavior disorder in Parkinson's disease - Is there a gender difference? *Park. Relat. Disord.* 19, 120–122. <https://doi.org/10.1016/j.parkreldis.2012.05.027>
- Blanc, F., Colloby, S.J., Philippi, N., De Pétigny, X., Jung, B., Demuynck, C., Phillipps, C., Anthony, P., Thomas, A., Bing, F., Lamy, J., Martin-Hunyadi, C., O'Brien, J.T., Cretin, B., McKeith, I., Armspach, J.P., Taylor, J.P., 2015. Cortical thickness in dementia with lewy bodies and alzheimer's disease: A comparison of prodromal and dementia stages. *PLoS One* 10, 1–18. <https://doi.org/10.1371/journal.pone.0127396>
- Bodkin, C.L., Schenck, C.H., 2009. Rapid eye movement sleep behavior disorder in women: relevance to general and specialty medical practice. *J. Womens. Health (Larchmt)*. 18, 1955–1963. <https://doi.org/10.1089/jwh.2008.1348>
- Boeve, B.F., Dickson, D.W., Olson, E.J., Shepard, J.W., Silber, M.H., Ferman, T.J., Ahlskog, J.E., Benarroch, E.E., 2007. Insights into REM sleep behavior disorder pathophysiology in brainstem-predominant Lewy body disease. *Sleep Med.* 8, 60–64. <https://doi.org/10.1016/j.sleep.2006.08.017>
- Boeve, B.F., Molano, J.R., Ferman, T.J., Smith, G.E., Lin, S.C., Bieniek, K., Haidar, W., Tippmann-Peikert, M., Knopman, D.S., Graff-Radford, N.R., Lucas, J.A., Petersen, R.C., Silber, M.H., 2011. Validation of the Mayo Sleep Questionnaire to screen for REM sleep behavior disorder in an aging and dementia cohort. *Sleep Med.* 12, 445–453. <https://doi.org/10.1016/j.sleep.2010.12.009>
- Boeve, B.F., Silber, M.H., Ferman, T.J., Lin, S.C., Benarroch, E.E., Schmeichel, A.M., Ahlskog, J.E., Caselli, R.J., Jacobson, S., Sabbagh, M., Adler, C., Woodruff, B., Beach, T.G., Iranzo, A., Gelpi, E., Santamaria, J., Tolosa, E., Singer, C., Mash, D.C., Luca, C., Arnulf, I., Duyckaerts, C., Schenck, C.H., Mahowald, M.W., Dauvilliers, Y., Graff-Radford, N.R., Wszolek, Z.K., Parisi, J.E., Dugger, B., Murray, M.E., Dickson, D.W., 2013. Clinicopathologic correlations in 172 cases of rapid eye movement sleep behavior disorder with or without a coexisting neurologic disorder. *Sleep Med.* 14, 754–762. <https://doi.org/10.1016/j.sleep.2012.10.015>
- Borroni, B., Premi, E., Formenti, A., Turrone, R., Alberici, A., Cottini, E., Rizzetti, C., Gasparotti, R., Padovani, A., 2015. Structural and functional imaging study in dementia with Lewy bodies and Parkinson's disease dementia. *Parkinsonism Relat. Disord.* 21, 1049–1055. <https://doi.org/10.1016/j.parkreldis.2015.06.013>
- Bourgouin, P., Rahayel, S., Gaubert, M., Postuma, R.B., 2018. Parkinsonism and Related Disorders Gray matter substrates of depressive and anxiety symptoms in idiopathic REM sleep behavior disorder. *Park. Relat. Disord.* 0–1. <https://doi.org/10.1016/j.parkreldis.2018.12.020>
- Braak, H., Tredici, K. Del, Rüb, U., de Vos, R. a. ., Jansen Steur, E.N., Braak, E., 2003. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiol. Aging* 24, 197–211. [https://doi.org/10.1016/S0197-4580\(02\)00065-9](https://doi.org/10.1016/S0197-4580(02)00065-9)
- Brown, R.K.J., Bohnen, N.I., Wong, K.K., Minoshima, S., Frey, K.A., 2014. Brain PET in suspected dementia: Patterns of altered FDG metabolism. *Radiographics* 34, 684–701. <https://doi.org/10.1148/rg.343135065>
- Bullmore, E., Sporns, O., 2009. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat. Rev. Neurosci.* 10, 186–98. <https://doi.org/10.1038/nrn2575>

- Büttner, T., Kuhn, W., Müller, T., Patzold, T., Heidbrink, K., Przuntek, H., 1995. Distorted color discrimination in “de novo” parkinsonian patients. *Neurology* 45, 386–387. <https://doi.org/10.1212/wnl.45.2.386>
- Byun, J.-I., Kim, H.-W., Kang, H., Cha, K.S., Sunwoo, J.-S., Shin, J.-W., Moon, J., Lee, S.-T., Jung, K.-H., Chu, K., Kim, M.-H., Shin, W.C., Lee, D.S., Schenck, C.H., Lee, S.K., Jung, K.-Y., 2020. Altered resting-state thalamo-occipital functional connectivity is associated with cognition in isolated rapid eye movement sleep behavior disorder. *Sleep Med.* 69, 198–203. <https://doi.org/10.1016/j.sleep.2020.01.010>
- Chen, S., Tan, H., Wu, Z., Sun, C., He, J., Li, X., Shao, M., 2014. Imaging of olfactory bulb and gray matter volumes in brain areas associated with olfactory function in patients with Parkinson’s disease and multiple system atrophy. *Eur. J. Radiol.* 83, 564–70. <https://doi.org/10.1016/j.ejrad.2013.11.024>
- Chow, N., Aarsland, D., Honarpisheh, H., Beyer, M.K., Somme, J.H., Elashoff, D., Rongve, A., Tysnes, O.B., Thompson, P.M., Apostolova, L.G., 2012. Comparing hippocampal atrophy in alzheimer’s dementia and dementia with lewy bodies. *Dement. Geriatr. Cogn. Disord.* 34, 44–50. <https://doi.org/10.1159/000339727>
- Cochen De Cock, V., Debs, R., Oudiette, D., Leu, S., Radji, F., Tiberge, M., Yu, H., Bayard, S., Roze, E., Vidailhet, M., Dauvilliers, Y., Rascol, O., Arnulf, I., 2011. The improvement of movement and speech during rapid eye movement sleep behaviour disorder in multiple system atrophy. *Brain* 134, 856–862. <https://doi.org/10.1093/brain/awq379>
- Cramer, C.K., Friedman, J.H., Amick, M.M., 2010. Olfaction and apathy in Parkinson’s disease. *Park. Relat. Disord.* 16, 124–126. <https://doi.org/10.1016/j.parkreldis.2009.09.004>
- Cummings, J.L., Mega, M., Gray, K., Rosenberg-Thompson, S., Carusi, D.A., Gornbein, J., 1994. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. *Neurology* 44, 2308–2314.
- Damholdt, M.F., Borghammer, P., Larsen, L., Østergaard, K., 2011. Odor identification deficits identify Parkinson’s disease patients with poor cognitive performance. *Mov. Disord.* 26, 2045–2050. <https://doi.org/10.1002/mds.23782>
- Dang-Vu, T.T., Gagnon, J.F., Vendette, M., Soucy, J.P., Postuma, R.B., Montplaisir, J., 2012. Hippocampal perfusion predicts impending neurodegeneration in REM sleep behavior disorder. *Neurology* 79, 2302–2306. <https://doi.org/10.1212/WNL.0b013e318278b658>
- Dayan, E., Browner, N., 2017. Alterations in striato-thalamo-pallidal intrinsic functional connectivity as a prodrome of Parkinson’s disease. *NeuroImage Clin.* 16, 313–318. <https://doi.org/10.1016/j.nicl.2017.08.003>
- Deeb, J., Shah, M., Muhammed, N., Gunasekera, R., Gannon, K., Findley, L.J., Hawkes, C.H., 2010. A basic smell test is as sensitive as a dopamine transporter scan: Comparison of olfaction, taste and DaTSCAN in the diagnosis of Parkinson’s disease. *Qjm* 103, 941–952. <https://doi.org/10.1093/qjmed/hcq142>
- Delazer, M., Högl, B., Zamarian, L., Wenter, J., Ehrmann, L., Gschliesser, V., Brandauer, E., Poewe, W., Frauscher, B., 2012. Decision Making and Executive Functions in REM Sleep Behavior Disorder 35, 667–673. <https://doi.org/http://dx.doi.org/10.5665/sleep.1828>
- Delli Pizzi, S., Franciotti, R., Bubbico, G., Thomas, A., Onofri, M., Bonanni, L., 2016. Atrophy of hippocampal subfields and adjacent extrahippocampal structures in dementia with Lewy bodies and Alzheimer’s disease. *Neurobiol. Aging* 40, 103–109. <https://doi.org/10.1016/j.neurobiolaging.2016.01.010>
- Delli Pizzi, S., Franciotti, R., Tartaro, A., Caulo, M., Thomas, A., Onofri, M., Bonanni, L., 2014. Structural Alteration of the Dorsal Visual Network in DLB Patients with Visual Hallucinations:

- A Cortical Thickness MRI Study. *PLoS One* 9, e86624. <https://doi.org/10.1371/journal.pone.0086624>
- De Marzi, R., Seppi, K., Högl, B., Müller, C., Scherfler, C., Stefani, A., Iranzo, A., Tolosa, E., Santamaria, J., Gizewski, E., Schocke, M., Skalla, E., Kremser, C., Poewe, W., 2016. Loss of dorsolateral nigral hyperintensity on 3.0 tesla susceptibility-weighted imaging in idiopathic rapid eye movement sleep behavior disorder. *Ann. Neurol.* 79, 1026–1030. <https://doi.org/10.1002/ana.24646>
- Doty, R.L., 2012a. Olfactory dysfunction in parkinson disease. *Nat. Publ. Gr.* 8, 329–339. <https://doi.org/10.1038/nrneurol.2012.80>
- Doty, R.L., Bromley, S.M., Stern, M.B., 1995. Olfactory Testing as an Aid in the Diagnosis of Parkinson's Disease: Development of Optimal Discrimination Criteria. *Neurodegeneration* 4, 93–97. <https://doi.org/10.1006/neur.1995.0011>
- Doty, R.L., Golbe, L., McKeown, D., Stern, M., Lehrach, C., Crawford, D., 1993. Olfactory testing differentiates between progressive supranuclear palsy and idiopathic Parkinson's disease. *Neurology* 43, 962–965.
- Doty, R.L., Kamath, V., 2014. The influences of age on olfaction: a review. *Front Psychol.* 5, <https://doi.org/10.3389/fpsyg.2014.00020>
- Doty, R.L., Stern, M., Pfeiffer, C., Gollomp, S., Hurtig, H., 1992. Bilateral olfactory dysfunction in early stage treated and untreated idiopathic Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 55, 138–142. <https://doi.org/10.1136/jnnp.55.2.138>
- Doty, R.L., 2012b. Olfaction in Parkinson's disease and related disorders. *Neurobiol. Dis.* 46, 527–552. <https://doi.org/10.1016/j.nbd.2011.10.026>
- Doty, R.L., 1995. *The Smell Identification Test. Administration Manual*, 3rd ed. Sensonics, Haddon Hts.
- Doty, R.L., Deems, D., Stellar, S., 1988. Olfactory dysfunction in parkinsonism: a general deficit unrelated to neurologic signs, disease stage, or disease duration. *Neurology* 38, 1237–1244.
- Doty, R.L., Ford, M., Preti, G., Huggins, G.R., 1975. Changes in the intensity and pleasantness of human vaginal odors during the menstrual cycle. *Science* 190, 1316–8.
- Doty, R.L., Green, P.A., Ram, C., Yankell, S.L., 1982. Communication of gender from human breath odors: Relationship to perceived intensity and pleasantness. *Horm. Behav.* 16, 13–22. [https://doi.org/10.1016/0018-506X\(82\)90002-2](https://doi.org/10.1016/0018-506X(82)90002-2)
- Doty, R.L., Orndorff, M.M., Leyden, J., Kligman, A., 1978. Communication of gender from human axillary odors: relationship to perceived intensity and hedonicity. *Behav. Biol.* 23, 373–80.
- Douaud, G., Smith, S., Jenkinson, M., Behrens, T., Johansen-Berg, H., Vickers, J., James, S., Voets, N., Watkins, K., Matthews, P.M., James, A., 2007. Anatomically related grey and white matter abnormalities in adolescent-onset schizophrenia. *Brain* 130, 2375–2386. <https://doi.org/10.1093/brain/awm184>
- Double, K.L., Rowe, D.B., Hayes, M., Chan, D.K.Y., Blackie, J., Corbett, A., Joffe, R., Fung, V.S., Morris, J., Halliday, G.M., 2003. Identifying the pattern of olfactory deficits in Parkinson disease using the brief smell identification test. *Arch. Neurol.* 60, 545–549. <https://doi.org/10.1001/archneur.60.4.545>
- Dubois, B., Burn, D., Goetz, C., Aarsland, D., Brown, R.G., Broe, G.A., Dickson, D., Duyckaerts, C., Cummings, J., Gauthier, S., Korczyn, A., Lees, A., Levy, R., Litvan, I., Mizuno, Y., McKeith, I.G., Olanow, C.W., Poewe, W., Sampaio, C., Tolosa, E., Emre, M., 2007. Diagnostic procedures for Parkinson's disease dementia: recommendations from the movement disorder society task force. *Mov. Disord.* 22, 2314–24. <https://doi.org/10.1002/mds.21844>
- Dušek, P., Ibarburu, V.L. y. L., Bezdicek, O., Dall'antonia, I., Dostálová, S., Kovalská, P., Krupička, R., Nepožitek, J., Nikolai, T., Novotný, M., Peřinová, P., Rusz, J., Serranová, T., Tykalová, T.,

- Ulmanová, O., Mecková, Z., Ptáčník, V., Trnka, J., Zogala, D., Růžička, E., Šonka, K., 2019. Relations of non-motor symptoms and dopamine transporter binding in REM sleep behavior disorder. *Sci. Rep.* 9, 1–10. <https://doi.org/10.1038/s41598-019-51710-y>
- Ehrminger, M., Latimier, A., Pyatigorskaya, N., Garcia-Lorenzo, D., Leu-Semenescu, S., Vidailhet, M., Lehericy, S., Arnulf, I., 2016. The coeruleus/subcoeruleus complex in idiopathic rapid eye movement sleep behaviour disorder. *Brain* 139, 1180–1188. <https://doi.org/10.1093/brain/aww006>
- Eidelberg, D., 2009. Metabolic brain networks in neurodegenerative disorders: a functional imaging approach. *Trends Neurosci.* 32, 548–557. <https://doi.org/10.1016/j.tins.2009.06.003>
- Eidelberg, D., Moeller, J.R., Dhawan, V., Spetsieris, P., Takikawa, S., Ishikawa, T., Chaly, T., Robeson, W., Margouleff, D., Przedborski, S., Fahn, S., 1994. The metabolic topography of Parkinsonism. *J. Cereb. Blood Flow Metab.* 14, 783–801. <https://doi.org/10.1038/jcbfm.1994.99>
- Eisensehr, I., Linke, R., Noachtar, S., Schwarz, J., Gildehaus, F.J., Tatsch, K., 2000. Reduced striatal dopamine transporters in idiopathic rapid eye movement sleep behaviour disorder. Comparison with Parkinson's disease and controls. *Brain* 123, 1155–1160. <https://doi.org/10.1093/brain/123.6.1155>
- Eisensehr, I., Linke, R., Tatsch, K., Kharraz, B., Gildehaus, J.F., Wetter, C.T., Trenkwalder, C., Schwarz, J., Noachtar, S., 2003. Increased muscle activity during rapid eye movement sleep correlates with decrease of striatal presynaptic dopamine transporters. IPT and IBZM SPECT imaging in subclinical and clinically manifest idiopathic REM sleep behavior disorder, Parkinson's disease. *Sleep* 26, 507–512. <https://doi.org/10.1093/sleep/26.5.507>
- Elmore, T.M., Castriotta, R.J., Hendley, K.L., Aalbers, B.M., Furr-Stimming, E., Hood, A.J., Suescun, J., Beurlot, M.R., Hendley, R.T., Schiess, M.C., 2013. Altered nigrostriatal and nigrocortical functional connectivity in rapid eye movement sleep behavior disorder. *Sleep* 36, 1885–1892. <https://doi.org/10.5665/sleep.3222>
- Elmore, T. M., Hood, A.J., Castriotta, R.J., Stimming, E.F., Bick, R.J., Schiess, M.C., 2010a. Reduced volume of the putamen in REM sleep behavior disorder patients. *Park. Relat. Disord.* 16, 645–649. <https://doi.org/10.1016/j.parkreldis.2010.08.014>
- Elmore, T. M., Hood, A.J., Castriotta, R.J., Stimming, E.F., Bick, R.J., Schiess, M.C., 2010b. Reduced volume of the putamen in REM sleep behavior disorder patients. *Park. Relat. Disord.* 16, 645–649. <https://doi.org/10.1016/j.parkreldis.2010.08.014>
- Ezzati, A., Katz, M.J., Zammit, A.R., Lipton, M.L., Zimmerman, M.E., Sliwinski, M.J., Lipton, R.B., Korey, S.B., 2016. Differential association of left and right hippocampal volumes with verbal episodic and spatial memory in older adults HHS Public Access. *Neuropsychologia* 93, 380–385. <https://doi.org/10.1016/j.neuropsychologia.2016.08.016>
- Fahn, S., Elton, R., 1987. UPDRS Program Member Unified Parkinson's Disease Rating Scale, in: Fahn, S, Marsden, CD, Goldstein, M, Calne, D. (Ed.), *Recent Developments in Parkinson's Disease*, Vol. 2. Macmillan Healthcare Information, Florham Park, NJ, pp. 153-163,293-304.
- Fan, L., Li, H., Zhuo, J., Zhang, Y., Wang, J., Chen, L., Yang, Z., Chu, C., Xie, S., Laird, A.R., Fox, P.T., Eickhoff, S.B., Yu, C., Jiang, T., 2016. The Human Brainnetome Atlas: A New Brain Atlas Based on Connectional Architecture. *Cereb. Cortex* 26, 3508–3526. <https://doi.org/10.1093/cercor/bhw157>
- Fantini, M.L., Farini, E., Ortelli, P., Zucconi, M., Manconi, M., Cappa, S., Ferini-Strambi, L., 2011. Longitudinal study of cognitive function in idiopathic REM sleep behavior disorder. *Sleep* 34, 619–625. <https://doi.org/10.1093/sleep/34.5.619>
- Fantini, M.L., Postuma, R.B., Montplaisir, J., Ferini-Strambi, L., 2006. Olfactory deficit in idiopathic rapid eye movements sleep behavior disorder. *Brain Res. Bull.* 70, 386–390. <https://doi.org/10.1016/j.brainresbull.2006.07.008>

- Fenelon, G., 2000. Hallucinations in Parkinson's disease: Prevalence, phenomenology and risk factors. *Brain* 123, 733–745. <https://doi.org/10.1093/brain/123.4.733>
- Fereshtehnejad, S.M., Yao, C., Pelletier, A., Montplaisir, J.Y., Gagnon, J.F., Postuma, R.B., 2019. Evolution of prodromal Parkinson's disease and dementia with Lewy bodies: A prospective study. *Brain* 142, 2051–2067. <https://doi.org/10.1093/brain/awz111>
- Ferini-Strambi, L.F., Gioia, M.R. Di, Castronovo, V., Oldani, A., Zucconi, M., Cappa, S.F., 2004. Neuropsychological assessment in idiopathic REM sleep behavior disorder (RBD) Does the idiopathic form of RBD really exist? *Neurology* 62, 41-45.
- Ferman, T.J., Boeve, B.F., Smith, G.E., Lin, S.C., Silber, M.H., Pedraza, O., Wszolek, Z., Graff-Radford, N.R., Uitti, R., Van Gerpen, J., Pao, W., Knopman, D., Pankratz, V.S., Kantarci, K., Boot, B., Parisi, J.E., Dugger, B.N., Fujishiro, H., Petersen, R.C., Dickson, D.W., 2011. Inclusion of RBD improves the diagnostic classification of dementia with Lewy bodies. *Neurology* 77, 875–882. <https://doi.org/10.1212/WNL.0b013e31822c9148>
- Fernández-Santiago, R., Iranzo, A., Gaig, C., Serradell, M., Fernández, M., Tolosa, E., Santamaría, J., Ezquerra, M., 2016. Absence of LRRK2 mutations in a cohort of patients with idiopathic REM sleep behavior disorder. *Neurology* 86, 1072 LP – 1073. <https://doi.org/10.1212/WNL.0000000000002304>
- Fischl, B., Dale, A.M., 2000. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc. Natl. Acad. Sci. U.S.A.* 97, 11050–5. <https://doi.org/10.1073/pnas.200033797>
- Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J. Psychiatr. Res.* 12, 189–198.
- Forn, C., Belloch, V., Bustamante, J.C., Garbin, G., Parcet-Ibars, M.À., Sanjuan, A., Ventura, N., Ávila, C., 2009. A Symbol Digit Modalities Test version suitable for functional MRI studies. *Neurosci. Lett.* 456, 11–14. <https://doi.org/10.1016/j.neulet.2009.03.081>
- Frauscher, B., Ehrmann, L., Zamarian, L., Auer, F., Mitterling, T., Gabelia, D., Brandauer, E., Delazer, M., Poewe, W., Högl, B., 2012. Validation of the Innsbruck REM sleep behavior disorder inventory. *Mov. Disord.* 27, 1673–1678. <https://doi.org/10.1002/mds.25223>
- Frye, R., Schwartz, B., Doty, R., 1990. Dose-related effects of cigarette smoking on olfactory function. *JAMA J. Am. Med. Assoc.* 263, 1233–1236.
- Fullard, M.E., Morley, J.F., Duda, J.E., 2017. Olfactory Dysfunction as an Early Biomarker in Parkinson's Disease. *Neurosci. Bull.* 33, 515–525. <https://doi.org/10.1007/s12264-017-0170-x>
- Fullard, M.E., Tran, B., Xie, S.X., Toledo, J.B., Scordia, C., Linder, C., Purri, R., Weintraub, D., Duda, J.E., Chahine, L.M., Morley, J.F., 2016. Olfactory impairment predicts cognitive decline in early Parkinson's disease. *Park. Relat. Disord.* 25, 45–51. <https://doi.org/10.1016/j.parkreldis.2016.02.013>
- Gagnon, J.-F., Vendette, M., Postuma, R.B., Desjardins, C., Jessica Massicotte-Marquez, Panisset, M., Montplaisir, J., 2009. Mild Cognitive Impairment in Rapid Eye Movement Sleep Behavior Disorder and Parkinson's Disease. *Ann. Neurol.* 66, 39–47. <https://doi.org/https://doi.org/10.1002/ana.21680>
- Galbiati, A., Carli, G., Dodich, A., Marelli, S., Caterina, P., Cerami, C., Zucconi, M., Ferini-Strambi, L., 2019a. Qualitative scoring of the pentagon test: A tool for the identification of subtle cognitive deficits in isolated rem sleep behavior disorder patients. *Arch. Clin. Neuropsychol.* 34, 1113–1120. <https://doi.org/10.1093/arclin/acz024>

- Galbiati, A., Verga, L., Giora, E., Zucconi, M., Ferini-Strambi, L., 2019b. The risk of neurodegeneration in REM sleep behavior disorder: A systematic review and meta-analysis of longitudinal studies. *Sleep Med. Rev.* 43, 37–46. <https://doi.org/10.1016/j.smrv.2018.09.008>
- Gan-Or, Z., Mirelman, A., Postuma, R.B., Arnulf, I., Bar-Shira, A., Dauvilliers, Y., Desautels, A., Gagnon, J.-F., Leblond, C.S., Frauscher, B., Alcalay, R.N., Saunders-Pullman, R., Bressman, S.B., Marder, K., Monaca, C., Högl, B., Orr-Urtreger, A., Dion, P.A., Montplaisir, J.Y., Giladi, N., Rouleau, G.A., 2015. GBA mutations are associated with Rapid Eye Movement Sleep Behavior Disorder. *Ann. Clin. Transl. Neurol.* 2, 941–945. <https://doi.org/10.1002/acn3.228>
- Garcia-Diaz, A.I., Segura, B., Baggio, H.C., Marti, M.J., Valldeoriola, F., Compta, Y., Bargallo, N., Uribe, C., Campabadal, A., Abos, A., Junque, C., 2018a. Structural Brain Correlations of Visuospatial and Visuo-perceptual Tests in Parkinson's Disease. *J. Int. Neuropsychol. Soc.* 24, 33–44. <https://doi.org/10.1017/S1355617717000583>
- Garcia-Diaz, A.I., Segura, B., Baggio, H.C., Marti, M.J., Valldeoriola, F., Compta, Y., Vendrell, P., Bargallo, N., Tolosa, E., Junque, C., 2014. Structural MRI correlates of the MMSE and pentagon copying test in Parkinson's disease. *Parkinsonism Relat. Disord.* 20, 1405–1410. <https://doi.org/10.1016/j.parkreldis.2014.10.014>
- Garcia-Diaz, A.I., Segura, B., Baggio, H.C., Uribe, C., Campabadal, A., Abos, A., Marti, M.-J., Valldeoriola, F., Compta, Y., Bargallo, N., Junque, C., 2018b. Cortical thinning correlates of changes in visuospatial and visuo-perceptual performance in Parkinson's disease: A 4-year follow-up. *Parkinsonism Relat. Disord.* 46, 62–68. <https://doi.org/10.1016/j.parkreldis.2017.11.003>
- García-Lorenzo, D., Longo-Dos Santos, C., Ewencyk, C., Leu-Semenescu, S., Gallea, C., Quattrocchi, G., Pita Lobo, P., Poupon, C., Benali, H., Arnulf, I., Vidailhet, M., Lehericy, S., 2013. The coeruleus/subcoeruleus complex in rapid eye movement sleep behaviour disorders in Parkinson's disease. *Brain* 136, 2120–2129. <https://doi.org/10.1093/brain/awt152>
- Ge, J., Wu, P., Peng, S., Yu, H., Zhang, H., Guan, Y., Eidelberg, D., Zuo, C., Ma, Y., Wang, J., 2015. Assessing cerebral glucose metabolism in patients with idiopathic rapid eye movement sleep behavior disorder. *J. Cereb. Blood Flow Metab.* 35, 2062–2069. <https://doi.org/10.1038/jcbfm.2015.173>
- Génier Marchand, D., Montplaisir, J., Postuma, R.B., Rahayel, S., Gagnon, J.F., 2017. Detecting the cognitive prodrome of dementia with lewy bodies: A prospective study of REM sleep behavior disorder. *Sleep* 40. <https://doi.org/10.1093/sleep/zsw014>
- Génier Marchand, D., Postuma, R.B., Escudier, F., De Roy, J., Pelletier, A., Montplaisir, J., Gagnon, J.-F., 2018. How does dementia with Lewy bodies start? prodromal cognitive changes in REM sleep behavior disorder. *Ann. Neurol.* 83, 1016–1026. <https://doi.org/10.1002/ana.25239>
- Gnanalingham, K.K., Byrne, E.J., Thornton, A., 1996. Clock-face drawing to differentiate Lewy body and Alzheimer type dementia syndromes. *Lancet* (London, England). [https://doi.org/10.1016/s0140-6736\(96\)91254-4](https://doi.org/10.1016/s0140-6736(96)91254-4)
- Goetz, C.G., Tilley, B.C., Shaftman, S.R., Stebbins, G.T., Fahn, S., Martinez-Martin, P., Poewe, W., Sampaio, C., Stern, M.B., Dodel, R., Dubois, B., Holloway, R., Jankovic, J., Kulisevsky, J., Lang, A.E., Lees, A., Leurgans, S., LeWitt, P.A., Nyenhuis, D., Olanow, C.W., Rascol, O., Schrag, A., Teresi, J.A., van Hilten, J.J., LaPelle, N.; Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov Disord.* 2008;23(15):2129–2170. doi:10.1002/mds.22340
- Goldstein, D.S., Holmes, C., Benth, O., Sato, T., Moak, J., Sharabi, Y., Imrich, R., Conant, S., Eldadah, B.A., 2008. Biomarkers to detect central dopamine deficiency and distinguish Parkinson disease from multiple system atrophy. *Parkinsonism Relat. Disord.* 14, 600–607. <https://doi.org/10.1016/j.parkreldis.2008.01.010>

- Goldstein, D.S., Sewell, L., Holmes, C., 2010. Association of anosmia with autonomic failure in Parkinson disease. *Neurology* 74, 245–251. <https://doi.org/10.1212/WNL.0b013e3181ca014c>
- Good, C.D., Johnsrude, I.S., Ashburner, J., Henson, R.N.A., Friston, K.J., Frackowiak, R.S.J., 2001. A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage* 14, 21–36. <https://doi.org/10.1006/nimg.2001.0786>
- Haba-Rubio, J., Frauscher, B., Marques-Vidal, P., Toriel, J., Tobback, N., Andries, D., Preisig, M., Vollenweider, P., Postuma, R., Heinzer, R., 2018. Prevalence and determinants of rapid eye movement sleep behavior disorder in the general population. *Sleep* 41, 1–8. <https://doi.org/10.1093/sleep/zsx197>
- Haehner, A., Boesveldt, S., Berendse, H.W., Mackay-Sim, A., Fleischmann, J., Silburn, P.A., Johnston, A.N., Mellick, G.D., Herting, B., Reichmann, H., Hummel, T., 2009. Prevalence of smell loss in Parkinson's disease - a multicenter study. *Parkinsonism Relat. Disord.* 15, 490–4. <https://doi.org/10.1016/j.parkreldis.2008.12.005>
- Haehner, A., Hummel, T., Hummel, C., Sommer, U., Junghanns, S., Reichmann, H., 2007. Olfactory loss may be a first sign of idiopathic Parkinson's disease. *Mov. Disord.* 22, 839–842. <https://doi.org/10.1002/mds.21413>
- Haehner, A., Hummel, T., Reichmann, H., 2014. A clinical approach towards smell loss in Parkinson's disease. *J. Parkinsons. Dis.* 4, 189–195. <https://doi.org/10.3233/JPD-130278>
- Hagler, D.J., Saygin, A.P., Sereno, M.I., 2006. Smoothing and cluster thresholding for cortical surface-based group analysis of fMRI data. *Neuroimage* 33, 1093–103. <https://doi.org/10.1016/j.neuroimage.2006.07.036>
- Hansen, D., Ling, H., Lashley, T., Holton, J.L., Warner, T.T., 2019. Review: Clinical, neuropathological and genetic features of Lewy body dementias. *Neuropathol. Appl. Neurobiol.* 1–20. <https://doi.org/10.1111/nan.12554>
- Hanyu, H., Inoue, Y., Sakurai, H., Kanetaka, H., Nakamura, M., Miyamoto, T., Sasai, T., Iwamoto, T., 2012. Voxel-based magnetic resonance imaging study of structural brain changes in patients with idiopathic REM sleep behavior disorder. *Park. Relat. Disord.* 18, 136–139. <https://doi.org/10.1016/j.parkreldis.2011.08.023>
- Hanyu, H., Inoue, Y., Sakurai, H., Kanetaka, H., Nakamura, M., Miyamoto, T., Sasai, T., Iwamoto, T., 2011. Regional cerebral blood flow changes in patients with idiopathic REM sleep behavior disorder. *Eur. J. Neurol.* 18, 784–788. <https://doi.org/10.1111/j.1468-1331.2010.03283.x>
- Harding, A.J., Lakay, B., Halliday, G.M., 2002a. Selective hippocampal neuron loss in dementia with Lewy bodies. *Ann. Neurol.* 51, 125–128. <https://doi.org/10.1002/ana.10071>
- Harding, A.J., Stimson, E., Henderson, J.M., Halliday, G.M., 2002b. Clinical correlates of selective pathology in the amygdala of patients with Parkinson's disease. *Brain* 125, 2431–2445. <https://doi.org/10.1093/brain/awf251>
- Hawkes, C.H., Shephard, B.C., Daniel, S.E., 1997. Olfactory dysfunction in Parkinson's disease. *J Neurol Neurosurg Psychiatry* 62, 436–446. <https://doi.org/10.1038/nrneurol.2012.80>
- Haxby, J. V., Hoffman, E.A., Gobbini, M.I., 2002. Human neural systems for face recognition and social communication. *Biol. Psychiatry* 51, 59–67. [https://doi.org/10.1016/s0006-3223\(01\)01330-0](https://doi.org/10.1016/s0006-3223(01)01330-0)
- Her, S., Cha, K.S., Choi, J.W., Kim, H., Byun, J.I., Sunwoo, J.S., Kim, T.J., Lim, J.A., Jung, K.Y., Kim, K.H., 2019. Impaired visuospatial attention revealed by theta- and beta-band cortical activities in idiopathic REM sleep behavior disorder patients. *Clin. Neurophysiol.* 130, 1962–1970. <https://doi.org/10.1016/j.clinph.2019.07.030>

- Herting, B., Schulze, S., Reichmann, H., Haehner, A., Hummel, T., 2008. A longitudinal study of olfactory function in patients with idiopathic Parkinson's disease. *J. Neurol.* 255, 367–70. <https://doi.org/10.1007/s00415-008-0665-5>
- Hoehn, M.M., Yahr, M.D., 1967. Parkinsonism: onset, progression and mortality. *Neurology* 17, 427–442.
- Högl, B., Stefani, A., Videnovic, A., 2018. Idiopathic REM sleep behaviour disorder and neurodegeneration - An update. *Nat. Rev. Neurol.* 14, 40–56. <https://doi.org/10.1038/nrneurol.2017.157>
- Hohenfeld, C., Werner, C.J., Reetz, K., 2018. Resting-state connectivity in neurodegenerative disorders: Is there potential for an imaging biomarker? *NeuroImage Clin.* 18, 849–870. <https://doi.org/10.1016/j.nicl.2018.03.013>
- Holtbernd, F., Gagnon, J.-F., Postuma, R.B., Ma, Y., Tang, C.C., Feigin, A., Dhawan, V., Vendette, M., Soucy, J.-P., Eidelberg, D., Montplaisir, J., 2014. Abnormal metabolic network activity in REM sleep behavior disorder. *Neurology* 82, 620–627. <https://doi.org/10.1212/WNL.0000000000000130>
- Huang, S.F., Chen, K., Wu, J.J., Liu, F.T., Zhao, J., Lin, W., Guo, S.S., Wang, Y.X., Wang, Y., Luo, S.S., Sun, Y.M., Ding, Z.T., Yu, H., Wang, J., 2016. Odor identification test in idiopathic REM-behavior disorder and Parkinson's disease in China. *PLoS One* 11, 1–13. <https://doi.org/10.1371/journal.pone.0160199>
- Hubert, H.B., Fabsitz, R.R., Feinleib, M., Brown, K.S., 1980. Olfactory sensitivity in humans: genetic versus environmental control. *Science* 208, 607–9.
- Hughes, A.J., Daniel, S.E., Kilford, L., Lees, A.J., 1992. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J. Neurol. Neurosurg. Psychiatry* 55, 181–4. <https://doi.org/10.1136/jnnp.55.3.181>
- Hummel, T., Kobal, G., Gudziol, H., Mackay-Sim, A., 2007. Normative data for the “Sniffin” Sticks” including tests of odor identification, odor discrimination, and olfactory thresholds: An upgrade based on a group of more than 3,000 subjects.” *Eur. Arch. Oto-Rhino-Laryngology* 264, 237–243. <https://doi.org/10.1007/s00405-006-0173-0>
- Ibarretxe-Bilbao, N., Junque, C., Marti, M.J., Valldeoriola, F., Vendrell, P., Bargallo, N., Zarei, M., Tolosa, E., 2010. Olfactory impairment in Parkinson's disease and white matter abnormalities in central olfactory areas: A voxel-based diffusion tensor imaging study. *Mov. Disord.* 25, 1888–1894. <https://doi.org/10.1002/mds.23208>
- Iglesias, J.E., Augustinack, J.C., Nguyen, K., Player, C.M., Player, A., Wright, M., Roy, N., Frosch, M.P., McKee, A.C., Wald, L.L., Fischl, B., Van Leemput, K., 2015. A computational atlas of the hippocampal formation using ex vivo, ultra-high resolution MRI: Application to adaptive segmentation of in vivo MRI. *Neuroimage* 115, 117–137. <https://doi.org/10.1016/j.neuroimage.2015.04.042>
- Iranzo, A., Gelpi, E., Tolosa, E., Molinuevo, J.L., Serradell, M., Gaig, C., Santamaria, J., 2014a. Neuropathology of prodromal Lewy body disease. *Mov. Disord.* 29, 410–415. <https://doi.org/10.1002/mds.25825>
- Iranzo, A., Isetta, V., Molinuevo, J.L., Serradell, M., Navajas, D., Farre, R., Santamaria, J., 2010a. Electroencephalographic slowing heralds mild cognitive impairment in idiopathic REM sleep behavior disorder. *Sleep Med.* 11, 534–539. <https://doi.org/10.1016/j.sleep.2010.03.006>
- Iranzo, A., Lomeña, F., Stockner, H., Valldeoriola, F., Vilaseca, I., Salamero, M., Molinuevo, J.L., Serradell, M., Duch, J., Pavía, J., Gallego, J., Seppi, K., Högl, B., Tolosa, E., Poewe, W., Santamaria, J., 2010b. Decreased striatal dopamine transporter uptake and substantia nigra hyperechogenicity as risk markers of synucleinopathy in patients with idiopathic rapid-eye-

- movement sleep behaviour disorder: A prospective study. *Lancet Neurol.* 9, 1070–1077. [https://doi.org/10.1016/S1474-4422\(10\)70216-7](https://doi.org/10.1016/S1474-4422(10)70216-7)
- Iranzo, A., Molinuevo, J.L., Santamaría, J., Serradell, M., Martí, M.J., Valldeoriola, F., Tolosa, E., 2006. Rapid-eye-movement sleep behaviour disorder as an early marker for a neurodegenerative disorder: a descriptive study. *Lancet Neurol.* 5, 572–577. [https://doi.org/10.1016/S1474-4422\(06\)70476-8](https://doi.org/10.1016/S1474-4422(06)70476-8)
- Iranzo, A., Ratti, P.L., Casanova-Molla, J., Serradell, M., Vilaseca, I., Santamaria, J., 2009. Excessive muscle activity increases over time in idiopathic REM sleep behavior disorder. *Sleep* 32, 1149–1153. <https://doi.org/10.1093/sleep/32.9.1149>
- Iranzo, A., Santamaria, J., Pujol, J., Moreno, A., Deus, J., Tolosa, E., 2002. Brainstem proton magnetic resonance spectroscopy in idiopathic REM sleep behavior disorder. *Sleep* 25, 867–70.
- Iranzo, A., Santamaria, J., Tolosa, E., 2016. Idiopathic rapid eye movement sleep behaviour disorder: Diagnosis, management, and the need for neuroprotective interventions. *Lancet Neurol.* 15, 405–419. [https://doi.org/10.1016/S1474-4422\(16\)00057-0](https://doi.org/10.1016/S1474-4422(16)00057-0)
- Iranzo, A., Santamaría, J., Valldeoriola, F., Serradell, M., Salamero, M., Gaig, C., Niñerola-Baizán, A., Sánchez-Valle, R., Lladó, A., De Marzi, R., Stefani, A., Seppi, K., Pavia, J., Högl, B., Poewe, W., Tolosa, E., Lomeña, F., 2017a. Dopamine transporter imaging deficit predicts early transition to synucleinopathy in idiopathic rapid eye movement sleep behavior disorder. *Ann. Neurol.* 82, 419–428. <https://doi.org/10.1002/ana.25026>
- Iranzo, A., Serradell, M., Vilaseca, I., Valldeoriola, F., Salamero, M., Molina, C., Santamaria, J., Tolosa, E., 2013. Longitudinal assessment of olfactory function in idiopathic REM sleep behavior disorder. *Park. Relat. Disord.* 19, 600–604. <https://doi.org/10.1016/j.parkreldis.2013.02.009>
- Iranzo, A., Stefani, A., Niñerola-Baizan, A., Stokner, H., Serradell, M., Vilas, D., Holzkecht, E., Gaig, C., Pavia, J., Lomeña, F., Reyes, D., Seppi, K., Santamaria, J., Högl, B., Tolosa, E., Poewe, W., 2020. Left-hemispheric predominance of nigrostriatal deficit in isolated REM sleep behavior disorder. *Neurology* 94, e1605–e1613. <https://doi.org/10.1212/WNL.00000000000009246>
- Iranzo, A., Stefani, A., Serradell, M., Martí, M.J., Lomeña, F., Mahlknecht, P., Stockner, H., Gaig, C., Fernández-Arcos, A., Poewe, W., Tolosa, E., Högl, B., Santamaria, J., 2017b. Characterization of patients with longstanding idiopathic REM sleep behavior disorder. *Neurology* 89, 242 LP – 248. <https://doi.org/10.1212/WNL.00000000000004121>
- Iranzo, A., Stockner, H., Serradell, M., Seppi, K., Valldeoriola, F., Frauscher, B., Molinuevo, J.L., Vilaseca, I., Mitterling, T., Gaig, C., Vilas, D., Santamaria, J., Högl, B., Tolosa, E., Poewe, W., 2014b. Five-year follow-up of substantia nigra echogenicity in idiopathic REM sleep behavior disorder. *Mov. Disord.* 29, 1774–1780. <https://doi.org/10.1002/mds.26055>
- Iranzo, A., Valldeoriola, F., Lomeña, F., Molinuevo, J.L., Serradell, M., Salamero, M., Cot, A., Ros, D., Pavía, J., Santamaria, J., Tolosa, E., 2011. Serial dopamine transporter imaging of nigrostriatal function in patients with idiopathic rapid-eye-movement sleep behaviour disorder: A prospective study. *Lancet Neurol.* 10, 797–805. [https://doi.org/10.1016/S1474-4422\(11\)70152-1](https://doi.org/10.1016/S1474-4422(11)70152-1)
- Iwanami, M., Miyamoto, T., Miyamoto, M., Hirata, K., Takada, E., 2010. Relevance of substantia nigra hyperechogenicity and reduced odor identification in idiopathic REM sleep behavior disorder. *Sleep Med.* 11, 361–365. <https://doi.org/10.1016/j.sleep.2009.12.006>
- Joyner, R.E., 1964. Effect of cigarette smoking on olfactory acuity. *Arch. Otolaryngol.* 80, 576–9.
- Jozwiak, N., Postuma, R.B., Montplaisir, J., Latreille, V., Panisset, M., Chouinard, S., Bourgouin, P.-A., Gagnon, J.-F., 2017. REM Sleep Behavior Disorder and Cognitive Impairment in Parkinson's Disease. *Sleep* 40. <https://doi.org/10.1093/sleep/zsx101>

- Kaasinen, V., Vahlberg, T., 2017. Striatal dopamine in Parkinson disease: A meta-analysis of imaging studies. *Ann. Neurol.* 82, 873–882. <https://doi.org/10.1002/ana.25103>
- Kaplan, E., Goodglass, H., Weintraub, S., 1983. Boston Naming Test, in: *The Corsini Encyclopedia of Psychology*. John Wiley & Sons, Inc., Hoboken, NJ, USA. <https://doi.org/10.1002/9780470479216.corpsy0139>
- Kikuchi, A., Baba, T., Hasegawa, T., Sugeno, N., Konno, M., Takeda, A., 2011. Differentiating Parkinson's disease from multiple system atrophy by [123I] meta-iodobenzylguanidine myocardial scintigraphy and olfactory test. *Parkinsonism Relat. Disord.* 17, 698–700. <https://doi.org/10.1016/j.parkreldis.2011.07.011>
- Kim, Y.K., Yoon, I.Y., Kim, J.M., Jeong, S.H., Kim, K.W., Shin, Y.K., Kim, B.S., Kim, S.E., 2010. The implication of nigrostriatal dopaminergic degeneration in the pathogenesis of REM sleep behavior disorder. *Eur. J. Neurol.* 17, 487–492. <https://doi.org/10.1111/j.1468-1331.2009.02854.x>
- Knudsen, K., Fedorova, T.D., Hansen, A.K., Sommerauer, M., Otto, M., Svendsen, K.B., Nahimi, A., Stokholm, M.G., Pavese, N., Beier, C.P., Brooks, D.J., Borghammer, P., 2018. In-vivo staging of pathology in REM sleep behaviour disorder: a multimodality imaging case-control study. *Lancet Neurol.* 17, 618–628. [https://doi.org/10.1016/S1474-4422\(18\)30162-5](https://doi.org/10.1016/S1474-4422(18)30162-5)
- Kondo, H., Matsuda, T., Hashiba, M., B.S., 1998. A study of the relationship between the T&T olfactometer and the University of Pennsylvania Smell Identification Test in a Japanese population. *Am. J. Rhinol. Allergy* 2, 353–8.
- Krismer, F., Pinter, B., Mueller, C., Mahlknecht, P., Nocker, M., Reiter, E., Djamshidian-Tehrani, A., Boesch, S.M., Wenning, G.K., Scherfler, C., Poewe, W., Seppi, K., 2017. Sniffing the diagnosis: Olfactory testing in neurodegenerative parkinsonism. *Park. Relat. Disord.* 35, 36–41. <https://doi.org/10.1016/j.parkreldis.2016.11.010>
- Lee, D.H., Oh, J.S., Ham, J.H., Lee, J.J., Lee, I., Lee, P.H., Kim, J.S., Sohn, Y.H., 2015. Is normosmic Parkinson disease a unique clinical phenotype? *Neurology* 85, 1270–1275. <https://doi.org/10.1212/WNL.0000000000001999>
- Lee, E.-Y., Eslinger, P.J., Du, G., Kong, L., Lewis, M.M., Huang, X., 2014. Olfactory-related cortical atrophy is associated with olfactory dysfunction in Parkinson's disease. *Mov. Disord.* 29, 1205–8. <https://doi.org/10.1002/mds.25829>
- Lee, J.-Y., Yoon, E.J., Kim, Y.K., Shin, C.W., Nam, H., Jeong, J.M., Kim, H.-J., Jeon, B., 2019. Nonmotor and Dopamine Transporter Change in REM Sleep Behavior Disorder by Olfactory Impairment. *J. Mov. Disord.* 12, 103–112. <https://doi.org/10.14802/jmd.18061>
- Lee, J.E., Cho, K.H., Ham, J.H., Song, S.K., Sohn, Y.H., Lee, P.H., 2014. Olfactory performance acts as a cognitive reserve in non-demented patients with Parkinson's disease. *Parkinsonism Relat. Disord.* 20, 186–91.
- Lee, J.H., Han, Y.H., Cho, J.W., Lee, J.S., Lee, S.J., Kim, D.J., Kim, Tae Hyoung, Kang, B.M., Kim, Tae Hyung, Mun, C.W., 2014. Evaluation of brain iron content in idiopathic REM sleep behavior disorder using quantitative magnetic resonance imaging. *Park. Relat. Disord.* 20, 776–778. <https://doi.org/10.1016/j.parkreldis.2014.03.023>
- Lenka, A., George, L., Arumugham, S.S., Hegde, S., Reddy, V., Kamble, N., Yadav, R., Pal, P.K., 2017. Predictors of onset of psychosis in patients with Parkinson's disease: Who gets it early? *Park. Relat. Disord.* 44, 91–94. <https://doi.org/10.1016/j.parkreldis.2017.09.015>
- Lenka, A., Pagonabarraga, J., Kumar, P., Bejr-Kasem, H., Kulisevsky, J., 2019. Minor hallucinations in Parkinson disease. *Neurology* 93, 259–266. DOI 10.1212/WNL.0000000000007913
- Lerche, S., Machetanz, G., Roeben, B., Wurster, I., Zimmermann, M., von Thaler, A.K., Liepelt-Scarfone, I., Eschweiler, G.W., Fallgatter, A., Metzger, F., Maetzler, W., Berg, D., Brockmann, K.,

2018. Deterioration of executive dysfunction in elderly with REM sleep behavior disorder (RBD). *Neurobiol. Aging* 70, 242–246. <https://doi.org/10.1016/j.neurobiolaging.2018.06.029>
- Levy, J.A., Chelune, G.J., 2007. Cognitive-behavioral profiles of neurodegenerative dementias: Beyond alzheimer's disease. *J. Geriatr. Psychiatry Neurol.* 20, 227–238. <https://doi.org/10.1177/0891988707308806>
- Lezak, M.D., 2012. *Neuropsychological assessment*. Oxford University Press.
- Li, S.X., Wing, Y.K., Lam, S.P., Zhang, J., Yu, M.W.M., Ho, C.K.W., Tsoh, J., Mok, V., 2010. Validation of a new REM sleep behavior disorder questionnaire (RBDQ-HK). *Sleep Med.* 11, 43–48. <https://doi.org/10.1016/j.sleep.2009.06.008>
- Li, X., Wang, K., Jia, S., Zhou, Z., Jin, Y., Zhang, X., Hou, C., Zheng, W., Rong, P., Jiao, J., 2018. The prospective memory of patients with idiopathic REM sleep behavior disorder. *Sleep Med.* 47, 19–24. <https://doi.org/10.1016/j.sleep.2018.03.019>
- Li, Y., Kang, W., Yang, Q., Zhang, Lina, Zhang, Linyuan, Dong, F., Chen, S., Liu, J., 2017. Predictive markers for early conversion of iRBD to neurodegenerative synucleinopathy diseases. *Neurology* 88, 1493–1500. <https://doi.org/10.1212/WNL.0000000000003838>
- Liguori, C., Ruffini, R., Olivola, E., Chiaravalloti, A., Izzi, F., Stefani, A., Pierantozzi, M., Biagio, N., Modugno, N., Centonze, D., Schillaci, O., 2019. Cerebral glucose metabolism in idiopathic REM sleep behavior disorder is different from tau-related and alfa-synuclein-related neurodegenerative disorders: A brain [18F]FDG PET study. *Parkinsonism Relat. Disord.* 64, 97–105.
- Litvan, I., Goldman, J.G., Tröster, A.I., Schmand, B. a, Weintraub, D., Petersen, R.C., Mollenhauer, B., Adler, C.H., Marder, K., Williams-Gray, C.H., Aarsland, D., Kulisevsky, J., Rodriguez-Oroz, M.C., Burn, D.J., Barker, R. a, Emre, M., 2012. Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines. *Mov. Disord.* 27, 349–56. <https://doi.org/10.1002/mds.24893>
- Liu, G., Zong, G., Doty, R., Sun, Q., 2016. Prevalence and risk factors of taste and smell impairment in a nationwide representative sample of the US population: A cross-sectional study. *BMJ Open* 6, 8–11. <https://doi.org/10.1136/bmjopen-2016-013246>
- Mahlknecht, P., Iranzo, A., Högl, B., Frauscher, B., Müller, C., Santamaría, J., Tolosa, E., Serradell, M., Mitterling, T., Gschliesser, V., Goebel, G., Brugger, F., Scherfler, C., Poewe, W., Seppi, K., 2015. Olfactory dysfunction predicts early transition to a Lewy body disease in idiopathic RBD. *Neurology* 84, 654–658. <https://doi.org/10.1212/WNL.0000000000001265>
- Mahlknecht, P., Pechlaner, R., Boesveldt, S., Volc, D., Pinter, B., Reiter, E., Müller, C., Krismer, F., Berendse, H.W., van Hilten, J.J., Wuschitz, A., Schimetta, W., Högl, B., Djamshidian, A., Nocker, M., Göbel, G., Gasperi, A., Kiechl, S., Willeit, J., Poewe, W., Seppi, K., 2016. Optimizing odor identification testing as quick and accurate diagnostic tool for Parkinson's disease. *Mov. Disord.* 31, 1408–1413. <https://doi.org/10.1002/mds.26637>
- Mak, E., Gabel, S., Su, L., Williams, G.B., Arnold, R., Passamonti, L., Vazquez Rodríguez, P., Surendranathan, A., Bevan-Jones, W.R., Rowe, J.B., O'Brien, J.T., 2017. Multi-modal MRI investigation of volumetric and microstructural changes in the hippocampus and its subfields in mild cognitive impairment, Alzheimer's disease, and dementia with Lewy bodies. *Int. Psychogeriatrics* 29, 545–555. <https://doi.org/10.1017/S1041610216002143>
- Mak, E., Su, L., Williams, G.B., Watson, R., Firbank, M.J., Blamire, A.M., O'Brien, J.T., 2015. Progressive cortical thinning and subcortical atrophy in dementia with Lewy bodies and Alzheimer's disease. *Neurobiol. Aging* 36, 1743–1750. <https://doi.org/10.1016/j.neurobiolaging.2014.12.038>
- Marcone, S., Gagnon, J.F., Desjardins, C., David, A.C., Postuma, R.B., Montplaisir, J., Joubert, S., Rouleau, I., 2019. Prospective memory in idiopathic REM sleep behavior disorder with or

- without mild cognitive impairment: A preliminary study. *Clin. Neuropsychol.* 33, 571–593. <https://doi.org/10.1080/13854046.2018.1435825>
- Marques, A., Dujardin, K., Boucart, M., Pins, D., Delliaux, M., Defebvre, L., Derambure, P., Monaca, C., 2010. REM sleep behaviour disorder and visuoperceptive dysfunction: A disorder of the ventral visual stream? *J. Neurol.* 257, 383–391. <https://doi.org/10.1007/s00415-009-5328-7>
- Massicotte-Marquez, J., Décary, A., Gagnon, J.-F., Vendette, M., Mathieu, A., Postuma, R.B., Carrier, J., Montplaisir, J., Petersen, R.C., 2008. Executive dysfunction and memory impairment in idiopathic REM sleep behavior disorder. *Neurology* 70, 1250–7. <https://doi.org/10.1212/01.wnl.0000286943.79593.a6>
- Mazza, S., Soucy, J.P., Gravel, P., Michaud, M., Postuma, R., Massicotte-Marquez, J., Decary, A., Montplaisir, J., 2006. Assessing whole brain perfusion changes in patients with REM sleep behavior disorder. *Neurology* 67, 1618–1622. <https://doi.org/10.1212/01.wnl.0000242879.39415.49>
- McKeith, I.G., Boeve, B.F., Dickson, D.W., Halliday, G., Taylor, J.-P., Weintraub, D., Aarsland, D., Galvin, J., Attems, J., Ballard, C.G., Bayston, A., Beach, T.G., Blanc, F., Bohnen, N., Bonanni, L., Bras, J., Brundin, P., Burn, D., Chen-Plotkin, A., Duda, J.E., El-Agnaf, O., Feldman, H., Ferman, T.J., ffytche, D., Fujishiro, H., Galasko, D., Goldman, J.G., Gomperts, S.N., Graff-Radford, N.R., Honig, L.S., Iranzo, A., Kantarci, K., Kaufer, D., Kukull, W., Lee, V.M.Y., Leverenz, J.B., Lewis, S., Lippa, C., Lunde, A., Masellis, M., Masliah, E., McLean, P., Mollenhauer, B., Montine, T.J., Moreno, E., Mori, E., Murray, M., O'Brien, J.T., Orimo, S., Postuma, R.B., Ramaswamy, S., Ross, O.A., Salmon, D.P., Singleton, A., Taylor, A., Thomas, A., Tiraboschi, P., Toledo, J.B., Trojanowski, J.Q., Tsuang, D., Walker, Z., Yamada, M., Kosaka, K., 2017. Diagnosis and management of dementia with Lewy bodies. *Neurology* 89, 88–100. <https://doi.org/10.1212/WNL.0000000000004058>
- McKinnon, J., Evidente, V., Driver-Dunckley, E., Premkumar, A., Hentz, J., Shill, H., Sabbagh, M., Caviness, J., Connor, D., Adler, C., 2010. Olfaction in the Elderly: A Cross-Sectional Analysis Comparing Parkinson's Disease with Controls and Other Disorders. *Int. J. Neurosci.* 120, 36–39. <https://doi.org/10.3109/00207450903428954>
- McShane, R.H., Nagy, Z., Esiri, M.M., King, E., Joachim, C., Sullivan, N., Smith, A.D., 2001. Anosmia in dementia is associated with Lewy bodies rather than Alzheimer's pathology. *J. Neurol. Neurosurg. Psychiatry* 70, 739–743. <https://doi.org/10.1136/jnnp.70.6.739>
- Meles, S.K., Renken, R.J., Janzen, A., Vadasz, D., Pagani, M., Arnaldi, D., Morbelli, S., Nobili, F., Mayer, G., Leenders, K.L., Oertel, W.H., Sittig-Wiegand, E., Depboylu, C., Reetz, K., Overeem, S., Pijpers, A., Reesink, F.E., Van Laar, T., Teune, L.K., Höffken, H., Luster, M., Timmermann, L., Kesper, K., Adriaanse, S.M., Booij, J., Sambuceti, G., Girtler, N., Jonsson, C., 2018. The Metabolic Pattern of Idiopathic REM Sleep Behavior Disorder Reflects Early-Stage Parkinson Disease. *J. Nucl. Med.* 59, 1437–1444. <https://doi.org/10.2967/jnumed.117.202242>
- Meles, S.K., Vadasz, D., Renken, R.J., Sittig-Wiegand, E., Mayer, G., Depboylu, C., Reetz, K., Overeem, S., Pijpers, A., Reesink, F.E., van Laar, T., Heinen, L., Teune, L.K., Höffken, H., Luster, M., Kesper, K., Adriaanse, S.M., Booij, J., Leenders, K.L., Oertel, W.H., 2017. FDG PET, dopamine transporter SPECT, and olfaction: Combining biomarkers in REM sleep behavior disorder. *Mov. Disord.* 32, 1482–1486. <https://doi.org/10.1002/mds.27094>
- Meusel, T., Westermann, B., Fuhr, P., Hummel, T., Welge-Lüssen, A., 2010. The course of olfactory deficits in patients with Parkinson's disease-A study based on psychophysical and electrophysiological measures. *Neurosci. Lett.* 486, 166–170. <https://doi.org/10.1016/j.neulet.2010.09.044>
- Miyamoto, M., Miyamoto, T., Saitou, J., Sato, T., 2020. Longitudinal study of striatal aromatic L-amino acid decarboxylase activity in patients with idiopathic rapid eye movement sleep behavior disorder. *Sleep Med.* 68, 50–56. <https://doi.org/10.1016/j.sleep.2019.09.013>

- Miyamoto, T., Miyamoto, M., Iwanami, M., Hirata, K., 2011. Idiopathic REM sleep behavior disorder: Implications for the pathogenesis of lewy body diseases. *Parkinsons. Dis.* 2011. <https://doi.org/10.4061/2011/941268>
- Miyamoto, T., Miyamoto, M., Iwanami, M., Hirata, K., Kobayashi, M., Nakamura, M., Inoue, Y., 2010. Olfactory dysfunction in idiopathic REM sleep behavior disorder. *Sleep Med.* 11, 458–461. <https://doi.org/10.1016/j.sleep.2009.09.013>
- Miyamoto, T., Miyamoto, M., Iwanami, M., Suzuki, K., Inoue, Y., Hirata, K., 2009. Odor identification test as an indicator of idiopathic REM sleep behavior disorder. *Mov. Disord.* 24, 268–273. <https://doi.org/10.1002/mds.22361>
- Moessnang, C., Frank, G., Bogdahn, U., Winkler, J., Greenlee, M.W., Klucken, J., 2011. Altered activation patterns within the olfactory network in Parkinson's disease. *Cereb. Cortex* 21, 1246–53. <https://doi.org/10.1093/cercor/bhq202>
- Mori, E., Shimomura, T., Fujimori, M., Hirono, N., Imamura, T., Hashimoto, M., Tanimukai, S., Kazui, H., Hanihara, T., 2000. Visuo-perceptual Impairment in Dementia With Lewy Bodies 57, 489–493.
- Müller, A., Müngersdorf, M., Reichmann, H., Strehle, G., Hummel, T., 2002a. Olfactory function in Parkinsonian syndromes. *J. Clin. Neurosci. Off. J. Neurosurg. Soc. Australas.* 9, 521–524. <https://doi.org/10.1054/jocn.2001.1071>
- Müller, A., Reichmann, H., Livermore, A., Hummel, T., 2002b. Olfactory function in idiopathic Parkinson's disease (IPD): results from cross-sectional studies in IPD patients and long-term follow-up of de-novo IPD patients. *J. Neural Transm.* 190, 805–811.
- Muntean, M.-L., Sixel-Döring, F., Trenkwalder, C., 2013. No Difference in Sleep and RBD between Different Types of Patients with Multiple System Atrophy: A Pilot Video-Polysomnographical Study. *Sleep Disord.* 2013, 258390. <https://doi.org/10.1155/2013/258390>
- Nepozitek, J., Dostalova, S., Dusek, Petr, Kemlink, D., Prihodova, I., Ibarburu Lorenzo Y Losada, V., Friedrich, L., Bezdicek, O., Nikolai, T., Perinova, P., Dall'Antonia, I., Dusek, Pavel, Ruml, M., Ruzicka, E., Sonka, K., 2019. Simultaneous tonic and phasic REM sleep without atonia best predicts early phenocconversion to neurodegenerative disease in idiopathic REM sleep behavior disorder. *Sleep* 42, 1–7. <https://doi.org/10.1093/sleep/zsz132>
- Nomura, T., Inoue, Y., Kagimura, T., Nakashima, K., 2013. Clinical significance of REM sleep behavior disorder in Parkinson's disease. *Sleep Med.* 14, 131–135. <https://doi.org/10.1016/j.sleep.2012.10.011>
- Ogihara, H., Kobayashi, M., Nishida, K., Kitano, M., Takeuchi, K., 2011. Applicability of the Cross-Culturally Modified University of Pennsylvania Smell Identification Test in a Japanese Population. *Am. J. Rhinol. Allergy* 25, 404–410. <https://doi.org/10.2500/ajra.2011.25.3658>
- Ohlhauser, L., Smart, C.M., Gawryluk, J.R., 2019. Tract-Based Spatial Statistics Reveal Lower White Matter Integrity Specific to Idiopathic Rapid Eye Movement Sleep Behavior Disorder as a Proxy for Prodromal Parkinson's Disease. *J. Parkinsons. Dis.* 9, 723–731. <https://doi.org/10.3233/JPD-191688>
- Olichney, J.M., Murphy, C., Hofstetter, C.R., Foster, K., Hansen, L.A., Thal, L.J., Katzman, R., 2005. Anosmia is very common in the Lewy body variant of Alzheimer's disease. *J. Neurol. Neurosurg. Psychiatry* 76, 1342–1347. <https://doi.org/10.1136/jnnp.2003.032003>
- Olson, E.J., Boeve, B.F., Silber, M.H., 2000. Rapid eye movement sleep behaviour disorder: Demographic, clinical and laboratory findings in 93 cases. *Brain* 123, 331–339. <https://doi.org/10.1093/brain/123.2.331>

- Pagano, G., De Micco, R., Yousaf, T., Wilson, H., Chandra, A., Politis, M., 2018. REM behavior disorder predicts motor progression and cognitive decline in Parkinson disease. *Neurology* 91, e894 LP-e905. <https://doi.org/10.1212/WNL.0000000000006134>
- Pagonabarraga, J., Martinez-Horta, S., Fernández de Bobadilla, R., Pérez, J., Ribosa-Nogué, R., Marín, J., Pascual-Sedano, B., García, C., Gironell, A., Kulisevsky, J., 2016. Minor hallucinations occur in drug-naïve Parkinson's disease patients, even from the premotor phase. *Mov. Disord.* 31, 45–52. <https://doi.org/10.1002/mds.26432>
- Pagonabarraga, J., Soriano-Mas, C., Llebaria, G., López-Solà, M., Pujol, J., Kulisevsky, J., 2014. Neural correlates of minor hallucinations in non-demented patients with Parkinson's disease. *Parkinsonism Relat. Disord.* 20, 290–296. <https://doi.org/10.1016/j.parkreldis.2013.11.017>
- Palma, J.-A., Fernandez-Cordon, C., Coon, E.A., Low, P.A., Miglis, M.G., Jaradeh, S., Bhaumik, A.K., Dayalu, P., Urrestarazu, E., Iriarte, J., Biaggioni, I., Kaufmann, H., 2015. Prevalence of REM sleep behavior disorder in multiple system atrophy: a multicenter study and meta-analysis. *Clin. Auton. Res.* 25, 69–75. <https://doi.org/10.1007/s10286-015-0279-9>
- Park, J.-W., Kwon, D.-Y., Choi, J.H., Park, M.-H., Yoon, H.-K., 2018. Olfactory dysfunctions in drug-naïve Parkinson's disease with mild cognitive impairment. *Parkinsonism Relat. Disord.* 46, 69–73. <https://doi.org/10.1016/j.parkreldis.2017.11.334>
- Park, K.M., Lee, H.J., Lee, B.I., Kim, S.E., 2018. Alterations of the brain network in idiopathic rapid eye movement sleep behavior disorder: structural connectivity analysis. *Sleep Breath.* 23, 587–593. <https://doi.org/10.1007/s11325-018-1737-0>
- Patenaude, B., Smith, S.M., Kennedy, D.N., Jenkinson, M., 2011. A Bayesian model of shape and appearance for subcortical brain segmentation. *Neuroimage* 56, 907–922. <https://doi.org/10.1016/j.neuroimage.2011.02.046>
- Pearce, R.K., Hawkes, C.H., Daniel, S.E., 1995. The anterior olfactory nucleus in Parkinson's disease. *Mov. Disord.* 10, 283–7. <https://doi.org/10.1002/mds.870100309>
- Peever, J., Luppi, P.H., Montplaisir, J., 2014. Breakdown in REM sleep circuitry underlies REM sleep behavior disorder. *Trends Neurosci.* 37, 279–288. <https://doi.org/10.1016/j.tins.2014.02.009>
- Peraza, L.R., Colloby, S.J., Deboys, L., O'Brien, J.T., Kaiser, M., Taylor, J.P., 2016. Regional functional synchronizations in dementia with Lewy bodies and Alzheimer's disease. *Int. Psychogeriatrics* 28, 1143–1151. <https://doi.org/10.1017/S1041610216000429>
- Peraza, L.R., Kaiser, M., Firbank, M., Graziadio, S., Bonanni, L., Onofrj, M., Colloby, S.J., Blamire, A., O'Brien, J., Taylor, J.P., 2014. FMRI resting state networks and their association with cognitive fluctuations in dementia with Lewy bodies. *NeuroImage Clin.* 4, 558–565. <https://doi.org/10.1016/j.nicl.2014.03.013>
- Peraza, L.R., Taylor, J.P., Kaiser, M., 2015. Divergent brain functional network alterations in dementia with Lewy bodies and Alzheimer's disease. *Neurobiol. Aging* 36, 2458–2467. <https://doi.org/10.1016/j.neurobiolaging.2015.05.015>
- Pereira, J.B., Junqué, C., Martí, M.-J., Ramirez-Ruiz, B., Bargalló, N., Tolosa, E., 2009a. Neuroanatomical substrate of visuospatial and visuoperceptual impairment in Parkinson's disease. *Mov. Disord.* 24, 1193–9. <https://doi.org/10.1002/mds.22560>
- Pereira, J.B., Junqué, C., Martí, M.J., Ramirez-Ruiz, B., Bartrés-Faz, D., Tolosa, E., 2009b. Structural brain correlates of verbal fluency in Parkinson's disease. *Neuroreport* 20, 741–4. <https://doi.org/10.1097/WNR.0b013e328329370b>
- Pereira, J.B., Weintraub, D., Chahine, L., Aarsland, D., Hansson, O., Westman, E., 2019. Cortical thinning in patients with REM sleep behavior disorder is associated with clinical progression. *npj Park. Dis.* 3, 5:7. <https://doi.org/10.1038/s41531-019-0079-3>

- Petersen, R.C., Doody, R., Kurz, A., Mohs, R.C., Morris, J.C., Rabins, P. V., Ritchie, K., Rossor, M., Thal, L., Winblad, B., 2001. Current concepts in mild cognitive impairment. *Arch. Neurol.* 58, 1985–1992. <https://doi.org/10.1001/archneur.58.12.1985>
- Picillo, M., Pellicchia, M.T., Erro, R., Amboni, M., Vitale, C., Iavarone, A., Moccia, M., Allocca, R., Orefice, G., Barone, P., 2014. The use of University of Pennsylvania Smell Identification Test in the diagnosis of Parkinson's disease in Italy. *Neurol. Sci.* 35, 379–83. <https://doi.org/10.1007/s10072-013-1522-6>
- Plomhause, L., Dujardin, K., Boucart, M., Herlin, V., Defebvre, L., Derambure, P., Charley, C.M., 2014. Impaired visual perception in rapid eye movement sleep behavior disorder. *Neuropsychology* 28, 388–393. <https://doi.org/10.1037/neu0000006>
- Posner, M.I., 1980. Orienting of attention. *Q. J. Exp. Psychol.* 32, 3–25. <https://doi.org/10.1080/00335558008248231>
- Postuma, R.B., Gagnon, J.F., 2010. Cognition and olfaction in Parkinson's disease. *Brain* 133, 1–2. <https://doi.org/10.1093/brain/awq225>
- Postuma, R.B., 2016. Resting state MRI: a new marker of prodromal neurodegeneration? *Brain* 139, 2104–2112. <https://doi.org/doi:10.1093/brain/aww131> References
- Postuma, R.B., Aarsland, D., Barone, P., Burn, D.J., Hawkes, C.H., Oertel, W., Ziemssen, T., 2012a. Identifying prodromal Parkinson's disease: Pre-Motor disorders in Parkinson's disease. *Mov. Disord.* 27, 617–626. <https://doi.org/10.1002/mds.24996>
- Postuma, R.B., Arnulf, I., Hogl, B., Iranzo, A., Miyamoto, T., Dauvilliers, Y., Oertel, W., Ju, Y. El, Puligheddu, M., Jennum, P., Pelletier, A., Wolfson, C., Leu-Semenescu, S., Frauscher, B., Miyamoto, M., Cochen De Cock, V., Unger, M.M., Stiasny-Kolster, K., Livia Fantini, M., Montplaisir, J.Y., 2012b. A single-question screen for rapid eye movement sleep behavior disorder: A multicenter validation study. *Mov. Disord.* 27, 913–916. <https://doi.org/10.1002/mds.25037>
- Postuma, R.B., Berg, D., Stern, M., Poewe, W., Marek, K., Litvan, I., 2015a. CME MDS Clinical Diagnostic Criteria for Parkinson ' s Disease Centrality of Motor Syndrome — Parkinsonism and PD Criteria Benchmark — The Expert Examination 30. <https://doi.org/10.1002/mds.26424>
- Postuma, R.B., Bertrand, J.A., Montplaisir, J., Desjardins, C., Vendette, M., Rios Romenets, S., Panisset, M., Gagnon, J.F., 2012c. Rapid eye movement sleep behavior disorder and risk of dementia in Parkinson's disease: A prospective study. *Mov. Disord.* 27, 720–726. <https://doi.org/10.1002/mds.24939>
- Postuma, R.B., Gagnon, J.-F., Vendette, M., Desjardins, C., Montplaisir, J.Y., 2011. Olfaction and color vision identify impending neurodegeneration in rapid eye movement sleep behavior disorder. *Ann. Neurol.* 69, 811–818. <https://doi.org/10.1002/ana.22282>
- Postuma, R.B., Gagnon, J.F., Bertrand, J.A., Génier Marchand, D., Montplaisir, J.Y., 2015b. Parkinson risk in idiopathic REM sleep behavior disorder: Preparing for neuroprotective trials. *Neurology* 84, 1104–1113. <https://doi.org/10.1212/WNL.0000000000001364>
- Postuma, R.B., Gagnon, J.F., Vendette, M., Montplaisir, J.Y., 2009. Markers of neurodegeneration in idiopathic rapid eye movement sleep behaviour disorder and Parkinson's disease. *Brain* 132, 3298–3307. <https://doi.org/10.1093/brain/awp244>
- Postuma, R.B., Iranzo, A., Hogl, B., Arnulf, I., Ferini-Strambi, L., Manni, R., Miyamoto, T., Oertel, W., Dauvilliers, Y., Ju, Y. El, Puligheddu, M., Sonka, K., Pelletier, A., Santamaria, J., Frauscher, B., Leu-Semenescu, S., Zucconi, M., Terzaghi, M., Miyamoto, M., Unger, M.M., Carlander, B., Fantini, M.L., Montplaisir, J.Y., 2015c. Risk factors for neurodegeneration in idiopathic rapid eye movement sleep behavior disorder: A multicenter study. *Ann. Neurol.* 77, 830–839. <https://doi.org/10.1002/ana.24385>

- Postuma, R.B., Iranzo, A., Hu, M., Högl, B., Boeve, B.F., Manni, R., Oertel, W.H., Arnulf, I., Ferini-Strambi, L., Puligheddu, M., Antelmi, E., Cochen De Cock, V., Arnaldi, D., Mollenhauer, B., Videnovic, A., Sonka, K., Jung, K.Y., Kunz, D., Dauvilliers, Y., Provini, F., Lewis, S.J., Buskova, J., Pavlova, M., Heidebreder, A., Montplaisir, J.Y., Santamaria, J., Barber, T.R., Stefani, A., Louis, S.E.K., Terzaghi, M., Janzen, A., Leu-Semenescu, S., Plazzi, G., Nobili, F., Sixel-Doering, F., Dusek, P., Bes, F., Cortelli, P., Ehgoetz Martens, K., Gagnon, J.F., Gaig, C., Zucconi, M., Trenkwalder, C., Gan-Or, Z., Lo, C., Rolinski, M., Mahlke, P., Holzke, E., Boeve, A.R., Teigen, L.N., Toscano, G., Mayer, G., Morbelli, S., Dawson, B., Pelletier, A., 2019. Risk and predictors of dementia and parkinsonism in idiopathic REM sleep behaviour disorder: A multicentre study. *Brain* 142, 744–759. <https://doi.org/10.1093/brain/awz030>
- Postuma, R.B., Lang, A.E., Gagnon, J.F., Pelletier, A., Montplaisir, J.Y., 2012d. How does parkinsonism start? Prodromal parkinsonism motor changes in idiopathic REM sleep behaviour disorder. *Brain* 135, 1860–1870. <https://doi.org/10.1093/brain/aws093>
- Postuma, R.B., Lang, A.E., Massicotte-Marquez, J., Montplaisir, J., 2006. Potential early markers of Parkinson disease in idiopathic REM sleep behavior disorder. *Neurology* 66, 845–851. <https://doi.org/10.1212/01.wnl.0000203648.80727.5b>
- Power, J.D., Barnes, K.A., Snyder, A.Z., Schlaggar, B.L., Petersen, S.E., 2012. Spurious but systematic correlations in functional connectivity MRI networks arise from subject motion. *Neuroimage* 59, 2142–2154. <https://doi.org/10.1016/j.neuroimage.2011.10.018>
- Price, M.J., Feldman, R.G., Adelberg, D., Kayne, H., 1992. Abnormalities in color vision and contrast sensitivity in Parkinson's disease. *Neurology* 42, 887 LP – 887. <https://doi.org/10.1212/WNL.42.4.887>
- Pruim, R.H.R., Mennes, M., van Rooij, D., Llera, A., Buitelaar, J.K., Beckmann, C.F., 2015. ICA-AROMA: A robust ICA-based strategy for removing motion artifacts from fMRI data. *Neuroimage* 112, 267–277. <https://doi.org/10.1016/j.neuroimage.2015.02.064>
- Puschmann, A., 2013. Monogenic Parkinson's disease and parkinsonism: clinical phenotypes and frequencies of known mutations. *Parkinsonism Relat. Disord.* 19, 407–415. <https://doi.org/10.1016/j.parkreldis.2013.01.020>
- Pyatigorskaya, N., Gaurav, R., Arnaldi, D., Leu-Semenescu, S., Yahia-Cherif, L., Valabregue, R., Vidailhet, M., Arnulf, I., Lehericy, S., 2017. Magnetic Resonance Imaging Biomarkers to Assess Substantia Nigra Damage in Idiopathic Rapid Eye Movement Sleep Behavior Disorder. *Sleep* 40. <https://doi.org/10.1093/sleep/zsx149>
- Rahayel, S., Gaubert, M., Postuma, R.B., Montplaisir, J., Carrier, J., Monchi, O., Remillard-Pelchat, D., Bourgouin, P.A., Panisset, M., Chouinard, S., Joubert, S., Gagnon, J.F., 2019. Brain atrophy in Parkinson's disease with polysomnographyconfirmed REM sleep behavior disorder. *Sleep* 42, 1–12. <https://doi.org/10.1093/sleep/zsz062>
- Rahayel, S., Montplaisir, J., Monchi, O., Bedetti, C., Postuma, R.B., Brambati, S., Carrier, J., Joubert, S., Latreille, V., Jubault, T., Gagnon, J.F., 2014. Patterns of cortical thinning in idiopathic rapid eye movement sleep behavior disorder. *Mov. Disord.* 30, 680–687. <https://doi.org/10.1002/mds.25820>
- Rahayel, S., Postuma, R.B., Montplaisir, J., Bedetti, C., Brambati, S., Carrier, J., Monchi, O., Bourgouin, P.A., Gaubert, M., Gagnon, J.F., 2018a. Abnormal Gray Matter Shape, Thickness, and Volume in the Motor Cortico-Subcortical Loop in Idiopathic Rapid Eye Movement Sleep Behavior Disorder: Association with Clinical and Motor Features. *Cereb. Cortex* 28, 658–671. <https://doi.org/10.1093/cercor/bhx137>
- Rahayel, S., Postuma, R.B., Montplaisir, J., Génier Marchand, D., Escudier, F., Gaubert, M., Bourgouin, P.-A., Carrier, J., Monchi, O., Joubert, S., Blanc, F., Gagnon, J.-F., 2018b. Cortical and subcortical gray matter bases of cognitive deficits in REM sleep behavior disorder. *Neurology* 90, e1759–e1770. [https://doi.org/10.1016/S0306-4522\(99\)00316-4](https://doi.org/10.1016/S0306-4522(99)00316-4)

- Ramírez-Ruiz, B., Junqué, C., Martí, M.-J., Valldeoriola, F., Tolosa, E., 2006. Neuropsychological deficits in Parkinson's disease patients with visual hallucinations. *Mov. Disord.* 21, 1483–7. <https://doi.org/10.1002/mds.20965>
- Ramírez-Ruiz, B., Martí, M.-J., Tolosa, E., Giménez, M., Bargalló, N., Valldeoriola, F., Junqué, C., 2007. Cerebral atrophy in Parkinson's disease patients with visual hallucinations. *Eur. J. Neurol.* 14, 750–6. <https://doi.org/10.1111/j.1468-1331.2007.01768.x>
- Reuter, M., Fischl, B., 2011. Avoiding asymmetry-induced bias in longitudinal image processing. *Neuroimage* 57, 19–21. <https://doi.org/10.1016/j.neuroimage.2011.02.076>
- Reuter, M., Rosas, H.D., Fischl, B., 2010. Highly accurate inverse consistent registration: A robust approach. *Neuroimage* 53, 1181–1196. <https://doi.org/10.1016/j.neuroimage.2010.07.020>
- Rodrigues-Brazète, J., Gagnon, J.-F., Postuma, R.B., Bertrand, J.-A., Petit, D., Montplaisir, J., 2016. Electroencephalogram slowing predicts neurodegeneration in rapid eye movement sleep behavior disorder. *Neurobiol. Aging* 37, 74–81. <https://doi.org/10.1016/j.neurobiolaging.2015.10.007>
- Rodríguez-Violante, M., Gonzalez-Latapi, P., Camacho-Ordoñez, A., Martínez-Ramírez, D., Morales-Briceño, H., Cervantes-Arriaga, A., 2014a. Comparing the accuracy of different smell identification tests in Parkinson's disease: Relevance of cultural aspects. *Clin. Neurol. Neurosurg.* 123, 9–14. <https://doi.org/10.1016/j.clineuro.2014.04.030>
- Rodríguez-Violante, M., Gonzalez-Latapi, P., Camacho-Ordoñez, A., Martínez-Ramírez, D., Morales-Briceño, H., Cervantes-Arriaga, A., 2014b. Low specificity and sensitivity of smell identification testing for the diagnosis of Parkinson's disease. *Arq. Neuropsiquiatr.* 72, 33–37. <https://doi.org/10.1590/0004-282X20130190>
- Rodríguez-Violante, M., Lees, A.J., Cervantes-Arriaga, A., Corona, T., Silveira-Moriyama, L., 2011. Use of smell test identification in Parkinson's disease in Mexico: A matched case-control study. *Mov. Disord.* 26, 173–176. <https://doi.org/10.1002/mds.23354>
- Rolinski, M., Griffanti, L., Piccini, P., Roussakis, A.A., Szewczyk-Krolikowski, K., Menke, R.A., Quinnell, T., Zaiwalla, Z., Klein, J.C., Mackay, C.E., Hu, M.T.M., 2016a. Basal ganglia dysfunction in idiopathic REM sleep behaviour disorder parallels that in early Parkinson's disease. *Brain* 139, 2224–2234. <https://doi.org/10.1093/brain/aww124>
- Rolinski, M., Szewczyk-Krolikowski, K., Tomlinson, P.R., Nithi, K., Talbot, K., Ben-Shlomo, Y., Hu, M.T., 2014. REM sleep behaviour disorder is associated with worse quality of life and other non-motor features in early Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 85, 560–6.
- Rolinski, M., Zokaei, N., Baig, F., Giehl, K., Quinnell, T., Zaiwalla, Z., Mackay, C.E., Husain, M., Hu, M.T.M., 2016b. Visual short-term memory deficits in REM sleep behaviour disorder mirror those in Parkinson's disease. *Brain* 139, 47–53. <https://doi.org/10.1093/brain/awv334>
- Ross, G.W., Petrovitch, H., Abbott, R.D., Tanner, C.M., Popper, J., Masaki, K., Launer, L., White, L.R., 2008. Association of olfactory dysfunction with risk for future Parkinson's disease. *Ann. Neurol.* 63, 167–173. <https://doi.org/10.1002/ana.21291>
- Royall, D.R., Cordes, J.A., Polk, M., 1998. CLOX: An executive clock drawing task. *J. Neurol. Neurosurg. Psychiatry* 64, 588–594. <https://doi.org/10.1136/jnnp.64.5.588>
- Rubinov, M., Sporns, O., 2010. Complex network measures of brain connectivity: uses and interpretations. *Neuroimage* 52, 1059–69. <https://doi.org/10.1016/j.neuroimage.2009.10.003>
- Rumeau, C., Nguyen, D.T., Jankowski, R., 2016. How to assess olfactory performance with the Sniffin' Sticks test®. *Eur Ann Otorhinolaryngol Head Neck Dis.* 133, 203–206. <https://doi.org/10.1016/j.anorl.2015.08.004>

- Sabattoli, F., Boccardi, M., Galluzzi, S., Treves, A., Thompson, P.M., Frisoni, G.B., 2008. Hippocampal shape differences in dementia with Lewy bodies. *Neuroimage* 41, 699–705. <https://doi.org/10.1016/j.neuroimage.2008.02.060>
- Sakurai, H., Hanyu, H., Inoue, Y., Kanetaka, H., Nakamura, M., Miyamoto, T., Sasai, T., Iwamoto, T., 2014. Longitudinal study of regional cerebral blood flow in elderly patients with idiopathic rapid eye movement sleep behavior disorder. *Geriatr. Gerontol. Int.* 14, 115–120. <https://doi.org/10.1111/ggi.12068>
- Salmon, D.P., Bondi, M.W., 2009. Neuropsychological Assessment of Dementia. *Annu. Rev. Psychol.* 60, 257–282. <https://doi.org/10.1146/annurev.psych.57.102904.190024>
- Sasai-Sakuma, T., Nishio, Y., Yokoi, K., Mori, E., Inoue, Y., 2017. Pareidolias in REM sleep behavior disorder: A possible predictive marker of lewy body diseases? *Sleep* 40. <https://doi.org/10.1093/sleep/zsw045>
- Sasai, T., Matsuura, M., Inoue, Y., 2013. Electroencephalographic Findings Related With Mild Cognitive Impairment in Idiopathic Rapid Eye Movement Sleep Behavior Disorder. *Sleep* 36, 1893–1899. <https://doi.org/10.5665/sleep.3224>
- Sasai, T., Miyamoto, T., Miyamoto, M., Iwanami, M., Abe, T., Matsuura, M., Inoue, Y., 2012. Impaired decision-making in idiopathic REM sleep behavior disorder. *Sleep Med.* 13, 301–306. <https://doi.org/10.1016/j.sleep.2011.09.012>
- Savic, I., Gulyas, B., Larsson, M., Roland, P., 2000. Olfactory functions are mediated by parallel and hierarchical processing. *Neuron* 26, 735–745. [https://doi.org/10.1016/S0896-6273\(00\)81209-X](https://doi.org/10.1016/S0896-6273(00)81209-X)
- Schenck, C.H., Bundlie, S.R., Ettinger, M.G., Mahowald, M.W., 1986. Chronic behavioral disorders of human REM sleep: A new category of parasomnia. *Sleep* 9, 293–308. <https://doi.org/10.1093/sleep/9.2.293>
- Schenck, C.H., Bundlie, S.R., Mahowald, M.W., 1996. Delayed emergence of a parkinsonian disorder in 38% of 29 older men initially diagnosed with idiopathic rapid eye movement sleep behavior disorder. *Neurology* 46, 388 LP – 393. <https://doi.org/10.1212/WNL.46.2.388>
- Schenck, C.H., Hurwitz, T.D., Mahowald, M.W., 1993. REM sleep behaviour disorder: an update on a series of 96 patients and a review of the world literature. *J. Sleep Res.* 2, 224–231. <https://doi.org/10.1111/j.1365-2869.1993.tb00093.x>
- Schenck, C.H., Mahowald, M.W., 2002. REM sleep behavior disorder: clinical, developmental, and neuroscience perspectives 16 years after its formal identification in Sleep. *Sleep* 25, 120–38.
- Scherfler, C., Frauscher, B., Schocke, M., Iranzo, A., Gschliesser, V., Seppi, K., Santamaria, J., Tolosa, E., Högl, B., Poewe, W., 2011. White and gray matter abnormalities in idiopathic rapid eye movement sleep behavior disorder: A diffusion-tensor imaging and voxel-based morphometry study. *Ann. Neurol.* 69, 400–407. <https://doi.org/10.1002/ana.22245>
- Scherfler, C., Seppi, K., Mair, K.J., Donnemiller, E., Virgolini, I., Wenning, G.K., Poewe, W., 2012. Left hemispheric predominance of nigrostriatal dysfunction in Parkinson's disease. *Brain* 135, 3348–3354. <https://doi.org/10.1093/brain/aws253>
- Ségonne, F., Pacheco, J., Fischl, B., 2007. Geometrically accurate topology-correction of cortical surfaces using nonseparating loops. *IEEE Trans. Med. Imaging* 26, 518–29. <https://doi.org/10.1109/TMI.2006.887364>
- Segura, B., Baggio, H.C., Marti, M.J., Valldeoriola, F., Compta, Y., Garcia-Diaz, A.I., Vendrell, P., Bargallo, N., Tolosa, E., Junque, C., 2014. Cortical thinning associated with mild cognitive impairment in Parkinson's disease. *Mov. Disord.* 29, 1495–1503. <https://doi.org/10.1002/mds.25982>

- Sengoku, R., Matsushima, S., Bono, K., Sakuta, K., Yamazaki, M., Miyagawa, S., Komatsu, T., Mitsumura, H., Kono, Y., Kamiyama, T., Ito, K., Mochio, S., Iguchi, Y., 2015. Olfactory function combined with morphology distinguishes Parkinson's disease. *Park. Relat. Disord.* 21, 771–777. <https://doi.org/10.1016/j.parkreldis.2015.05.001>
- Shin, C., Lee, J.Y., Kim, Y.K., Nam, H., Yoon, E.J., Shin, S.A., Kim, H.J., Jeon, B., 2019. Cognitive decline in association with hyposmia in idiopathic rapid eye movement sleep behavior disorder: a prospective 2-year follow-up study. *Eur. J. Neurol.* 26, 1417–1420. <https://doi.org/10.1111/ene.14025>
- Shin, H.Y., Joo, E.Y., Kim, S.T., Dhong, H.-J., Cho, J.W., 2013. Comparison study of olfactory function and substantia nigra hyperechogenicity in idiopathic REM sleep behavior disorder, Parkinson's disease and normal control. *Neurol. Sci.* 34, 935–940. <https://doi.org/10.1007/s10072-012-1164-0>
- Shine, J.M., Halliday, G.M., Gilat, M., Matar, E., Bolitho, S.J., Carlos, M., Naismith, S.L., Lewis, S.J.G., 2014. The role of dysfunctional attentional control networks in visual misperceptions in Parkinson's disease. *Hum. Brain Mapp.* 35, 2206–2219. <https://doi.org/10.1002/hbm.22321>
- Silveira-Moriyama, L., Azevedo, A.M., Ranvaud, R., Barbosa, E.R., Doty, R.L., Lees, A.J., 2010a. Applying a new version of the Brazilian-Portuguese UPSIT smell test in Brazil. *Arq. Neuropsiquiatr.* 68, 700–705. <https://doi.org/10.1590/S0004-282X2010000500005>
- Silveira-Moriyama, L., de Jesus Carvalho, M., Katzenschlager, R., Petrie, A., Ranvaud, R., Barbosa, E.R., Lees, A.J., 2008. The use of smell identification tests in the diagnosis of Parkinson's disease in Brazil. *Mov. Disord.* 23, 2328–2334. <https://doi.org/10.1002/mds.22241>
- Silveira-Moriyama, L., Hughes, G., Church, A., Ayling, H., Williams, D.R., Petrie, A., Holton, J., Revesz, T., Kingsbury, A., Morris, H.R., Burn, D.J., Lees, A.J., 2010b. Hyposmia in progressive supranuclear palsy. *Mov. Disord.* 25, 570–577. <https://doi.org/10.1002/mds.22688>
- Silveira-Moriyama, L., Mathias, C., Mason, L., Best, C., Quinn, N.P., Lees, A.J., 2009. Hyposmia in pure autonomic failure. *Neurology* 72, 1677–1681. <https://doi.org/10.1212/WNL.0b013e3181a55fd2>
- Sixel-Döring, F., Schweitzer, M., Mollenhauer, B., Trenkwalder, C., 2011a. Intraindividual variability of REM sleep behavior disorder in Parkinson's disease: A comparative assessment using a new REM sleep behavior disorder severity scale (RBDSS) for clinical routine. *J. Clin. Sleep Med.* 7, 75–80. <https://doi.org/10.5664/jcsm.28044>
- Sixel-Döring, F., Trautmann, E., Mollenhauer, B., Trenkwalder, C., 2011b. Associated factors for REM sleep behavior disorder in Parkinson disease. *Neurology* 77, 1048 LP – 1054. <https://doi.org/10.1212/WNL.0b013e31822e560e>
- Sixel-Döring, F., Trautmann, E., Mollenhauer, B., Trenkwalder, C., 2014. Rapid Eye Movement Sleep Behavioral Events: A New Marker for Neurodegeneration in Early Parkinson Disease? *Sleep* 37, 431–438. <http://dx.doi.org/10.5665/sleep.3468>
- Sixel-Döring, F., Zimmermann, J., Wegener, A., Mollenhauer, B., Trenkwalder, C., 2016. The Evolution of REM Sleep Behavior Disorder in Early Parkinson Disease. *Sleep* 39, 1737–1742. <https://doi.org/10.5665/sleep.6102>
- Sled, J.G., Zijdenbos, A.P., Evans, A.C., 1998. A nonparametric method for automatic correction of intensity nonuniformity in MRI data. *IEEE Trans. Med. Imaging* 17, 87–97. <https://doi.org/10.1109/42.668698>
- Smith, A., 2000. Symbol digit modalities test: Manual, 8th ed. Western Psychological Services, Los Angeles.
- Smith, S.M., Jenkinson, M., Woolrich, M.W., Beckmann, C.F., Behrens, T.E.J., Johansen-Berg, H., Bannister, P.R., De Luca, M., Drobnjak, I., Flitney, D.E., Niazy, R.K., Saunders, J., Vickers, J., Zhang,

- Y., De Stefano, N., Brady, J.M., Matthews, P.M., 2004. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 23 Suppl 1, S208-19. <https://doi.org/10.1016/j.neuroimage.2004.07.051>
- Smith, S.M., Nichols, T.E., 2009. Threshold-free cluster enhancement: Addressing problems of smoothing, threshold dependence and localisation in cluster inference. *Neuroimage* 44, 83–98. <https://doi.org/10.1016/j.neuroimage.2008.03.061>
- Sorokowska, A., Schriever, V.A., Gudziol, V., Hummel, C., Hähner, A., Iannilli, E., Sinding, C., Aziz, M., Seo, H.S., Negoias, S., Hummel, T., 2015. Changes of olfactory abilities in relation to age: odor identification in more than 1400 people aged 4 to 80 years. *Eur. Arch. Oto-Rhino-Laryngology* 272, 1937–1944. <https://doi.org/10.1007/s00405-014-3263-4>
- Sourty, M., Thoraval, L., Roquet, D., Armspach, J.-P., Foucher, J., Blanc, F., 2016. Identifying Dynamic Functional Connectivity Changes in Dementia with Lewy Bodies Based on Product Hidden Markov Models. *Front. Comput. Neurosci.* 10, 1–11. <https://doi.org/10.3389/fncom.2016.00060>
- Starkstein, S.E., Mayberg, H.S., Preziosi, T.J., Andrezejewski, P., Leiguarda, R., Robinson, R.G., 1992. Reliability, validity, and clinical correlates of apathy in Parkinson's disease. *J. Neuropsychiatry Clin. Neurosci.* 4, 134–139. <https://doi.org/10.1176/jnp.4.2.134>
- Stiasny-Kolster, K., Doerr, Y., Möller, J.C., Höffken, H., Behr, T.M., Oertel, W.H., Mayer, G., 2005. Combination of “idiopathic” REM sleep behaviour disorder and olfactory dysfunction as possible indicator for α -synucleinopathy demonstrated by dopamine transporter FP-CIT-SPECT. *Brain* 128, 126–137. <https://doi.org/10.1093/brain/awh322>
- Stiasny-Kolster, K., Mayer, G., Schäfer, S., Möller, J.C., Heinzel-Gutenbrunner, M., Oertel, W.H., 2007. The REM sleep behavior disorder screening questionnaire - A new diagnostic instrument. *Mov. Disord.* 22, 2386–2393. <https://doi.org/10.1002/mds.21740>
- Stroop, J.R., 1935. Studies of interference in serial verbal reactions. *J. Exp. Psychol.* 18, 643–662. <https://doi.org/10.1037/h0054651>
- Sun, J., Lai, Z., Ma, J., Gao, L., Chen, M., Chen, J., Fang, J., Fan, Y., Bao, Y., Zhang, D., Chan, P., Yang, Q., Ye, C., Wu, T., Ma, T., 2019. Quantitative evaluation of iron content in idiopathic rapid eye movement sleep behavior disorder. *Mov. Disord.* 1, 1–8. <https://doi.org/10.1002/mds.27929>
- Suzuki, M., Hashimoto, M., Yoshioka, M., Murakami, M., Kawasaki, K., Urashima, M., 2011. The odor stick identification test for Japanese differentiates Parkinson's disease from multiple system atrophy and progressive supra nuclear palsy. *BMC Neurol.* 11, 157. <https://doi.org/10.1186/1471-2377-11-157>
- Telesford, Q.K., Simpson, S.L., Burdette, J.H., Hayasaka, S., Laurienti, P.J., 2011. The Brain as a Complex System: Using Network Science as a Tool for Understanding the Brain. *Brain Connect.* 1, 295–308. <https://doi.org/10.1089/brain.2011.0055>
- Terzaghi, M., Sinforiani, E., Zucchella, C., Zambrelli, E., Pasotti, C., Rustioni, V., Manni, R., 2008. Cognitive performance in REM sleep behaviour disorder: a possible early marker of neurodegenerative disease? *Sleep Med.* 9, 343–351. <https://doi.org/10.1016/j.sleep.2007.06.013>
- Terzaghi, M., Toscano, G., Casoni, F., Picascia, M., Arnaldi, D., Rustioni, V., Versino, M., Sinforiani, E., Manni, R., 2019. Assessment of cognitive profile as a prodromal marker of the evolution of rapid eye movement sleep behavior disorder. *Sleep* 42, 1–8. <https://doi.org/10.1093/sleep/zsz103>
- Terzaghi, M., Zucchella, C., Rustioni, V., Sinforiani, E., Manni, R., 2013. Cognitive Performances and Mild Cognitive Impairment in Idiopathic Rapid Eye Movement Sleep Behavior Disorder: Results of a Longitudinal Follow-Up Study. *Sleep* 36, 1527–1532. <https://doi.org/10.5665/sleep.3050>

- Teune, L.K., Renken, R.J., De Jong, B.M., Willemsen, A.T., Van Osch, M.J., Roerdink, J.B.T.M., Dierckx, R.A., Leenders, K.L., 2014. Parkinson's disease-related perfusion and glucose metabolic brain patterns identified with PCASL-MRI and FDG-PET imaging. *NeuroImage Clin.* 5, 240–244. <https://doi.org/10.1016/j.nicl.2014.06.007>
- Teune, L.K., Renken, R.J., Mudali, D., De Jong, B.M., Dierckx, R.A., Roerdink, J.B.T.M., Leenders, K.L., 2013. Validation of parkinsonian disease-related metabolic brain patterns. *Mov. Disord.* 28, 547–551. <https://doi.org/10.1002/mds.25361>
- Thorpy MJ, C., Diagnostic Classification Steering Committee, 1990. International classification of sleep disorders: diagnostic and coding manual. Rochester, MN.
- Tiraboschi, P., Salmon, D.P., Hansen, L.A., Hofstetter, R.C., Thal, L.J., Corey-Bloom, J., 2006. What best differentiates Lewy body from Alzheimer's disease in early-stage dementia? *Brain* 129, 729–735. <https://doi.org/10.1093/brain/awh725>
- Tissingh, G., Berendse, H.W., Bergmans, P., DeWaard, R., Drukarch, B., Stoof, J.C., Wolters, E.C., 2001. Loss of olfaction in de novo and treated Parkinson's disease: possible implications for early diagnosis. *Mov. Disord.* 16, 41–6.
- Tomlinson, C.L., Stowe, R., Patel, S., Rick, C., Gray, R., Clarke, C.E., 2010. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Mov. Disord.* 25, 2649–2653. <https://doi.org/10.1002/mds.23429>
- Uchiyama, M., Isse, K., Tanaka, K., Yokota, N., Hamamoto, M., Aida, S., Ito, Y., Yoshimura, M., Okawa, M., 1995. Incidental Lewy body disease in a patient with REM sleep behavior disorder. *Neurology* 45, 709 LP – 712. <https://doi.org/10.1212/WNL.45.4.709>
- Uitti, R.J., Baba, Y., Whaley, N.R., Wszolek, Z.K., Putzke, J.D., 2005. Parkinson disease: Handedness predicts asymmetry. *Neurology* 64, 1925–1930. <https://doi.org/10.1212/01.WNL.0000163993.82388.C8>
- Unger, M.M., Belke, M., Menzler, K., Heverhagen, J.T., Keil, B., Stiasny-Kolster, K., Rosenow, F., Diederich, N.J., Mayer, G., Möller, J.C., Oertel, W.H., Knake, S., 2010. Diffusion tensor imaging in idiopathic REM sleep behavior disorder reveals microstructural changes in the brainstem, substantia nigra, olfactory region, and other brain regions. *Sleep* 33, 767–773. <https://doi.org/10.1093/sleep/33.6.767>
- United Nations, Department of Economic and Social Affairs, P.D., 2019. World Population Prospects 2019.
- Uribe, C., Segura, B., Baggio, H.C., Abos, A., Marti, M.J., Valldeoriola, F., Compta, Y., Bargallo, N., Junque, C., 2016. Patterns of cortical thinning in nondemented Parkinson's disease patients. *Mov. Disord.* 31, 699–708. <https://doi.org/10.1002/mds.26590>
- Vendette, M., Gagnon, J.-F., Soucy, J.-P., Gosselin, N., Postuma, R.B., Tuineag, M., Godin, I., Montplaisir, J., 2011. Brain perfusion and markers of neurodegeneration in rapid eye movement sleep behavior disorder. *Mov. Disord.* 26, 1717–1724. <https://doi.org/10.1002/mds.23721>
- Vendette, M., Montplaisir, J., Gosselin, N., Soucy, J.-P., Postuma, R.B., Dang-Vu, T.T., Gagnon, J., 2012. Brain perfusion anomalies in rapid eye movement sleep behavior disorder with mild cognitive impairment. *Mov. Disord.* 27, 1255–1261. <https://doi.org/10.1002/mds.25034>
- Vossel, S., Geng, J.J., Fink, G.R., 2014. Dorsal and Ventral Attention Systems. *Neurosci.* 20, 150–159. <https://doi.org/10.1177/1073858413494269>
- Wan, Y., Luo, Y., Gan, J., Hu, R., Zhou, M., Liu, Z., 2016. Clinical markers of neurodegeneration in Chinese patients with idiopathic rapid eye movement sleep behavior disorder. *Clin. Neurol. Neurosurg.* 150, 105–109. <https://doi.org/10.1016/j.clineuro.2016.09.002>

- Wang, J., You, H., Liu, J.F., Ni, D.F., Zhang, Z.X., Guan, J., 2011. Association of olfactory bulb volume and olfactory sulcus depth with olfactory function in patients with Parkinson disease. *Am. J. Neuroradiol.* 32, 677–681. <https://doi.org/10.3174/ajnr.A2350>
- Watson, R., Colloby, S.J., Blamire, A.M., O'Brien, J.T., 2015. Assessment of Regional Gray Matter Loss in Dementia with Lewy Bodies: A Surface-Based MRI Analysis. *Am. J. Geriatr. Psychiatry* 23, 38–46. <https://doi.org/10.1016/j.jagp.2014.07.005>
- Wattendorf, E., Welge-Lüssen, A., Fiedler, K., Bilecen, D., Wolfensberger, M., Fuhr, P., Hummel, T., Westermann, B., 2009. Olfactory impairment predicts brain atrophy in Parkinson's disease. *J. Neurosci.* 29, 15410–3. <https://doi.org/10.1523/JNEUROSCI.1909-09.2009>
- Wechsler, D., 1999. Escala de inteligencia de Weschler para adultos-III. TEA, Madrid.
- Weintraub, D., Chahine, L.M., Hawkins, K.A., Siderowf, A., Eberly, S., Oakes, D., Seibyl, J., Stern, M.B., Marek, K., Jennings, D., 2017. Cognition and the course of prodromal Parkinson's disease. *Mov. Disord.* 32, 1640–1645. <https://doi.org/10.1002/mds.27189>
- Wen, M.C., Heng, H.S.E., Hsu, J.L., Xu, Z., Liew, G.M., Au, W.L., Chan, L.L., Tan, L.C.S., Tan, E.K., 2017. Structural connectome alterations in prodromal and de novo Parkinson's disease patients. *Park. Relat. Disord.* 45, 21–27. <https://doi.org/10.1016/j.parkreldis.2017.09.019>
- Wenning, G.K., Shephard, B., Hawkes, C., Petrukevitch, A., Lees, A., Quinn, N., 1995. Olfactory function in atypical parkinsonian syndromes. *Acta Neurol. Scand.* 91, 247–250. <https://doi.org/10.1111/j.1600-0404.1995.tb06998.x>
- Westermann, B., Wattendorf, E., Schwerdtfeger, U., Husner, A., Fuhr, P., Gratzl, O., Hummel, T., Bilecen, D., Welge-Lüssen, A., Welge-Lussen, A., 2008. Functional imaging of the cerebral olfactory system in patients with Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry* 79, 19–24. <https://doi.org/10.1136/jnnp.2006.113860>
- Westervelt, H.J., Stern, R.A., Tremont, G., 2003. Odor identification deficits in diffuse lewy body disease. *Cogn. Behav. Neurol. Off. J. Soc. Behav. Cogn. Neurol.* 16, 93–99. <https://doi.org/10.1097/00146965-200306000-00002>
- Williams, D.R., Lees, A.J., 2005. Visual hallucinations in the diagnosis of idiopathic Parkinson's disease: A retrospective autopsy study. *Lancet Neurol.* 4, 605–610. [https://doi.org/10.1016/S1474-4422\(05\)70146-0](https://doi.org/10.1016/S1474-4422(05)70146-0)
- Winblad, B., Palmer, K., Kivipelto, M., Jelic, V., Fratiglioni, L., Wahlund, L.O., Nordberg, A., Bäckman, L., Albert, M., Almkvist, O., Arai, H., Basun, H., Blennow, K., De Leon, M., Decarli, C., Erkinjuntti, T., Jacobini, E., Graff, C., Hardy, J., Jack, C., Jorm, A., Ritchie, K., Van Duijn, C., Visser, P., Petersen, R.C., 2004. Mild cognitive impairment - Beyond controversies, towards a consensus: Report of the International Working Group on Mild Cognitive Impairment. *J. Intern. Med.* 256, 240–246. <https://doi.org/10.1111/j.1365-2796.2004.01380.x>
- Winkler, A.M., Ridgway, G.R., Webster, M.A., Smith, S.M., Nichols, T.E., 2014. Permutation inference for the general linear model. *Neuroimage* 92, 381–397. <https://doi.org/10.1016/J.NEUROIMAGE.2014.01.060>
- Wu, P., Yu, H., Peng, S., Dauvilliers, Y., Wang, J., Ge, J., Zhang, H., Eidelberg, D., Ma, Y., Zuo, C., 2014. Consistent abnormalities in metabolic network activity in idiopathic rapid eye movement sleep behaviour disorder. *Brain* 137, 3122–3128. <https://doi.org/10.1093/brain/awu290>
- Wu, X., Yu, C., Fan, F., Zhang, K., Zhu, C., Wu, T., Li, K., Chan, P., 2011. Correlation between progressive changes in piriform cortex and olfactory performance in early Parkinson's disease. *Eur. Neurol.* 66, 98–105. <https://doi.org/10.1159/000329371>
- Yamada, G., Ueki, Y., Oishi, N., Oguri, T., Fukui, A., Nakayama, M., Sano, Y., Kandori, A., Kan, H., Arai, N., Sakurai, K., Wada, I., Matsukawa, N., 2019. Nigrostriatal dopaminergic dysfunction and

- altered functional connectivity in rem sleep behavior disorder with mild motor impairment. *Front. Neurol.* 10, 1–12. <https://doi.org/10.3389/fneur.2019.00802>
- Yoon, E.J., Lee, J.Y., Nam, H., Kim, H.J., Jeon, B., Jeong, J.M., Kim, Y.K., 2019. A new metabolic network correlated with olfactory and executive dysfunctions in idiopathic rapid eye movement sleep behavior disorder. *J. Clin. Neurol.* 15, 175–183. <https://doi.org/10.3988/jcn.2019.15.2.175>
- Yoon, J.H., Kim, M., Moon, S.Y., Yong, S.W., Hong, J.M., 2015. Olfactory function and neuropsychological profile to differentiate dementia with Lewy bodies from Alzheimer's disease in patients with mild cognitive impairment: A 5-year follow-up study. *J. Neurol. Sci.* 355, 174–179. <https://doi.org/10.1016/j.jns.2015.06.013>
- Youn, S., Kim, T., Yoon, I.Y., Jeong, J., Kim, H.Y., Han, J.W., Kim, J.M., Kim, K.W., 2016. Progression of cognitive impairments in idiopathic REM sleep behaviour disorder. *J. Neurol. Neurosurg. Psychiatry* 87, 890–896. <https://doi.org/10.1136/jnnp-2015-311437>
- Yücepur, C., Özücer, B., Değirmenci, N., Yıldırım, Y., Veyseller, B., Ozturan, O., 2012. University of Pennsylvania smell identification test: application to Turkish population. *Kulak Burun Bogaz Ihtis. Derg.* 22, 77–80.
- Zalesky, A., Fornito, A., Bullmore, E.T., 2010. Network-based statistic: Identifying differences in brain networks. *Neuroimage* 53, 1197–1207. <https://doi.org/10.1016/j.neuroimage.2010.06.041>
- Zhang, J., Xu, C.Y., Liu, J., 2017. Meta-analysis on the prevalence of REM sleep behavior disorder symptoms in Parkinson's disease. *BMC Neurol.* 17, 4–9. <https://doi.org/10.1186/s12883-017-0795-4>
- Zhang, X., Song, Z., Ye, J., Fu, Y., Wang, J., Su, L., Zhu, X., Zhang, M., Cheng, Y., Wu, W., Xue, R., 2019. Polysomnographic and neuropsychological characteristics of rapid eye movement sleep behavior disorder patients. *Brain Behav.* 9, 1–9. <https://doi.org/10.1002/brb3.1220>
- Zhou, J., Zhang, J., Li, Y., Du, L., Li, Z., Lei, F., Wing, Y.K., Kushida, C.A., Zhou, D., Tang, X., 2015. Gender differences in REM sleep behavior disorder: A clinical and polysomnographic study in China. *Sleep Med.* 16, 414–418. <https://doi.org/10.1016/j.sleep.2014.10.020>
- Zoetmulder, M., Nikolic, M., Biernat, H., Korbo, L., Friberg, L., Jennum, P., 2016. Increased motor activity during REM sleep is linked with dopamine function in idiopathic REM sleep behavior disorder and Parkinson disease. *J. Clin. Sleep Med.* 12, 895–903. <https://doi.org/10.5664/jcsm.5896>

