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NEUROPSYCHOBIOLOGY OF TRAIT–STATE FACTORS OF DEPRESSIVE DISORDERS AND NEUROTICISM

Thesis presented by
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to obtain the Degree of Doctor in Neurosciences.

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declare and confirm that they have supervised and guided the PhD thesis entitled NEUROPSYCHOBIOLOGY OF TRAIT–STATE FACTORS OF DEPRESSIVE DISORDERS AND NEUROTICISM, *presented by* ***Maria J Portella Moll****, and that the work has been carried out in both Universities. They hereby assert that this thesis fulfils the requirements to be defended for the Degree of Doctor.*

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The reasonable man adapts himself to the world;
the unreasonable one persists in trying to adapt the world to himself.
Therefore, all progress depends on the unreasonable man.

George Bernard Shaw
Irish dramatist and socialist (1856 - 1950)

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CONTENTS

Foreword

This dissertation, presented to obtain the degree of Doctor in Neurosciences of the University of Barcelona, is the result of different works carried out during a 4-year period at the Institut Clínic de Psiquiatria i Psicologia, Fundació Clínic per a la Recerca Biomèdica, and at the Department of Psychiatry, Warneford Hospital, University of Oxford. During this period, I have obtained the *Diploma d'Estudis Avançats* (DEA) through the Neurosciences Program of the School of Medicine at the University of Barcelona. The following articles have been published in national and international journals, as a result of the work performed, with a global impact factor (IF) of 9.507 (Isiknowledge, JCR 2003):

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Portella MJ, Goodwin GM, Flint J, Cowen P, Harmer CJ. Neuroticism as a psychological vulnerability factor for mood disorders. British Association for Psychopharmacology. Harrogate, UK. 2004 *Journal of Psychopharmacology* 18 (3): A12-A12 (2004) Meeting Abstract.

Glossary of Abbreviations

5-HT	*Serotonin*
ACTH	*Adrenocorticotrophic Hormone*
ANOVA	*Analysis of Variance*
ARAS	*Ascending Reticulo-Cortical Activation System*
ARG	*Andreas-Retzius Gyrus*
AUC	*Area Under the Curve*
BDI	*Beck Depression Inventory*
BDNF	*Brain-derived Neurotrophic Factor*
BOLD	*Blood-Oxygen level dependent*
CA	*Cornu Ammonis*
CNS	*Central Nervous System*
CRF	*Corticotrophin Releasing Hormone*
CRH	*Cortisol Releasing Factor*
CV	*Coefficient of Variation*
DLPFC	*Dorsolateral Prefrontal Cortex*
E	*Epinephrine*
EC	*Entorhinal Cortex*
ECG	*Electrocardiographic*
ECT	*Electroconvulsive Therapy*
EMD	*Elderly Major Depression*
EMG	*Electromyography*
EPQ	*Eysenck Personality Questionnaire*
EPS	*Emotion Potentiated Startle*
FG	*Fasciolar Gyrus*
fMRI	*Functional Magnetic Resonance Imaging*
GR	*Glucocorticoid Receptor*
HDRS	*Hamilton Depression Rating Scale*
HPA	*Hypothalamic-Pituitary-Adrenal*
LM I	*Immediate Logical Memory*
LM II	*Delayed Logical Memory*
MDD	*Major Depressive Disorder*
MMSE	*Mini-Mental State Examination*
MRI	*Magnetic Resonance Imaging*
N	*Neuroticism*
NE	*Norepinephrine*
NMDA	*N-methyl-D-aspartate*
PET	*Positron Emission Tomography*
ROI	*Region of Interest*
RVIP	*Rapid Visual Information Processing*
SD	*Standard Deviation*
SNS	*Sympathetic Nervous System*
SNSRI	*Selective Norepinephrine Serotonine Reuptake Inhibitor*
SPECT	*Single-photon Emission Computed Tomography*
SSRI	*Selective Serotonine Reuptake Inhibitor*
STAI	*State and Trait Anxiety Inventory*
TE	*Time to Echo*
TMT-A	*Trail Making Test, form A*
TOL	*Tower of London*
TR	*Repetition Time*
VM I	*Immediate Visual Memory*
VM II	*Delayed Visual Memory*

1. INTRODUCTION

1.1. APPROACH

Major depressive disorders present a significant mental health concern to our society. According to the DSM-IV, the lifetime risk for major depressive disorder (MDD) is between 5% and 12% for men and between 10% and 25% for women (American Psychiatric Association, 1994). Although MDD might develop at any age, the average age of onset is in the 30s. It is accepted that the essential feature of depression is either low mood or the loss of interest or pleasure, but depressed subjects can also display irritability and/or anxiety (APA, 1994). Also, they often have other neurologically mediated symptoms (insomnia, fatigue…) and significant social and interpersonal difficulties. In addition, people experiencing depression show a strong cognitive impairment that, together with the rest of symptoms, can be fairly disabling.

Huge efforts have been done to untangle the aetiology, the course, the broad spectrum, and many other characteristics of MDD. Many of these efforts have provided a better knowledge of the disease and, nowadays, there is a partial agreement of what MDD is, what kind of treatments are useful or useless, which cognitive functions may be impaired, and which brain structures may be involved in MDD. However, to date it is not well established whether MDD is a unique mental disorder or consists of several subtypes differentiated by age of onset, psychotic characteristics, course of the illness, etc…

The cognitive impairment in the acute phase of MDD has been widely characterised in young people: psychomotor slowness, memory deficits and executive dysfunction. However, this consistency is not apparent when MDD is studied in elder patients. Previous studies have mainly used screening tests instead of comprehensive neuropsychological batteries. Therefore, the neuropsychological profile of elderly MDD in the acute phase, and more importantly its cognitive status after treatment, remains to be ascertained. It can be hypothesised that residual cognitive deficits might persist even when the depressive episode has remitted in the elderly, since a few recent studies have reported this in younger patients. Thus, the cognitive impairment associated to MDD could be considered as a trait characteristic, instead of a temporal state or a consequence of the illness.

Among the vulnerability factors for the development of MDD that have been proposed, neuroticism (N) is one of the most accepted, since many studies have identified a relationship between high scores on N and MDD. N is believed to play a crucial role in the way in which the body and the brain respond to emotional material or situations. Convergent results from studies of mood disorders support a model in which the signs and symptoms of MDD emanate from a dysfunction within the limbic and other brain systems that modulate emotional behaviour. Given the connection between N and MDD, it is possible to raise the following question: might the

neurocognitive functioning observed in MDD be similar in people with this vulnerability factor but who have never become depressed? This is relevant to find out if high N people are at risk for developing MDD.

1.2. GENERAL OBJECTIVES OF THE THESIS

The general aim of this dissertation is to study the trait-state neurocognitive characteristics of depressive disorders. Here we focus on late-onset MDD and on N –as a vulnerability factor for MDD–. For this purpose, different tools such as neuropsychological instruments and neuroimaging techniques are used in order to investigate the cognitive functioning and the brain characteristics of this illness and related topics. This dissertation consists of different studies designed to shed new light on MDD and N. The challenge is to untangle the neuropsychological and biological mechanisms mediating the vulnerability and the development of depressive disorders. We investigate the neurocognitive functioning to determine the impact of enduring deficits of brain function.

First, we carry out a longitudinal study in elderly depressed patients to determine if cognitive impairment remits after treatment (Chapter 4.1). As it has been previously reported in young people suffering from MDD, cognitive impairment may be a cause or an added symptom, rather than a consequence, of the illness. By assessing the most important cognitive domains implicated in elderly depressed patients, in the acute phase and twelve months after pharmacotherapy, the objective of this study is to ascertain whether cognitive impairment in elderly MDD is a trait rather than a state characteristic of the illness. In parallel, we try to clarify the utility of the planning task used in the elder sample (TOL), since we do not observe planning deficits in these patients, apparently due to a ceiling effect when the original version by Shallice is administered (Annex A). This work is performed in a new sample of healthy volunteers.

Given that residual neuropsychological deficits are observed in elderly major depression, we planned to investigate those characteristics related to depressive disorders in healthy volunteers who might be at some risk for developing the illness. It might be thought that the neurocognitive functioning observed in MDD could be present even before the onset of the illness or in people at risk. To answer this, we have designed three studies to test some of the issues involved in MDD in healthy subjects with very high scores on N (compared to low scorers), considering N as a risk factor for depressive disorders. The sample is selected to exclude those volunteers with a past or current history of DSM-Axis 1 disorders. The objective of the first of these studies (Chapter 4.2) is to determine whether healthy people at risk for developing depression might show cognitive abnormalities when processing information, either neutral or emotional information. Studies of endocrine function in patients with MDD have identified dysregulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis; the aim of the next study (Chapter 4.3) is to examine waking cortisol in these healthy people, to test the hypothesis

that high N itself is associated with altered adrenocortical regulation. Finally, in Chapter 4.4 and Chapter 4.5 we examine structural and functional brain involvement in people very high or very low in N with no history of mood disorders. For this purpose, we take into account that subjects with depressive disorders show increased amygdala responses to negative facial expressions; volumetric differences of amygdali and hippocampi volumes compared to normal subjects; and increased responses to emotion potentiated startle (EPS). Our studies aim at finding out if high neurotic subjects show any of these abnormalities.

2. THEORETICAL FRAMEWORK OF DEPRESSIVE DISORDERS

2.1. INTRODUCTION

Affective disorders are leading causes of both morbidity and mortality. Depressive disorders, together with bipolar disorder, rank among the major causes of disability worldwide (Murray and Lopez, 1997). Depression is a common affective disorder characterised by persistent negative mood and selective deficits in cognitive, circadian, and motor functioning. Traditionally, affective disorders have been considered to be relapsing and remitting conditions characterised by complete inter-episode recovery. However, recent evidence about MDD may suggest that even during periods of euthymia, cognitive impairments known to be present during mood episodes may still persist, either in young (Weiland-Fiedler *et al.*, 2004) and elder patients (Nebes *et al.*, 2000).

Theories implicating specific neurochemical and neuropeptide systems, focal lesions in specific brain regions, and selective dysfunction of known pathways have been proposed, and many are supported by a growing number of clinical and basic studies demonstrating anatomic, neurochemical, genetic, endocrine, sleep, and selective cognitive abnormalities in depressed patients. Despite these advances, a unifying neurobiological mechanism for major affective illness has not yet been identified.

To date it is not well established if depressive disorders are a unique mental disorder or consists of several subtypes differentiated by age of onset, psychotic characteristics, melancholia, course of the illness, etc… In this respect, comprehensive models for the causes of depressive disorders have addressed the question of how distinct risk factors interact in the aetiology of the illness (Kendler *et al.*, 2004), and what is the prognostic significance of these risk factors on the major subtypes of depression (Angst *et al.*, 2000).

2.2. MAJOR DEPRESSIVE DISORDER

2.2.1. Clinical features

The diagnosis of primary major depression (DSM-IV) is based on the presence of a persistent negative mood state in association with disturbances in attention, motivation, motor and mental speed, sleep, appetite, and libido, as well as anhedonia, anxiety, excessive or inappropriate guilt, recurrent thoughts of death with suicidal ideations and perhaps suicide attempts (Mayberg *et al.* in Yudofsky & Hales, 1997). It would be naïve to consider MDD as a consequence of a single brain location, lesion or neurochemical system; rather, the associated impairment of cognition

and somatic functions suggests that MDD is a composite disorder affecting discrete but functionally interconnected limbic, paralimbic, and neocortical circuits. The fact that depression has a biologic aetiology is suggested by many different studies, although precise biological mechanisms for the increased vulnerability of women or the relative constancy of age at onset are unknown.

The influence of environmental factors in the aetiology of depression is equally complex. No correlations between depression and socioeconomic status, education, or specific lifestyle have been demonstrated. Although stress is often seen as a precipitant, the relationship between stress and vulnerability to, or precipitation of, a depressive disorder is far from clear (Mayberg *et al.* in Yudofsky and Hales, 1997).

2.2.2. Pathophysiology of Major Depressive Disorder

No single neurotransmitter abnormality can fully explain the pathophysiology of depression or its associated constellation of mood, motor, cognitive, and somatic features (Bauer & Frazer, 1994). Even when a peripheral chemical marker for depression is identified, it still must be interpreted in the context of multiple neuroreceptor subtypes, second-messenger effects, and regionally specific regulatory mechanisms (Mayberg *et al.* in Yudofsky, 1997). Nevertheless, alterations in many neurochemical systems have been reported, and the most known are the monoamine system and the neuroendocrine system.

The Monoamine Theory

The monoamine theory for pathophysiology of MDD is the first recognised neurobiological theory. It states that depression is caused by a deficit of monoamine transmitters at certain parts of the brain. Some of the amines are Epinephrine (E), Norepinephrine (NE) and Serotonin (5-HT). These neurotransmitters affect a range of symptoms such as vigilance, motivation, euphoria, appetite, and impulsivity. NE and 5-HT have been the most extensively investigated, mainly because of the well-characterised effects of most typical antidepressant drugs on them. The original catecholamine hypothesis of affective disorders, which was proposed in the sixties, focused specific attention on the noradrenergic system. With the clinical introduction of selective 5-HT reuptake inhibitors (SSRI), more recent work shifted attention to the serotonergic system. The findings on antidepressant effects suggest that individual modulation of either NA or 5-HT neurotransmission is beneficial in treating depression (Slattery *et al.*, 2004). Furthermore, post-mortem studies have addressed the levels of neurotransmitters in depression and their receptors and transporters. One of the more consistent findings are elevated levels of 5-HT_2 receptors in the frontal cortex of suicide victims (see Coyle and Duman, 2003 for review). Adding more evidence to this theory, the presence of dense projections from the subgenual cingulate to the serotonin-rich brainstem dorsal raphe nucleus suggests that this cortical area plays some role in

the regulation of serotonergic activity, an activity that may be impaired in depression (Mayberg *et al.*, 1999). Depletion studies have been performed in a further attempt to elucidate the role of NA and 5-HT in depression. In an SSRI treated group, tryptophan depletion causes a relapse in depressive symptoms and specially in those whose symptoms had remitted for under 2 weeks while having little effect on patients treated with mainly noradrenergic drugs (Bremmer *et al.* 1997).

Although this theory is widely used to explain the pathophysiology of MDD, it is not ideal. The effect of the antidepressant drugs that elevates the state of mood (SSRI, selective NE 5-HT reuptake inhibitors –SNSRI–) and post-mortem studies of depressed patients provide confirming evidence of this theory. But also, there is inconclusive evidence against it. As latter examples of this, it has been found that some NE and 5-HT antagonists have minimal effect on mood. Depletion studies also remain equivocal: control patients subjected to tryptophan depletion do not demonstrate a lowering of mood. Similarly, untreated depressives subjected to this paradigm do not demonstrate any worsening of symptoms (Slattery *et al.*, 2004). Besides, a primary dopaminergic mechanism for depression is generally considered to be unlikely (Mayberg *et al.* in Yudofsky and Hales, 1997). Also, amphetamine and cocaine have no direct effect by their own on mood, neither L-Dopa drugs; however, the mood-enhancing properties and clinical utility of methylphenidate in treating some depressed patients is well documented, although dopaminergic stimulation alone is inadequate in alleviating all depressive symptoms. Despite considerable advances in the treatment of mood disorders during previous decades, there remains an urgent need to identify compounds that will successfully treat mood episodes (including the associated cognitive impairments) and prevent their recurrence.

The Neuroendocrine Theory

As documented by Mackin and Young (2004) both Kraepelin and Freud in the beginning of last century regarded endocrinology as potentially important in the causation and treatment of psychiatric disorders. The role of dysfunctional endocrine systems in the pathogenesis of affective disorders has been the focus of research for many decades. Studies of endocrine function in patients with depression have identified dysregulation of the Hypothalamic-Pituitary-Adrenal (HPA) axis. Cortisol, a glucocorticoid released from the adrenal cortex, is the end product of the HPA axis. A variety of stressors, both physical and psychological, cause the neurosecretory cells within the paraventricular nucleus of the hypothalamus to secrete corticotrophin-releasing hormone (CRH), which acts on the anterior lobe of the pituitary gland to secrete adrenocorticotrophic hormone (ACTH).

Defects in the HPA axis may cause increased secretion of cortisol. Hypercortisolaemia is associated with alterations in glucocorticoid receptors (GRs) and neurotoxicity in the hippocampus, which is an important structure of the limbic system. The hippocampus normally exhibits an inhibitory effect on the HPA axis through feedback inhibition. The inhibitory effect

may be impaired when GRs are altered, leading to increased cortisol production and increased neurotoxicity in the hippocampus. A dysfunctional hippocampus may result in some of the depressive symptoms. This could be due to the fact that glucocorticoids inhibit neurons from using glucose, or by increasing the rate of calcium entry into cells, which in large concentrations is toxic. In addition, glucocorticoids can decrease the expression of brain-derived neurotrophic factor (BDNF) necessary for cell proliferation in the hippocampus. In 1986 (Salposky *et al.*), it was postulated that a neurodegenerative effect of cortisol may underlie some of the cognitive deficits observed in MDD. More recently, it has been suggested that cognitive deficits may represent permanent and possibly irreversible hypercortisolaemia-induced damage to crucial neuronal circuits. An early re-establishment of normal HPA activity in mood disorders before permanent deficits in cognitive function occur may therefore be an important therapeutic goal (Mackin and Young, 2004).

While there is substantial evidence indicating that the hippocampus is particularly sensitive to elevation of glucocorticoids, the effects on other areas of the brain are less clear. Cortisol releasing factor (CRF) neurons have also been found in extrahypothalamic sites. CRF neurons from these sites have been found to project to major sites of origin of NE and 5HT neurons. Defects in the CRF system would then be expected to influence NE and 5HT neurons that directly affect mood and behaviour.

2.3. COGNITIVE FUNCTION IN MAJOR DEPRESSIVE DISORDER

Cognitive deficits are a common and potentially debilitating feature of affective disorders. In MDD, impairment is most often found in attention, memory, and psychomotor speed (Austin *et al.*, 1992; 2001). Cognitive deficits are of moderate intensity but can become severe in prolonged or intractable depression, adding to everyday functional disability (Mayberg *et al.* in Yudofsky and Hales, 1997). Besides, clinically significant anxiety, which further impairs cognitive efficiency, occurs in many patients with depression (Rathus and Reber, 1994).

Cognitive impairment is often more frequent and of greater severity in elderly patients with depression than in their younger counterparts. Several physiologic mechanisms had been proposed for this effect of age, including greater baseline neuronal loss in the elderly (McHugh and Folstein, 1979) and age-related decreases in regional monoamine neurotransmitters (Savard *et al.*, 1980).

2.3.1. Frontal lobe involvement in elderly major depression

(Translation of the published review by Portella *et al. Rev Neurol* 2002; 35(9): 891-4)

2.3.1.1. Introduction

Over the last decade there has been increased interest in assessing the cognitive state of patients with psychiatric disorders. Part of this interest has concerned major depression, a common disorder in the general population. In this regard, several studies have sought to determine whether affective disorders show a differential pattern in terms of alterations in cognitive functioning.

At present it seems difficult to find an accepted neuropsychological profile for depression as the different studies vary with respect to their chosen taxonomy of depressive disorders, the neuropsychological functions explored, and the age range of patients included in the sample, among other aspects. As yet there is no consensus in terms of determining whether there is a single depressive disorder or whether clinical findings refer to different disorders that depend, among other factors, on the age of onset and the presence or absence of external factors. It should be noted, however, that the current trend is towards considering different affective disorders with respect to criteria of severity, treatment response and evolution of the disorder (Parker, 2000). In this context, a number of studies have addressed what is referred to as late-onset or elderly major depression (EMD). The importance of this nosological entity resides both in the fact that depression is undoubtedly the most common psychiatric disorder among the elderly population (Cervilla, 2001), and with respect to treatment and evolution of the disorder, in the difficulty of diagnosing early dementia or affective disorders. Moreover, some studies have suggested that EMD may be associated with vascular disease (Thomas *et al*., 2000). Aetiological models of EMD have focused particularly on the presence of microvascular lesions in cerebral white matter, as observed in magnetic resonance imaging (MRI) studies (Hickie and Scott, 1998). Nevertheless, the proposed models so far debated in the literature are partial. One of them involves damage to the hippocampus and predicts deficits in semantic memory. Another concerns lesions in subcortical structures and the basal ganglia that are regarded as causing depression in subjects over the age of 45 (Reischies and Neu, 2000), and which may predict the irreversibility of cognitive deficits following remission of the depressive disorder.

A pattern of neuropsychological deficits is one of the characteristics of MDD; in the case of EMD this seems to be associated with the severity of the disorder (Butters *et al.*, 2000), which is closely linked to the deterioration suffered by the brain during normal ageing. Thus, considering EMD as a slightly separate nosological entity is consistent with the idea that the brain of an elderly individual deteriorates more than that of a young person, even in the absence of any neurological disorder (Cervilla, 2001).

2.3.1.2. Neuropsychology of Major Depressive Disorder

At present it is still difficult to speak of a specific and differential neuropsychological profile for MDD. Clearly, assessing the cognitive impairment in MDD may be a key factor in determining the degree to which a subject's daily activities are affected. Also important are the efforts to unite theories from cognitive neuropsychology with the anatomy and physiology of brain functioning. If depression is a brain dysfunction, as research on the biochemistry of psychiatric disorders suggests, then studying the associated neuropsychological deterioration may enable us to identify the neural substrates underlying the disorder (Austin et al., 2001); naturally, these substrates may be both anatomical and biochemical. The use of drugs in treating depression has been and continues to be of great importance in terms of discovering the neural pathways and chemical substances involved in mental disorders. However, although antidepressant medications have been used for decades, the neurobiological substrate underlying their effectiveness is yet to be fully understood. Indeed, although the effect of these drugs on monoamines is well established, more recent evidence suggests that they may also affect other neurotransmitter systems; for example, the continued use of antidepressants produces changes in N-methyl-D-aspartate (NMDA) glutamate receptors, exclusively in the cortex (Michael-Titus *et al.*, 2000). This provides evidence of the brain's interconnections, both anatomical and physiological, and their role in overall cerebral functioning.

There is wide agreement among researchers regarding both the presence of cognitive impairment during depressive disorders, and that the most affected functions are memory (Austin *et al.*, 1992; Basso and Bornstein, 1999) and psychomotor speed (Hart and Kwentus, 1987; Hickie *et al.*, 1999; Krishnan *et al.*, 1991; Lemelin and Baruch, 1998). These memory deficits and psychomotor slowing are associated with a greater severity of the depressive disorder (Beats *et al.*, 1996). Some studies have also reported residual cognitive impairment following remission of the illness (Marcos *et al.*, 1994).

Another neuropsychological aspect that has generated interest in studies of depressed patients is executive function, which encapsulates planning, sequencing, organisation and abstraction (Lezak, 1995). The conclusions drawn in the first studies looking at alterations in executive function were somewhat unclear, although in general significant deficits were found in patients with more severe depression (Silberman *et al.*, 1983). The pattern of executive deficits reported in more recent studies has, however, been more consistent (Beats *et al.*, 1996; Austin *et al.*, 1999; Brodaty *et al.*, 2001; Purcell *et al.*, 1997), and researchers have found deficits in frontal functions such as verbal fluency, working memory, and attentional switching, among others. It should be noted that the studies reporting executive dysfunction were conducted either with subjects over 60 years old or in those with severe depression. However, other authors have failed to find a specific executive dysfunction in depressed subjects (Elliot *et al.*, 1997), such

deficits also being found in young subjects with dysphoria or mild depression (Channon and Green, 1999).

Controversy has also arisen with respect to those neuropsychological deficits correlated with the degree of severity. Some researchers have used cognitive-behavioural paradigms of motivation to explain the neurocognitive deterioration observed in depression. Motivation has been defined as 'the ability to initiate an appropriate activity, either spontaneously or in response to environmental clues' (Lezak, 1995). The concept of motivation is based predominantly on studies of patients with frontal lobe lesions, where both motivation and affect are significantly affected. It seems that a motivational deficit has the potential to hinder performance on any neurocognitive task.

The neuropsychology of EMD has also been studied in detail and it is now widely accepted that the cognitive alterations affect immediate and medium-term memory (Austin *et al.*, 1992). Current research is addressing what has been termed the executive dysfunction-depression syndrome in EMD. This syndrome is relatively resistant to antidepressant treatment and has a poor long-term prognosis (Kalayam and Alexopoulos, 1999; Alexopoulos *et al.*, 2000). Some studies suggest that a fronto-striatal alteration contributes to the onset of depression as this disorder is common in patients with damage to subcortical structures—for example, in vascular dementia, Parkinson's disease, Huntington's chorea, progressive supranuclear paralysis and basal ganglia calcification (Sobin and Sackeim, 1997). Although the results of both structural and functional brain imaging studies remain somewhat confusing (Hickie *et al.*, 1999; Elliot *et al.*, 1997; Salloway *et al.*, 1996; Dolan *et al.*, 1994), they suggest in general that dysfunction of the fronto-striatal thalamic tracts is associated with MDD (Dunkin *et al.*, 2000). In this regard, single-photon emission computed Tomography (SPECT) neuroimaging studies have found hypoperfusion in the frontal lobes (Navarro *et al.*, 2001), and that this perfusion normalised with symptom remission in subjects affected by EMD (Navarro *et al.*, 2002). Nevertheless, although the neuropsychological deficits in EMD have been widely studied, few attempts have been made to integrate the neuropsychological and neuroimaging results for EMD into a comprehensive theoretical model (Austin *et al.*, 2001).

In light of the results described here, however, it is possible to establish a specific neuropsychological profile for MDD in young adult subjects, in which mnesic functions and psychomotor speed are affected. In EMD, in contrast, memory deficits and psychomotor slowing are accompanied by executive dysfunction. The results of neuroimaging studies are consistent with the above in that they report alterations in frontal and subcortical structures, especially in subjects over 50 years old. It should also be noted that magnetic resonance studies have found that adults over 45 show more fronto-subcortical lesions than do younger subjects, a finding that has been linked to age (Reischies and Neu, 2000).

2.3.1.3. Functional neuroanatomical pathways in Major Depressive Disorder

Given the results of neuropsychological and neuroimaging studies of MDD, it remains unclear whether there is a consensus on which to establish a neuroanatomical model involving frontal and temporal cortical areas, along with subcortical areas. Nevertheless, studies of cerebral functioning in healthy subjects suggest that the frontal lobes are responsible for overall brain functioning.

The frontal lobes have been divided into five neurofunctional areas (Alexander and Crutcher, 1990): motor cortex, premotor cortex, frontal operculum, prefrontal area, and the paraolfactory or subcallosum area—for a review of each area see Estévez *et al.* (2000). The prefrontal cortex comprises those areas that receive projections from thalamic nuclei (dorsomedial, ventral anterior, medial pulvinar and the suprageniculate nucleus) (Burruss *et al.*, 2000; Fuster, 1997), and can therefore be divided into three areas: dorsolateral, orbitofrontal and frontomedial. The dorsolateral prefrontal cortex (DLPFC) sends and receives information to and from associative areas in the parietal, occipital and temporal lobes, and is involved in functions such as concept formation, reasoning, executive functions (planning, sequencing, organisation, etc.) (Lezak, 1995), the generation of voluntary actions, and the management of objectives during the exploration and processing of secondary objectives (Koechlin *et al.*, 1999).

It was initially hypothesised (Cummings, 1993) that patients with depression showed impairment in the limbic system, this having repercussions on the autonomic and vegetative systems, as well as on mood. However, these studies did not refer to the cognitive symptomatology found in MDD. Subsequent findings from studies of cerebral activation provided strong support for the role of the limbic system—specifically the subgenual cingulate (Drevets *et al.*, 1997) with the DLPFC—in depression (Teasdale *et al.*, 1999; Mayberg *et al.*, 1999) and proposed that certain structures of the medial prefrontal cortex, including the anterior cingulate, may be involved in the cognitive induction of negative feelings in healthy subjects. These studies suggest that affect and cognition could be anatomically related to the dorsolateral and orbitofrontal regions. The key role of the amygdala in the limbic system and fronto-striatal tracts has also been widely studied, and the resulting neural model suggests that dysfunction in limbic structures and the prefrontal cortex would interfere with the amygdala's modulatory functions, thus leading to the incorrect processing of emotional stimuli.

The ways in which these two prefrontal regions (dorsolateral and orbitofrontal) and subcortical structures interact with the rest of the brain remain unclear. Many of these functional networks have been implicated in the pathogenesis of certain psychiatric disorders, including MDD (Austin *et al.*, 2001; Austin and Mitchell, 1995). One possibility is that the biochemical alterations associated with MDD, or other mental disorders, result in a loss of connections in both the cortico-subcortical and the cortico-cortical pathways. This hypothesis, based on latest research findings, could lead, as Austin *et al*. (2001) propose, to a review of the fronto-

subcortical networks that are thought to operate independently and in parallel (Alexander and Crutcher, 1990). Thus, given that the mental illness in EMD affects an elderly brain, it could be expected that what is observed in EMD is a global alteration of cerebral functioning, in contrast to what occurs in young adults with MDD.

Current research into the cognitive impairment found in EMD is focused on alterations in the prefrontal cortex, leading to executive dysfunction, that is, poor performance on frontal neuropsychological tasks (Lezak, 1995). This executive dysfunction (Alexopoulos *et al.*, 2000) appears to be related to the development of the depressive disorder and with the lack of response to pharmacological treatment with SSRIs (fluoxetine) (Alexopoulos, 2001). However, the executive dysfunction in these studies is determined solely on the basis of mental status tests. The main finding of these studies is a role for the DLPFC, along with its functional connections to the rest of the brain.

It is widely accepted that the DLPFC, along with the other prefrontal regions, sends and receives information to and from almost the whole of the brain (Fuster, 2000). Indeed, the model of cerebral functioning used in these studies suggests that the neuropsychological profile of EMD cannot be determined without taking into account the DLPFC region. Thus, it could be hypothesised that the dorsolateral prefrontal area and its vast number of cerebral connections constitute the basis for the cognitive impairment in EMD. In other words, patients with EMD present a neuropsychological alteration that affects, on the one hand, temporal areas and the hippocampus (memory problems, psychomotor slowing, learning difficulties (Austin *et al.*, 2001)) and, on the other, frontal areas (perseveration, poor sequencing and planning) (Butters *et al.*, 2000).

According to the cerebral model of Fuster (2000) the DLPFC plays a role in the temporal organisation of action, this being necessary for any task. Stuss and Alexander (2000) also refer to the temporal mediation required in carrying out an action, this being reflected in the construction of expectations that function as a kind of abstract memory, depending on the action to be performed. The temporal organisation of an action to be carried out can thus be outlined as a hierarchical organisation of memory networks in the posterior cortex (perceptual memory) and the anterior cortex (executive memory). Thus, the role of the DLPFC, at the highest point of the hierarchy, can be thought of as the interpreter in the formulation and execution of plans of action.

2.3.1.4. Summary

Research conducted to date remains far from completely conclusive; furthermore, it is difficult to compare study results as there are too many differences between subject's samples, the neuropsychological tests used, and the aetiological model on which the research is based.

Nevertheless, some basic aspects of EMD can be described on the basis of the studies reviewed here. Most of the studies that have found alterations to frontal functions were conducted with subjects over 50 years old, this being the cut-off age for a first episode of EMD. Neuroimaging studies report a pattern of cerebral alteration in subjects over 45, from which it is deduced that there may be comorbidity between the depressive disorder and cognitive deficits among the elderly; these alterations correspond to fronto-temporal subcortical structures.

In conclusion, efforts to determine the presence of differential neuropsychological profiles should be centred around studies that enable comparisons to be made between young and elderly depressed subjects, using similar neuropsychological instruments and based on an integrated aetiological model that includes all the connections between the frontal lobes and the rest of the brain. Further, investigations should address the role of cognitive impairment in MDD not as a secondary symptom of the illness, but as a symptom itself.

2.4. NEUROIMAGING STUDIES OF MAJOR DEPRESSIVE DISORDER

2.4.1. Brain Imaging Techniques

The variety in the location of identified lesions is due in part to the technical and theoretical limitations of the anatomic methods. The combination of functional and structural imaging techniques provides an alternative strategy to test the following: i) how similar mood symptoms occur with anatomically or neurochemically distinct disease states, and ii) why comparable lesions do not always result in comparable behavioural phenomena.

Currently, the two main brain-imaging technologies available are emission tomography, which includes positron emission tomography (PET) and SPECT, and magnetic resonance imaging (MRI). Emission tomography involves administration of compounds containing radioisotopes, which emit positrons (PET) or gamma rays (SPECT). PET and, in more limited manner, SPECT are used to measure a variety of physiologic variables in vivo, including regional brain blood flow, oxygen metabolism and glucose utilization, etc., applied to the study of neuropsychiatric diseases. The advantages and disadvantages of different brain imaging modalities can be checked in a review by Anand and Shekhar (2003, page 373).

2.4.1.1. Magnetic Resonance Imaging

MRI is based on the principle that brain tissue emits electromagnetic radiation when placed in a magnetic field and stimulated by pulses of radiofrequency waves. The released radiofrequency waves correspond to different molecules in the brain, which emit radiation at different frequencies. Signals from protons in water, the most prevalent compound in the brain, and

signals from lipids can be processed to create images showing dark and bright regions that reveal the structure of the brain.

Another application of MRI technology is functional MRI (fMRI). Functional MRI is based on the assumptions that the focal change in neuronal activity is closely coupled to changes in local blood flow and blood volume. Blood oxygen level-dependent (BOLD) fMRI measures changes in local concentrations of paramagnetic deoxyhemoglobin. BOLD fMRI uses haemoglobin as a convenient internal contrast agent, relying on the magnetization difference between oxy- and deoxyhemoglobin to create the fMRI signal (Arthurs and Boniface, 2002). Areas of increased activation are presumed to have decreases in deoxyhemoglobin due to increased blood flow relative to oxygen extraction. The BOLD imaging does not measure tissue perfusion or flow directly, however, because over 70% of the brain's blood lies within the microvascular capillaries and venules, the measurement of the magnetic susceptibility-induced T2* signal loss is thought to mainly reflect the regional deoxygenation state of the venous system (Smith, 1998).

2.4.2. Neuroimaging findings in Major Depressive Disorder

It is frequently but inconsistently found by structural MRI that there is an association of decreased volume, possibly due to neurodegeneration, in a number of brain regions, particularly in the hippocampus, amygdala, frontal cortex, striatum, and other limbic areas such as the subgenual cingulate cortex (Anand and Shekhar, 2003 for a review). A number of neuroimaging studies have examined specifically volumetric changes in temporolimbic brain regions of patients with MDD such as the hippocampus and the amygdala.

Several reports from different investigators have found hippocampal volume reduction in MDD patients (Bremner *et al.*, 2000; Sheline *et al.*, 1999; Mervaala *et al.*, 2000; and Campbell *et al.*, 2004 for a meta-analysis). As it has been previously pointed out, a definite pathophysiology related to hypercortisolaemia neurotoxicity has been postulated. However, this finding has also been found in anxiety disorder (such as post-traumatic stress disorder). A strong relationship exists between MRI hyperintensities, vascular disease and depression; therefore vascular pathology may underlie hyperintensities and may be of particular significance in late-onset depression. Thus, individuals with late-onset depression would have acquired biological factors besides exposure to hypercortisolaemia (Lloyd *et al.*, 2004).

Separate literature suggests that depressed individuals have smaller core amygdala nuclei than never-depressed individuals (Drevets, 2000; Sheline *et al.*, 1998). Siegle *et al.* (2003) showed that amygdala activity during emotional information processing is related to amygdala volume in depression due to a potential hypercortisolaemia effect of the amygdala hyperactivity observed in MDD. However, other studies have found higher amygdala volumes in patients with MDD compared to healthy controls (Frodl *et al.*, 2002; 2003; Bremner *et al.*, 2000).

Functional abnormalities have been found in the amygdala in MDD patients. In the left amygdala, healthy humans increase cerebral blood flow during exposure to pictures of faces expressing fear, but this response is blunted in depressed subjects. Sheline *et al.* (2001) reported that hemodynamic responses in the left amygdala were exaggerated in MDD subjects exposed to fearful or smiling faces that were displayed during 40 ms. Consistently, significant greater left amygdala activation in MDD are also observed in PET studies of increased resting metabolism and blood flow (Drevets *et al.*, 1992). The duration of the amygdala response to emotionally valenced stimuli is also abnormal in MDD. Siegle *et al.* (2002) reported that the elevation in hemodynamic activity occurring in the amygdala during exposure to sadly valenced words persisted for an abnormally long time in depressives relative to controls. Nevertheless, it is conceivable that the elevated amygdala activity in MDD reflects an exaggerated response to the stress, which may be mediated by the positive feedback between amygdala neuronal activity and secretion of CRH, cortisol, and NE, which appear dysregulated in MDD.

2.5. RISKS FACTORS FOR MAJOR DEPRESSIVE DISORDERS

There is overwhelming evidence that risk factors impact on the aetiology of depressive disorders. Kendler *et al.*, (1993) proposed a fitting model to predict the liability to major depression, which was able to predict 50.1% of the variance. The strongest predictors of this liability were, in descending order, stressful life events, genetic factors, previous history of major depression, and N. In a later study (Kendler *et al.*, 2004) found that the most potent risk factors for MDD were N, gender and stressful life events. In particular, MDD, together with dysthymia and seasonal affective disorder, are more common in women. Over the course of a lifetime, about one in five women will experience major depression or dysthymia, compared with about one in ten men (American Psychiatric Association, 1994). People with a family history of depressive disorders tend to be at increased risk of developing depression (Angst *et al.*, 2003). In addition, a history of one or more previous episodes of depression significantly increases the risk of a subsequent episode (Kessing *et al.*, 2004). A stressful change in life patterns can trigger a depressive episode. Such stressful events may include a serious loss, a difficult relationship, trauma, or financial problems. N has been interpreted as a marker of “psychobiological vulnerability”, and long-term difficulties (i.e. stressful life events) as a marker of “environmental vulnerability” (Ormel *et al.*, 2001). Special attention will be paid to N and to its relation with MDD through this thesis.

2.5.1. Neuroticism

2.5.1.1. Concept of Neuroticism

> *"High N scorer is generally anxious, worrying individual, moody and frequently depressed. He is likely to sleep badly, and to suffer from various psychosomatic disorders. He is overly emotional, reacting too strongly to all sorts of stimuli, and finds it difficult to get back on an even keel after each emotionally arousing experience. His strong emotional reactions interfere with his proper adjustment, making him react in irrational, sometimes rigid ways… If the high N individual has to be described in one word, one might say that he is a worrier; his main characteristic is a constant preoccupation with things that might go wrong, and a strong emotional reaction of anxiety to these thoughts".*
>
> *Hans Eysenck, 1947.*

Hans Eysenck (1967, 1987) was one of the pioneers in attempting to relate personality to biology. By using the statistic known as factor analysis, Eysenck concluded that all human traits can be broken down into two distinct primary dimensions of behaviour: extraversion-introversion and neuroticism-stability. Most experimental and theoretical statements concerning the biological substrates of personality are directly or indirectly related to his theory, whose basis of introversion-extraversion, neuroticism-stability, and socialization-psychoticism (Eysenck, 1987) has evolved from taxonomic work (Eysenck and Eysenck, 1964) to a proposed biological model (Eysenck, 1967) that has been the basis of a variety of suggested modifications (Cloninger, 1987; McCrae and Costa, 1990). Eysenck's theory is an impressive effort to conceptualise and test brain functioning and personality. It was however developed prior to modern methods of testing brain functioning and it might be wrong in some respects. One of its basic assumptions is that the human brain has excitatory and inhibitory neural mechanisms. Balance between the two produces a level of psychological arousal at any given moment. Eysenck hypothesised that this balance was regulated by the Ascending Reticulo-Cortical Activating System (ARAS), which is a structure in the brainstem thought to control cortical arousal. However, the ARAS is not such a general system as was first thought. Differing levels of arousal across different parts of the brain are normal. N is based on a separate biological system related to the visceral brain (the hippocampus-amygdala, cingulate, septum, and hypothalamus) that produces autonomic arousal. Eysenck (1967) believed that N reflected how sensitive a person's sympathetic nervous system (SNS) was. This system is part of one's autonomic or involuntary nervous system. The "fight or flight" response is often attributed to it. Eysenck (1987) found that more neurotic people had hypersensitive SNS, which suggests that constant worry reflects an always-active SNS.

Neuroticism was initially designed to measure emotionality (Eysenck and Eysenck, 1964) and has been identified as a major personality dimension by almost all subsequent investigators. In adulthood, neuroticism is stable over time (McCrae and Costa, 1990). Neuroticism (N) is one

of the most accepted vulnerability markers since many studies have identified a relationship between high scores on N and MDD.

2.5.1.2. Neuroticism as a risk factor for Major Depressive Disorder

Depression is a highly prevalent psychiatric disorder. There is a shared and continuous liability between personality characteristics such as N and depressive disorders. Neuroticism is heritable and it is genetically correlated with major depression (Fanous *et al.*, 2002). Adverse life events are known to precipitate the onset of depression and anxiety (Kendler *et al*., 1999; 2003). The complex interplay between life events and genetic liability is thought to be crucial to the aetiology of affective disorders.

Determining the nature of relationships between personality traits and the risk for depressive disorders is an important enterprise that may help clarify the aetiologies of the latter conditions. Following Kendler *et al*. (2004), it can be assumed that high levels of N are associated with risk for major depression. These authors found two ways by which the impact of N on MDD risk was greater for individuals at high levels of adversity: first, they found that N directly increased the risk of illness at every level of stress exposure; second they found that high N moderated the pathogenic effects of stress exposure. Kendler and co-authors (2004) concluded that individuals with low scores on N were much less sensitive to the depressogenic effects of adversity than those with high levels of N.

2.5.2. Stress and Major Depressive Disorder

The human body has a variety of stresses to cope with. These include not just emotional stress, but physical stress. The HPA axis evolved to help the body to coping with physical stress. When humans lived by hunting and gathering, the ability of the body to change from quiet waiting to sudden extreme physical exertion was crucial. This was essential not only to chase the prey, but also to avoid being prey (Allman, 2000). The HPA axis is also stimulated by emotional stress.

Hans Seyle (1936) proposed a model of general adaptation to stress and he assumed that an organism, when confronted with a stressor, would respond in a non-specific way. However, he gave a very limited role to psychological factors such as personality, subjective perceptions, etc., since he assumed that all responses to stress were uniform. This neglects the fact that many people experience negative effects of stress earlier in the sequence, or even before the event has happened. By contrast, Richard S. Lazarus (1974), taking into account a psychological view of stress, suggested that when people are confronted with a stressful event, they engage in a process of primary appraisal to determine the meaning of the event: positive, negative, or neutral. Also, people confronted with a stressful event assess harm, threat, or challenge, and consider whether they have sufficient resources and coping abilities to overcome the issues posed by the stressor.

Stressful life events such as the death of a loved one, relationship and/or marital problems, troubles at work, financial difficulties, unemployment… can trigger a depressive episode. Sometimes even positive life changes such as marriage, new relationship, job promotion, or moving can trigger a depressive episode. The association between life events and depression is, however, influenced by various person-environment interactions (Rutter *et al*. 1997). First, some individuals may be more vulnerable to depression following life events than others (person-environment interaction). Secondly, some individuals are more likely than others to experience life events in the first place (person-environment correlation). Previous research has suggested that vulnerability to both stressful life events and experiencing these events are partly under genetic control (Kendler *et al*., 1995). The brain changes in MDD patients may be related to the brain's response to stress (or bad experiences) in early life. Later in life, this may cause the brain to react to one or more new negative events. Without any negative life events, people may avoid the risk for developing depression.

As explained in Chapter 2.2.2, stress prompts the release of CRF from the hypothalamus. As already mentioned, CRF is a hormone that acts on the pituitary gland, yielding ACTH secretion. One of ACTH´s actions is to increase the production of cortisol from the adrenal glands. Cortisol is an essential stress hormone (corticosteroid) and is needed for survival. Also, cortisol inhibits the release of CRF and ACTH. Thus, cortisol feedbacks the loop and prevents excessive secretion of CRF, ACTH and cortisol. The feedback part of the loop is essential to stop the HPA axis going into overdrive. There is overwhelming evidence indicating that during periods of acute stress, glucocorticoids promote survival by mobilising energy reserves. Levels of cortisol rise with acute stress periods (either emotional or physical). Although glucocorticoid production is essential for survival, overproduction is associated with a significant disruption of cellular functioning, which, in turn, leads to widespread physiological dysfunction. When an organism is subjected to a long period of stress, some detrimental effects occur. Dehydroepiandrosterone (DHEA) levels fall, leaving the high cortisol levels unchecked. CRF remains raised, which produces poor sleep, decreased appetite, and low sex drive –all symptoms seen in depression. Prolonged levels of stress can produce structural changes in neurons from the hippocampus.

There is robust evidence demonstrating abnormalities of the HPA axis in MDD. These abnormalities include raised levels of CRF and deficits of the cortisol receptors in the feedback loop, so that the loop is constantly overactive, even in the presence of cortisol. Also, secretion of cortisol does not follow the usual daily changes that are seen in healthy people. These changes can be very subtle and are only seen using specialised and complex endocrine tests. Hypercortisolaemia caused by prolonged-stress periods might be central to the pathogenesis of depressive symptoms and cognitive deficits. Manipulation of the HPA axis has been shown to have therapeutic effects in both preclinical and clinical studies, and recent data suggest that

direct antagonism of GRs may be a future therapeutic strategy in the treatment of mood disorders (Mackin and Young, 2004).

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3. HYPOTHESES OF THE THESIS

The hypotheses raised for study in the present thesis are the following:

Hypothesis 1

Elderly major depressed patients show cognitive deficits in those cognitive domains known to be involved in MDD, as well as in executive function. This impairment will remain even after remission of the illness.

Hypothesis 2

Highly neurotic volunteers show deficits in cognitive functions and biases towards negative information in line with their vulnerability for developing mood and anxiety disorders.

Hypothesis 3

Healthy subjects with high scores on N –compared to subjects with low scores– have altered adrenocortical regulation, similar to the observed in MDD. This will be assessed by examining salivary cortisol levels at waking and throughout the day in high and low N subjects.

Hypothesis 4

Healthy volunteers high on N show similar amygdala responses to conscious facial expressions as the ones observed in subjects with affective disorders. This will be tested with a functional MRI block design.

Hypothesis 5

Healthy subjects with high scores on N show some abnormalities in medial temporal structures as it has been observed in subjects with depressive disorders: increased signal changes in amygdala functioning in unconscious facial expression recognition –fMRI study–, volumetric abnormalities of medial temporal lobe structures (hippocampus and amygdala) –quantitative MRI study–, and increased emotion potentiated startle responses to emotional pictures.

4. RESULTS OF THE THESIS

4.1. RESIDUAL COGNITIVE IMPAIRMENT IN LATE-LIFE DEPRESSION AFTER A 12-MONTH PERIOD FOLLOW-UP.

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4.1.1. Introduction

Late-life depression involves an impairment of cognitive functions such as memory and psychomotor speed in the acute phase of the disease (Abas *et al.,* 1990; Ilsley *et al.,* 1995; King *et al.,* 1998; Lemelin, S. & Baruch, P., 1998; Basso et al. 1999; Schatzberg *et al.,* 2000; Austin *et al.,* 2001). Impairment of frontal or executive functions in late-onset major depression has been more fully examined in recently years (Beats *et al.,* 1996; Lesser *et al.,* 1996; Kalayam *et al.,* 1999; Lockwood *et al.,* 2002) and is related to the severity of the depression (Goodwin, 1997; Taylor *et al.,* 2002) and it may be associated with vegetative symptoms (Palmer *et al.,* 1996).

It is still unclear if the cognitive impairment is a state characteristic of major depression in the elderly, or whether the cognitive impairment persists upon recovery. In elderly people who suffer from major depression, response to treatment may not be complete, and this could lead to the disease becoming chronic. Alexopoulos *et al.,* (2001) has reported residual cognitive impairment in geriatric depression, involving the executive functions of patients who did not respond to pharmacological treatment. Some studies have reported changes in cognitive functioning after treatment, but they used brief mental status test (Butters *et al.,* 2000; Alexopoulos *et al.,* 2000). However, little is known about the course of the cognitive impairment of the disease, beyond successful or unsuccessful treatment. Butters *et al.,* (2000) studied an elderly sample longitudinally (one-year follow-up), but they excluded those patients who did not response to treatment.

It has been proposed that there may be a vascular etiology of late-life depression (Hickie *et al.,* 1999). This question is still controversial because some neuroimaging studies have reported a normalisation of functionality brain impairment after remission in late-life depression (Navarro *et al.,* 2002), and some have shown chronic brain abnormalities (Sheline *et al.,* 1996; O'Brien *et al.,* 1998; Alexopoulos *et al.,* 2002).

This present study investigated the cognitive impairment of elderly major depressed patients as a prospective follow-up study. The main objective was to assess the most important cognitive domains implicated in late-life depression, in the acute phase and twelve months after pharmacotherapy. Furthermore, to establish whether there was long-term residual cognitive dysfunction, we assessed remitted and non-remitted patients after treatment.

4.1.2. Method

Study Group

The study was conducted at the Hospital Clínic of Barcelona. Forty-five right-handed in- and outpatients with unipolar major depression aged 60 years or over were recruited. To be eligible for inclusion, patients had to fulfil the DSM-IV criteria (American Psychiatry Association, 1994) for a current major depressive episode, with or without endogenous or psychotic features. We introduced the modification that the symptoms must have been present for at least one month. Only late-onset depressed subjects were included, that is, those in whom depression had begun after age 50 years. The patients underwent routine medical examination, electrocardiographic (ECG) recording and laboratory screening, including full blood count and thyroid function-tests. Thus, we excluded patients with significant abnormal biological findings on electrocardiographic recording or laboratory examination, those taking medications with potential central nervous system side effects, those with neurological disorders, and those with uncontrolled medical illness at the time of recruitment. Psychiatric exclusion criteria included any history of mania, hypomania or psychosis, current substance dependence, and electroconvulsive therapy (ECT) within 6 months of recruitment.

Elderly healthy comparison subjects (n=15) with no psychiatric history were recruited from the community. They had no personal history of psychiatric illness of substance abuse ascertained by the SADS-lifetime version (Endicott and Spitzer, 1986) and none had been prescribed psychotropic medication. The ethical committee of our hospital approved the study. All subjects signed informed consent statements.

Instruments

Clinical Assessment. Upon recruitment, all study candidates were assessed clinically. Depressive symptoms were rated on the 17-item Hamilton Depression rating Scale (HDRS, Hamilton 1960). All patients had to have a baseline HDRS score of 21 or greater. We also screened for reduced global cognitive function, defined as a score of 24 or lower on the Mini-Mental State Examination (MMSE, Folstein *et al.*, 1975), and dementia was discarded according to the DSM-IV criteria as a confounding variable of organic cognitive impairment. Vascular risk factors were quantified following Baldwin and Tomenson (1995), since the apparent relationship between vascular disease and high intensity lesions on magnetic resonance images of patients with elderly major depression has led investigators to suggest that vascular mechanisms may be the most important factor in the development of elderly major depression (Alexopoulos *et al.*, 1997, Krishnan *et al.*, 1997, Simpson *et al.*, 1998).

Neuropsychological Assessment. All subjects were evaluated by means of a test battery comprising the following instruments: Block Design, digit span (forward and backward) and digit symbol subtests of the Wechsler Adult Intelligence Scale (Wechsler, 1990); immediate

logical memory (LM I) and visual memory (VM I) and delayed logical memory (LM II) and delayed visual memory (VM II) of the Wechsler Memory Scale, revised (Wechsler, 1987); Trail Making Test, form A (TMT-A) (Reitan *et al.*, 1974) and the Tower of London (TOL) (Shallice, 1982). These instruments were selected to assess performance on the following neuropsychological domains, known to be involved in cognitive impairment of major depression: processing speed, executive function, verbal memory, visual memory, visuoperceptive function, and attention span. Block Design analyses the visuoperceptive function but it also implies a problem-solving component and so, it is a measure of planning and according to Lezak (1995) it can be considered as an executive test.

Study design. Patients underwent a minimum of a ten-day antidepressant medication wash-out and a minimum of two-day benzodiazepine medication washout. Baseline cognitive and clinical assessments were then conducted. After the baseline examinations, treatment was started (citalopram as the first treatment, nortriptyline as the second treatment when patients did not respond and ECT as the final chance of treatment), adjusted to standard therapeutic norms. All patients attended a monthly follow-up with the psychiatrist. Remission was defined as a 17-item HRDS score below 8 between the 6-month visit to the 12-month visit. After twelve months, all the patients (remitted and non-remitted) were neuropsychologically assessed with the same cognitive tasks. Antidepressant treatment was suspended temporarily for 10 days before the 12-month neuropsychological assessment. Control subjects underwent neuropsychological assessment at only one time-point.

Design and Data Analysis

This present work is a cross-sectional study for baseline and a longitudinal study for the follow-up of elderly depressed patients assessed before current pharmacological treatment started, and 12-months after the first evaluation. The final sample of the experimental group was of 30 subjects. Fifteen subjects were excluded from the cross-sectional and longitudinal study because they did not fulfil the complete assessment. These excluded patients did not differ from the included patients in any of the neuropsychological tasks, or in demographic variables. The control sample was of 15 healthy subjects for the cross-sectional part of the study. Demographic variables were explored to avoid differences between groups, by using Student's t-tests, and χ^2.

Student's t-tests compared the performance ratings of the two subject groups (control group and patients group) on each neuropsychological baseline task. In those comparisons where there were not similar variances, Satterthwaite's modification was taken into account. For the follow-up analysis we performed a mixed analysis of variance (ANOVA) for every neuropsychological task that included the between-subjects variable of group (remission *vs*. non-remission) and the within-subjects variable of time (baseline *vs*. follow-up assessments). For the comparisons, the

alpha level was set at 0.05. *P* values were adjusted by Bonferroni correction since a large number of comparisons were carried out ($p<0.004$). Two-tailed statistics were used throughout the study.

4.1.3. Results

Sample characteristics

The mean age was 72.07 years (*SD* = 5.93; range 65-84 years) in the patients group and 70.87 years (*SD* = 5.13; range 65-80 years) in the controls group. The baseline score of MMSE was 26.70 (*SD* = 0.95) and the final score was 28.70 (*SD* = 2.28). The percentage of women in the patients group was higher than in the control group, but this difference was not significant. There were no differences between the two groups in years of schooling, neither in age nor in vascular risk factor (see Table 4.1.1 for sample characteristics). There were no significant differences between remitted and non-remitted patients on a number of characteristics, including age ($t = 0.37$; $df = 28$; $p = 0.71$), years of schooling ($t = 1.196$; $df = 28$; $p = 0.24$) and baseline score on MMSE ($t = 0.961$; $df = 28$; $p = 0.35$). Regarding response to treatment, 20% (6) were fully responsive to citalopram, 30% (9) % responded fully to second-line treatment (nortryptiline), and 50% (15) were treated with ECT, being some of these patients (9) "non-remitters".

Baseline Comparisons

The baseline performance to neuropsychological tasks of the patients group differed statistically from the controls group in Block Design test, Digit Symbol task, LM I, LM II and VM II (see Table 4.1.2). Patients' scores were lower than control scores in every neuropsychological task.

	Patient's group	*Control Group*	χ^2/t	*p*
n	30	15		
Sex (%women)	73.3	60	3.016	0.082
Age	72.07 (5.93)	70.87 (5.13)	0.668	0.508
Vascular risk factor	1.27 (0.74)	1.47 (0.64)	-0.892	0.377
Years of schooling	5.60 (1.48)	6.27 (1.39)	-1.460	0.152
Baseline HRDS score	30.43 (6.31)	1.90 (1.48)	17.144	<0.001
Baseline MMSE score	26.70 (0.95)	29.00 (0.85)	-11. 355	<0.001

Table 4.1.1. *Sample characteristics at baseline of the two groups. Values represent mean (SD).*

	Patients	*Controls*	*t*	p^+
Vocabulary	38.70(11.65)	47.67(14.42)	-2.247	0.048
Block Design	17.86(7.17)	27.73(7.55)	-4.252	0.000
Digit Symbol	13.00(7.03)	28.43(13.87)	-3.902	0.001
TMT-A	113.19(61.14)	75.53(44.59)	2.083	0.044
LM I	5.47(2.80)	8.80(3.82)	-3.326	0.002
LM II	2.52(2.33)	6.67(3.83)	-3.856	0.001
VM I	3.55(2.61)	6.40(4.12)	-2.436	0.024
VM II	1.34(1.88)	5.07(4.54)	-3.042	0.008
TOL	24.79(6.94)	26.71(5.68)	-0.924	0.361
Digits Forward	4.60(1.19)	4.13(0.64)	1.708	0.095
Digits Backward	3.03(0.93)	3.47(1.06)	-1.409	0.166

Table 4.1.2. *Baseline comparisons on neuropsychological performance between control group and patients group. *Values are expressed in Mean (SD).*$^+\alpha$*=0.005, after Bonferroni adjustment for multiple comparisons.*

	Baseline remitted	*Baseline non-remitted*	*Follow-up remitted*	*Follow-up non-remitted*	*F**	p^+
MMSE	26.81(1.03)	26.44(0.72)	29.62(1.72)	26.43(2.13)	20.1	<0.001
Vocabulary	40.57(11.36)	34.33(11.78)	41.71(10.57)	32.78(13.53)	1.1	0.307
Block Design	18(6.69)	17.50(8.80)	21.52(5.69)	17.25(7.78)	2.4	0.133
Digit Symbol	13.47(7.28)	11.71(6.63)	17.47(8.46)	13.71(9.05)	0.6	0.434
TMT-A	107.65(34.2)	131.67(117.1)	95.65(29.02)	98.67(56.70)	0.6	0.465
LM I	5.33(1.93)	5.78(4.35)	5.62(2.73)	5.89(4.68)	0	0.869
LM II	2.26(1.67)	3.11(3.48)	3.29(3.00)	3.22(5.09)	1.1	0.313
VM I	4.05(2.84)	2.25(1.28)	5.29(3.39)	3.50(1.77)	0	0.990
VM II	1.57(2.06)	0.75(1.16)	2.48(3.12)	0.50(1.07)	2.3	0.142
TOL	24.28(6.61)	25.63(8.02)	23.94(6.58)	20.50(8.50)	2.7	0.112
Digits Forward	4.71(1.31)	4.33(0.87)	4.90(1.22)	3.89(0.60)	3.3	0.079
Digits Backward	3.10(0.94)	2.89(0.93)	2.71(0.85)	2.67(0.87)	0.3	0.584

Table 4.1.3. *Neuropsychological performance. Values are expressed in mean (SD).*$^+\alpha$*=0.004, after Bonferroni adjustment for multiple comparisons. *Group-by-time interactions. Remission (remitted and non-remitted patients) by baseline and follow-up stages.*

Follow-up comparisons

Of the 30 patients who completed the study, 21 (70%) were considered remitters. These patients had recovered from depression after treatment (HRDS<= 8), and 9 (30%) had not recovered after twelve months from baseline (HDRS>8). Non-remitted patients were maintained in the study. Five of the twenty-one-recovered patients suffered relapses within twelve months of the follow-up. None of the patients developed dementia during the study period.

Analysis of cognitive performance tasks did not yield either group-by-time interactions, or time effects or group effects in any of the neuropsychological tasks, except in the MMSE score, where it was a group-by-time interaction, a time effect (F =22.76; df = 1, 28; $p < 0.001$) and a group effect (F = 12.41; df = 1, 28; $p = 0.001$) (see Table 4.1.3).

4.1.4. Discussion

This study investigated the cognitive functioning of elderly major depression at baseline and twelve months after. We assessed depressed patients with cognitive impairment at the beginning of the study. The main finding was that cognitive impairment is a trait characteristic of elderly major depression, since after twelve months from baseline, there is a cognitive dysfunction in remitted patients and in non-remitted patients.

At baseline, patients showed impairment in functions such as visuoperception, processing speed, and memory. Previous studies have demonstrated cognitive impairment in the elderly major depression related to its severity (Hart *et al.,* 1987; Boone *et al.,* 1994). More recent studies have shown frontal impairment in elderly depressed patients, and they have related it to poorer response to antidepressant treatment (Kalayam *et al.,* 1999; Kiosses *et al.,* 2001; Alexopoulos *et al.,* 2002). Simpson *et al.,* 1998 had showed that the extrapyramidal and pyramidal signs characterized the resistant groups of late-life depression (subcortico-frontal type of the neuropsychological impairment). Accordingly, our results find that late-life depression is frequently associated with cognitive dysfunction. However, we have not found impairment in executive functioning at baseline, and probably, the main reason could be a ceiling effect on the planning task used in this study.

After twelve months, our results suggest that depressed elders with cognitive impairment before treatment had not experienced improvement in specific cognitive domains. Thus, impairment of cognitive function in remitted patients after drug therapy, observed in this study could be understood as a trait characteristic of elderly major depression. Although some functions such as logical memory, psychomotor speed and sequencing were slightly improved from baseline in the remitted group, none of them had significantly recovered. Regarding the executive functioning, performance on executive tasks of remitted and non-remitted patients was lower twelve months after -although the differences were not statistically significant-.

These findings add to the body of evidence that elderly depression is frequently related to cognitive impairment of visuoperception, processing speed, and memory functions at baseline. Moreover, major depression in elderly patients yields a residual cognitive impairment even with successful antidepressant therapy. Accordingly to the vascular etiology, changes in central nervous system (CNS), such as MRI hyperintensities or decreased parenchymal tissue and basal ganglia lesions (Krishnan *et al.,* 1991; Butters *et al.,* 2000; Thomas *et al.,* 2002) seen in elderly depressed patients, could lower their main domains of cognitive functioning. But it does not seem feasible to be related to a dementia progression since the global functioning, measured by MMSE, is increased or maintained in almost all the patients at 12 months.

In conclusion, our results suggest that cognitive impairment is a trait characteristic of elderly major depression, regardless of the successful response to treatment. The residual cognitive impairment may reflect non-progressive brain dysfunctions rather than progressive dementia. Further investigations should be planned to ascertain the role of structural and functional changes in the brain in the aetiology and pathophysiology of major depression with residual cognitive impairment. Besides, a revision of the TOL (wooden version) should be carried out to determine whether the lack of planning/executive disability could be explained by a ceiling effect.

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4.2. COGNITIVE FUNCTION IN NEUROTICISM

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(Submitted by Portella *et al. Br J Psychiatry*)

4.2.1. Introduction

High scores on measures of neuroticism (N) have been suggested as a predisposing factor for developing depressive disorders (Schmitz et al., 2003; Roberts and Kendler, 1999; Duggan et al., 1995) as well as anxiety disorders (Breslau et al., 1991; Clark et al., 1994). Neuroticism is also associated with genetic risk of depression (Sen et al., 2004) and shows some of the same neurobiological vulnerability markers such as heightened early morning salivary cortisol (Portella et al., 2005).

It is commonly accepted that major depression is associated with cognitive deficits, which affect memory, executive functioning and attention (Austin *et al.*, 2001; Landro *et al.*, 2001). Mnemonic dysfunction is consistent across most studies (Sweeney *et al.*, 2000; among others) and it has been associated with a reduction of the hippocampal volume (Sheline, 1996). The studies examining impairment in executive tasks have produced conflicting results, although in recent reports it is mainly accepted that the dorsolateral prefrontal cortex (DLPFC) is involved in the pathophysiology of major depression and its executive deficits (Brody *et al.*, 2001; Teasdale *et al.*, 1999). After recovery, a few studies have reported persistent neuropychological impairment of some functions in elder subjects (Portella *et al.*, 2003; Butters *et al.*, 2000).

Emotional processing biases towards negative/threat-relevant information are observed in patients with anxiety disorders (Harrison *et al.*, 2003; Paunovi *et al.*, 2002; Neidhardt *et al.*, 1999). Similar biases of interpretation and memory are believed to occur in depression, particularly when tapping into themes of guilt, failure and loss (Beck and Clark, 1988). Beck's diathesis-stress model proposes that latent negative schemas are apparent before the onset of depressive disorder and are triggered by stressors to which the individual is particularly susceptible (Beck, 1967). However, it is unclear whether biases in emotional processing and/or cognitive deficits precede depressive or anxiety disorders or occur as a response to them.

The aim of this study was to look at cognitive functioning in volunteers high (versus low) in N who are at increased risk of developing depression but who had never suffered from depression or anxiety disorders themselves. Therefore, volunteers high and low in N were assessed on measures of attention, executive function and emotional processing previously found to be affected by some psychiatric illnesses. It was hypothesized that highly neurotic volunteers would show deficits in cognitive function and biases towards negative information in line with their vulnerability for developing mood and anxiety disorders.

4.2.2. Method

Sample Characteristics and Subjective State

Forty-six healthy volunteers (aged 21-58) were selected on the basis of their score in N in the Eysenck Personality Questionnaire (EPQ). Individuals were chosen from a cohort of 20,427 families collected as part of an investigation into the genetic basis of personality (Fullerton *et al.,* 2003). For the present study we contacted unrelated individuals from the selected extremes. Therefore, twenty-two subjects with extremely high scores in N –high N– (mean = 21, *SD* = 1.33, range 19-23) and twenty-four subjects with extremely low scores –low N– (mean = 1.63, *SD* = 1.66, range 0-4) were included in the analysis. Both groups were matched in terms of age in years (high N group: mean = 38.6, *SD* = 12.7; low N group: mean = 37.9, *SD* = 14.2), gender (12 males in each group), and IQ (National Adult Reading Test score: mean = 115.5, *SD* = 6.86; and mean = 117.4, *SD* = 6.4, respectively).

On the basis of the structured clinical interview for DSM-IV (SCID-I) subjects were determined to be free of past or current axis I disorder. They had no current physical illness and had been free of medication for at least one month. To assess the impact of subjective state on emotional processing, State and Trait Anxiety Inventory (STAI; Spielberger *et al.,* 1970) and Beck Depression Inventory (BDI; Beck *et al.,* 1974) were collected. All subjects gave their written consent to participate in the study, which was approved by the local psychiatric ethical committee.

Cognitive Tasks

The subjects performed the Rapid Visual Information Processing (RVIP) task as a measure of sustained attention, and the Stockings on Cambridge (TOL) task to assess planning ability which is an executive function, -both tasks from the computerized neuropsychological battery (CANTAB, CeNeS Pharmaceuticals, Cambridge, U.K.)-.

Emotional Tasks

Facial Expression recognition task: it featured six emotions (anger, disgust, fear, surprise, happiness and sadness) taken form the Ekman and Friesen (1976) Pictures of Affect Series. These had been morphed between each prototype and neutral using graphic techniques described by Young *et al.* (1997). Briefly, this procedure involved taking a percentage of the shape and texture differences between the two standard images 0% (neutral) and 100% (full emotion) in 10% steps (Figure 4.2.1). Four examples of every emotion at each intensity were given. Each face was also given in a neutral expression, giving a total of 210 stimuli presentations. Each stimulus remained on the screen for 500 ms and was immediately replaced by a blank screen.

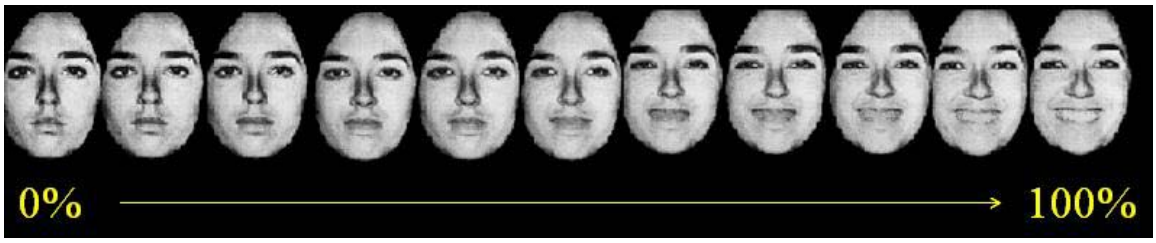

Figure 4.2.1. *Morphed faces using Young et al. (1997) technique.*

Subjects made their response by pressing labeled keys on the keyboard. The task was broken down into three parts, with a no-limit rest period between each to prevent fatigue. Subjects were asked to respond as quickly and as accurately as possible.

Emotional categorization and emotional memory: Sixty personality characteristics selected to be extremely unlikable or likable were presented on the computer screen for 500 ms (matched on word length, frequency and meaningfulness). The volunteers were asked to categorize these personality traits as likable or unlikable as quickly and as accurately as possible. Emotional memory was examined by a surprise test of recall of the personality traits used in the emotional categorization task as well as a test of emotional recognition. For this task the number of items recalled for each valence (minus false positives) was calculated.

Data Analysis

Both groups were compared in terms of age and IQ by using t-tests. Subjective ratings of mood were compared in both groups by means of one-way ANOVAs. Repeated measures ANOVAs were performed for all the tasks, with group and gender as between-group factors. A Huynh-Feldt correction was used where the assumption of sphericity was violated. Significant interactions were further analyzed by using simple main effect analyses. We conducted repeated measures ANCOVA, with scores on the BDI and STAI-state used as covariates.

4.3.3. Results

Subjective State

There were no differences between groups in terms of age and IQ ($p < 0.05$). High N subjects' scores were higher for BDI, STAI-state, STAI-trait ($F = 65.57$, $df = 1, 43$, $p < 0.001$; $F = 24.75$, $df = 1, 43$, $p < 0.001$; $F = 142.15$, $df = 1, 43$, $p < 0.001$, respectively). Mean values and standard deviations for each group are shown in Table 4.2.1.

	High N group		*Low N group*	
	Mean	SD	Mean	SD
BDI	10.5	4.9	1.61	1.8
STAI-State	38.95	10.2	26.87	5.5
STAI-Trait	48.95	7.8	26.61	4.4

Table 4.2.1. *Subjective state ratings at testing time. BDI = Beck's Depression Inventory, STAI = Spielberger State and Trait Anxiety Inventory.*

Performance on cognitive tasks

RVIP: High N volunteers showed impaired vigilance in the RVIP task. There was a significant interaction of blocks over time by group for RT in RVIP: $F = 3.49$, df = 3, 126, $P = 0.02$, with high N subjects showing a greater vigilance decrement (last block over time, high N *vs.* low N groups: mean difference 103.86, 95% CI 37.64–170.07, $P = 0.003$) (Figure 4.2.2). This difference was abolished when BDI and STAI-state scores were included as covariates ($F = 1.9$, df = 3, 135, $P = 0.13$), but a group effect was maintained ($F = 6.1$, df = 1, 43, $P = 0.02$). There was a similar trend for reduction in hits ($F = 2.87$, df = 1, 44, $P = 0.09$) but no change in false alarms in this task, and ANCOVA also showed no differences in false alarms and hits.

TOL: High N volunteers showed slowed planning in the TOL which was particularly evident with the most difficult problems (task difficulty by group: $F = 6.27$, df = 3, 129, $P = 0.002$). Latency in 5-move problems was significantly greater in high N volunteers (mean difference 13073.68, 95% CI 5320.21–20827.14, $P = 0.002$) (see Figure 4.2.2). Correct choices were similar in the two groups. This was not affected by the inclusion of STAI state and BDI scores as covariates ($F = 6.6$, df = 3, 128, $P < 0.001$).

Performance on emotional tasks

Facial Expression Recognition: There was a main effect for group in signal detection scores on the facial expression recognition task ($F = 5.94$, df = 1, 41, $P = 0.02$, NS interaction with group by gender), showing that high N subjects were less accurate than low N subjects across different facial expressions of emotion Specifically, these differences were significant for sad facial expressions (mean difference –0.0335, 95% CI –0.064 to –0.034, $P = 0.03$) and for surprised facial expressions (mean difference –0.043, 95% CI –0.072 to –0.013, $P = 0.006$). This effect appears to be related to the low grade mood symptoms in these volunteers since it did not survive the ANCOVA with anxiety and depression scores ($F = 0.78$, df = 5, 205, $P = 0.56$).

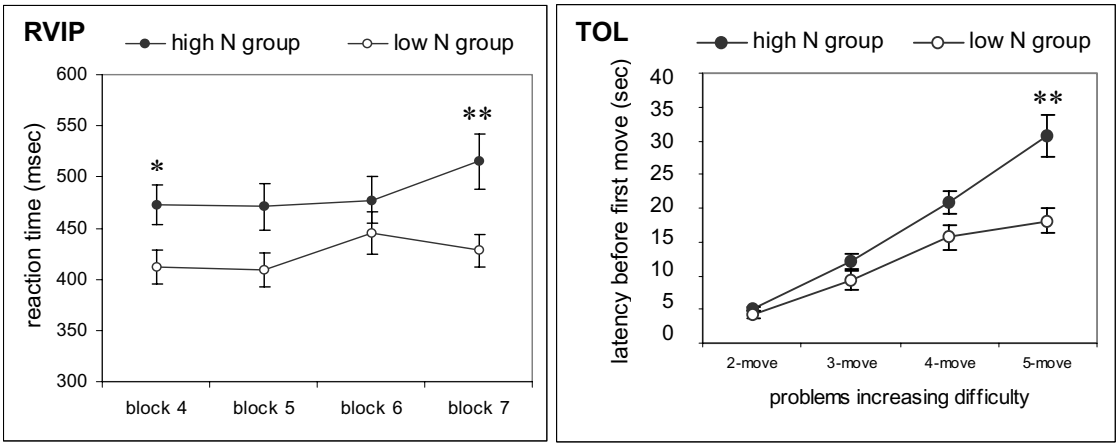

Figure 4.2.2. *Latency (milliseconds) recorded before response in RVIP over four blocks, and in TOL with increasing difficulty.* * *Significant differences between groups (p<0.05)* ** *Significant differences between groups at (p<0.01).*

Emotional Categorization Task: There was no effect on accuracy of word categorization, with all groups scoring highly (>95%). However, ANCOVA showed a marginal interaction (valence by group by gender) for reaction time (F = 3.76, df = 1, 42, P = 0.06), with the high N female volunteers showing similar reaction times for negative words and positive words (F = 4.25, df = 1, 42, P = 0.04) whereas low N females were quicker to respond to positive stimuli (mean difference 115.91, 95% CI 6.088–225.728, P = 0.04).

Emotional Recall: The surprise recall test of emotional words showed a significant interaction for valence (positive versus negative) by group by gender (F = 6.75, df = 1, 42, P = 0.01), when covariates were included (scores on BDI and STAI-state). Further analysis with female volunteers showed a significant interaction for valence by group (F = 6.21, df = 1, 20, P = 0.02), that is to say, high N females recalled more negative words and less positive words than low N females (mean difference –1.625, 95% CI –2.64 to –0.61, P = 0.002) (Figure 4.2.3). Post hoc analysis with male volunteers showed no effect.

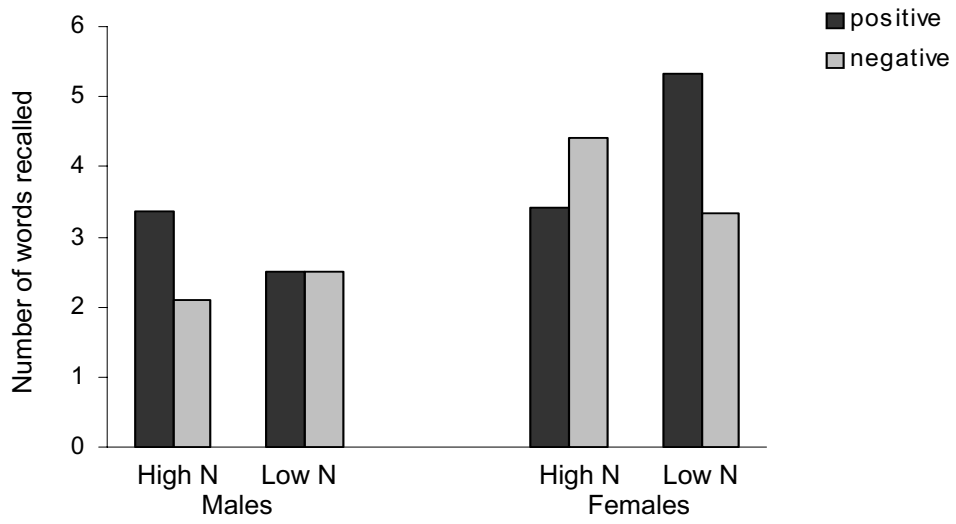

Figure 4.2.3. *Mean scores of emotional words recalled by surprise separated by gender.*

4.3.4. Discussion

These results suggest that volunteers with N, but without prior history of psychiatric disorder, show minor cognitive abnormalities on planning ability, sustained attention and emotional processing. Facial recognition and sustained attention appear to be affected by low grade symptoms (scores on BDI and STAI-state) at the assessment time, whereas increased latency for planning, increased reaction time for emotional information and negatively biased emotional recall (at least in women), seem to be a feature of N itself.

Depressed patients mainly exhibit problem-solving impairments, attention deficits and mnemonic dysfunction (Austin *et al*, 2001; Landro *et al*, 2001). It is becoming accepted that cognitive dysfunction must be understood as another feature of the depressive phenotype and some studies report enduring neuropsychological impairment even after recovery of the disease (Portella *et al*, 2003). It is nevertheless usually assumed that such impairments may be a consequence of illness episodes, hypercortisolemia etc. Ours is among the first reports to suggest that slowed planning is apparent in those at risk of developing depression and remains even when low grade symptoms were controlled for. Further work is required to assess whether the DLPFC, a key part of the neural circuitry underlying planning, is involved in these deficits observed in high risk participants and whether it may be related to poor problem solving skills.

By contrast, the impairment in sustained attention seen in high N volunteers was abolished by the inclusion of low grade anxious and depressive symptoms as covariates. Although this suggests that performance on this task may represent a state rather than a trait factor in depression and anxiety, high N subjects showed longer reaction times throughout the task. This would be partly in agreement with a recent study by Weiland-Fiedler *et al.* (2004),

where it was suggested that sustained attention deficits might represent a trait vulnerability marker for major depressive disorders.

The emotional processing differences that were observed in the high N versus low N volunteers resemble the negative biases of information processing seen in depression and anxiety disorders. Typically, depressed patients recall more negative emotional information and interpret emotional stimuli more negatively (Matt *et al*, 1992). Accordingly, we saw that high N females responded faster to negative words than low N females and also recalled more negative than positive words: female volunteers are likely to be at increased risk of developing depression compared to males (Lake *et al*, 2000).

Facial expression recognition was generally impaired in the high N volunteers, but again, differences between groups were abolished after controlling for low anxious-depressive symptomatology. Deficits in facial expression recognition have also been reported in depression (Rubinow *et al*, 1992) and have been suggested to contribute to the deficits in social interactions in this illness. Other studies suggest selective negative biases to detect facial expression in acute depressive episodes (Gur *et al*, 1992, Richards *et al* 2002). Bhagwagar *et al* (2004) also found a selectively greater recognition of fear in subjects with a previous history of depression compared to subjects without such a history. Since this increased negative recognition is seen in periods of full remission from depression, we have suggested that it is an enduring trait vulnerability marker. Also, the present results may imply an enduring 'scar' effect following sub-clinical episodes rather than a trait predisposition, since some significant effects seem to require low-grade symptoms. However, we screened our sample to exclude those volunteers with a past or current history of DSM-Axis 1 disorders. Since these volunteers were largely past the high risk age for depression and anxiety (with a mean age of 37 years), these volunteers may represent an unusual subset of highly resilient neurotic volunteers. Future studies should examine cognitive task performance in samples of younger neurotic volunteers to assess whether similar effects are found in a sample still at high prospective risk for anxiety and depression.

In conclusion, we have studied extremely high N healthy subjects, in comparison with extremely low N subjects. It is accepted that N represents a vulnerability factor for mood disorders. Our findings show that high scores on N are associated with abnormalities of cognitive functioning without past or current illness. Thus, neuropsychological impairments in planning and attentional domains may in part mediate trait vulnerability to depression. Emotional bias in high N subjects is similar to that observed in anxiety and depressive disorders, but is partly related to current anxious-depressive symptomatology. Accordingly we cannot be completely sure that it provides a mechanism for trait-related vulnerability to depression. The negative biases reported in acutely unwell or recovered subjects may be a consequence of illness, or reflect an abnormality only present in those subjects with high N truly at high risk of depression. In either case, the new challenge is to unpack the likely neuropsychological mechanisms mediating the vulnerability of high N subjects to depression.

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4.3. ENHANCED EARLY MORNING SALIVARY CORTISOL IN NEUROTICISM

(Results partly published by Portella *et al.* 2004. *J Psychopharmacol* 18(3): A11, suppl meeting abstract)
(Published by Portella *et al.* 2005. *Am J Psychiatry* 162(4): 807-809)

4.3.1. Introduction

Neuroticism is a dimensional measure of an individual's tendency to experience negative emotions, manifested at one extreme as anxiety, depression and moodiness, and at the other, as emotional stability. The heritability of neuroticism is well-established (Kirk *et al.*, 2000) and some studies have suggested that there is a substantial overlap between the genetic risk factors for neuroticism and major depression (Lake *et al.*, 2000). However, it is not known which biological correlates of neuroticism might give rise to the increased risk of clinical depression.

Major depression is often accompanied by hypothalamic-pituitary-adrenal (HPA) axis dysfunction (Pariante and Miller, 2001). Recent studies suggest that the increase in salivary cortisol that follows waking provides a reliable dynamic measure of adrenocortical activity (Pruessner *et al.*, 1997) and this response is increased in euthymic unmedicated patients with a history of depression (Bhagwagar *et al.*, 2003). However, it is unknown whether elevated waking cortisol is a risk factor for depression or a result of having been depressed or treated for depression in the past. The aim of this study was to examine waking cortisol in people high or low in neuroticism without history of mood disorder, to test the hypothesis that high neuroticism itself is associated with altered adrenocortical regulation.

4.3.2. Method

Thirty healthy volunteers (aged 21-57 years) were selected from their neuroticism score on the Eysenck Personality Questionnaire (EPQ). Individuals were chosen from a cohort of 20,427 families collected as part of an investigation into the genetic basis of personality (Fullerton *et al.*, 2003). We contacted unrelated individuals from the selected extremes: 15 subjects (7 males, mean age: 39.5, *SD* = 14.62) with extremely high scores in neuroticism –high N– (mean = 21.3, *SD* = 1.29, range 19-23) and 15 subjects (7 males, mean age: 46.9, *SD* = 8.97) with extremely low scores –low N– (mean = 0.53, *SD* = 1.13) were included in the analysis.

On the Structured Clinical Interview for DSM-IV, subjects were determined to be free of past or current history of axis I disorder. They had no current physical illness and had been free of medication for at least one month. The group with high neuroticism scores had a mean score on the Beck Depression Inventory of 9.14 (*SD* = 4.73), and the group with low neuroticism scores had a mean score of 3.07 (*SD* = 2.34). The study was approved by the local ethics committee. All subjects gave written informed consent.

Fasting saliva samples were collected in salivette tubes with the first sample taken immediately upon waking and continuing at 15-minute intervals for the next hour (Wüst *et al.*, 2000). After this they resumed normal activity. Subsequently, saliva samples were taken at 12:00, 18:00 and 22:00 hours, avoiding food for one hour previously. The premenstrual week was avoided for females. Salivary cortisol was measured with an in-house double antibody radioimmunoassay.

Salivary levels were analyzed with a two-way repeated measures analysis of variance (ANOVA) with "group" and "gender" as between-subjects factors and "time" (sampling time) as the main within-subjects factor with Huynh-Feldt correction (uncorrected df reported). Significant interactions were analyzed using post-hoc t-tests. The area under the curve (AUC) for the first sixty minutes after waking was measured by the trapezoid method, with subtraction of baseline cortisol secretion.

4.3.3. Results

There was no difference in age between groups ($t = -1.67$, $df = 28$, $p = 0.1$). The mean time of awakening did not differ between high N subjects (7:03am ± 41 minutes) and low N subjects (6:43am ± 49 minutes) ($t = 1.15$, $df = 28$, $p = 0.26$).

The ANOVA showed a significant group by time interaction ($F = 4.18$, $df = 4, 104$, $p = 0.005$). Post-hoc testing (Figure 4.3.1) showed a greater increase in salivary cortisol 30 minutes after waking in the high N group, and this difference from low N subjects was maintained for the next 30 minutes. After that, levels of cortisol were similar for both groups. There was no effect of gender ($F = 2.47$, $df = 1, 26$, $p = 0.13$; gender × time $F = 0.88$, $df = 4, 104$, $p = 0.48$).

The AUC for cortisol secretion was substantially greater in the high N group (mean = 14.92 nmol × hour/liter, $SD = 11.38$, versus mean=5.79 nmol × hour/liter, $SD = 9.46$; $t = 2.39$, $df = 28$, $p = 0.02$). In high N subjects there was a correlation between the AUC and neuroticism score ($r = 0.53$, $p = 0.04$); however BDI score did not correlate with cortisol AUC in the high N volunteers ($r = 0.26$, $p = 0.37$).

4.3.4. Discussion

High N subjects showed significantly greater levels of salivary cortisol between thirty and sixty minutes after waking. It is well-established that waking in the morning is followed by an adrenocortical response with brief ACTH and cortisol pulses in the majority of the subjects. The greater waking increase in free cortisol found in this study is similar to that reported in recovered depressed patients (Bhagwagar *et al.*, 2003). Also we have recently identified a similar abnormality in unmedicated patients with acute major depression (Bhagwagar *et al.*, in

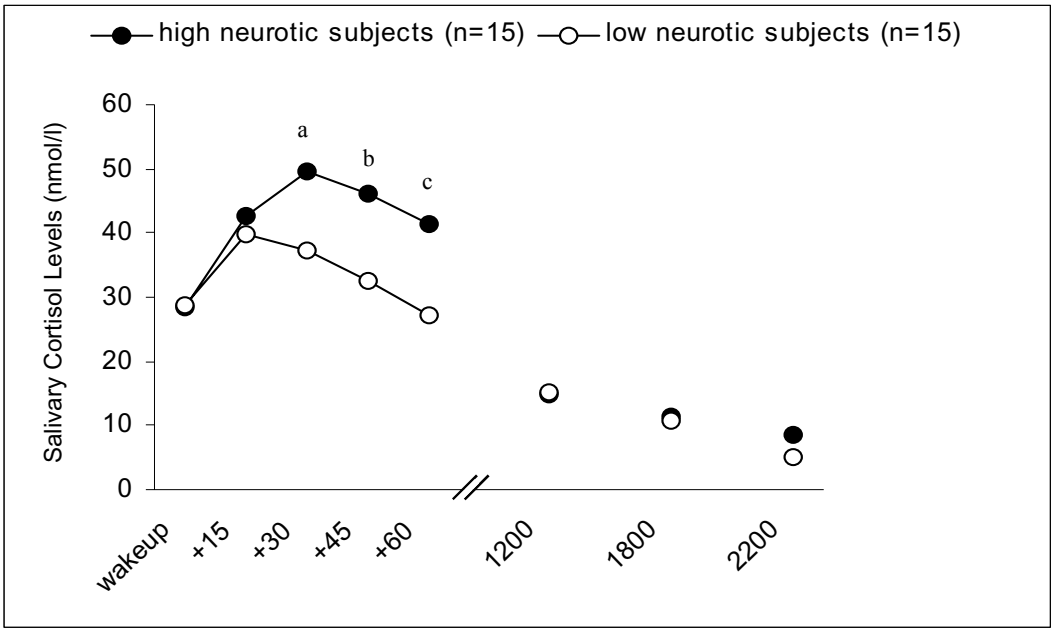

Figure 4.3.1. *Diurnal variation of salivary cortisol levels in high N and low N subjects. Values represent means .*[a] *Significant difference between groups (t = 2.33, df = 28, p = 0.03).* [b] *Significant difference between groups (t = 2.59, df = 28, p = 0.01).* [c] *Significant difference between groups (t = 2.74, df = 28, p = 0.01).*

preparation). The salivary cortisol response to waking represents only a single aspect of HPA axis function; thus we cannot conclude that neuroticism is associated with generally increased HPA axis activity. However, our observations are consistent with a greater ACTH response to waking or increased sensitivity of the adrenal gland to ACTH stimulation in high N subjects.

Our sample had no past or current history of depression suggesting that increased waking cortisol is a risk factor for, rather than a consequence of, depression. It is possible that this abnormal response is inherited as part of the trait of neuroticism and, like high N, waking salivary cortisol levels show significant heritability (Bartels *et al.*, 2003). Another possibility however, is that the elevated waking cortisol levels might be a consequence of experiencing more subjective stress.

Nonetheless, our data suggest that elevated morning cortisol levels can exist in the absence of major depression. In this respect, our findings could resemble the abnormal HPA axis activity seen in the first-degree relatives of depressed patients who have not experienced depression (Holsboer *et al.*, 1995). Longitudinal prospective studies initiated before the onset of depression are needed to untangle the relationship between neuroticism, cortisol hypersecretion and depression.

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4.4. AN FMRI STUDY OF HIGHLY NEUROTIC SUBJECTS' RESPONSES TO CONSCIOUS FACIAL EXPRESSIONS

(Submitted by Portella *et al., Biol Psychiatry*)

4.4.1. Introduction

Personality traits are believed to play a crucial role in the way in which the body and the brain respond to emotional material or situations and this may be important in the way in which the individual copes with stress. The centrality of stress reactivity to neuroticism is well established and many studies have described a relationship between high scores on measures of neuroticism and major depressive disorders as well as other psychiatric disorder (Stewart *et al.*, 2002). It is still unknown what functional correlates of neuroticism might give rise to the increased risk of experiencing mood disorders. One study has explored a possible vulnerability factor in highly neurotic people without history of mental illness and has showed an altered response of adrenocortical systems (Portella *et al.*, 2005) similar to the one observed in recovered depressed patients (Bhagwagar *et al.*, 2003). Functional and structural imaging studies have explored several brain structures involved in biased information processing in depressive disorders such as the orbitofrontal cortex, anterior cingulate, insula and amygdaloid complex (Siegle *et al.*, 2002, Frodl *et al.*, 2003, Hariri *et al.*, 2003) as well as their interconnections which mediate behavioural responses associated with distinct emotions. Furthermore, some studies have attempted to relate brain reactivity to emotional stimuli and personality traits such as extraversion and neuroticism (Canli *et al.*, 2001).

The amygdala is a major component of the limbic system located in the temporal lobe. As part of the limbic system, the amygdaloid complex has been implicated in brain functions including mainly emotion (LeDoux 1994, Gallagher and Chiba 1996), and learning and memory (Gallagher and Holland 1994). Nowadays, the amygdala is widely recognised as critical for processing emotional stimuli and forming emotional memory (Adolphs et al.,1994; McGaugh 2004 for a review), but there may be important individual differences in the biological basis of emotion: some psychological determinants of individual variability in emotional responsiveness have been identified, such as specific personality traits (Canli et al.,2001): they found that extraversion was correlated with brain reactivity to positive stimuli in cortical (frontal and temporal) and subcortical (amygdala, caudate, putamen among others) regions, and brain activation to negative pictures correlated significantly with subjects' neuroticism scores in left frontal and temporal cortical regions, but they failed to find a correlation between N and amygdaloid responses.

The aim of this study was to examine brain functioning in people very high or very low in neuroticism with no history of mood disorders. We hypothesise that highly neurotic people might show similar amygdala responses to facial expressions as the ones observed in subjects

with affective disorders (Sheline et al., 2001; Siegle et al., 2003). We believed that high and low scorers could be a better way to isolate differences and this would allow us to assess whether amygdala hyperacitivty occurs in people at risk of depression who have not actually been depressed themselves.

4.4.2. Method

Sample

Twenty-four healthy volunteers (aged 19-58 years) were selected on the basis of their score in neuroticism in the Eysenck Personality Questionnaire (EPQ). Individuals were chosen from a cohort of 20,427 families collected as part of an investigation into the genetic basis of personality (Fullerton *et al.,* 2003). For the current study we contacted unrelated individuals from the selected extremes. Therefore, 12 subjects with extremely high scores in neuroticism -high N- (mean=21.1, SD=1.25, range 19-23) and 12 subjects with extremely low scores -low N- (mean=1.42, SD=1.56, range 0-4) were included in the analysis. Both groups were matched on age (mean=39.83 years, SD=13.15 and mean=40.67 years, SD=12.84), gender (12 males –6 high N and 6 low N-, and 12 females) and IQ (National Adult Reading Test score: mean=115, SD=7.7, and mean=117, SD=6).

All subjects were healthy, with no past history of psychiatric or neurological illness and were not taking any medication (except the contraceptive pill). In order to assess subjects' mood, they completed the Beck Depression Inventory (BDI: Beck, 1979), and the State-Trait Anxiety Inventory (STAI: Spielberger, 1983). All subjects gave written informed consent for study participation, which was approved by the local ethics committee.

Stimulus and task design

The study was designed to compare amygdala activation (evoked neural responses to presentations of fearful and happy faces) between the two groups of subjects in a block design. The experiment consisted of one session which was composed of 17 blocks (fear faces, rest, happy faces: A-C-B-C-A...) (Figure 4.4.1). Each block was 33 seconds long, alternating rest and task. Stimuli were generated from facial photographs of 8 actors (4 men, 4 women) from the Ekman and Friesen series of emotional faces (Ekman and Friesen, 1976). Four blocks each of fearful and happy faces alternated with fixation blocks in a fixed order. 192 volumes were acquired, one every three seconds.

In order to maintain their attention on the faces from both tasks, subjects were instructed to discriminate the gender of faces by pressing two keys (one for male and one for female) on a pad. They could use either hands or both -no instructions were given, but subjects were asked to be consistent throughout the sessions-. The stimuli were generated on a personal computer and projected onto a half transparent screen by a projector. The subjects observed the stimuli through

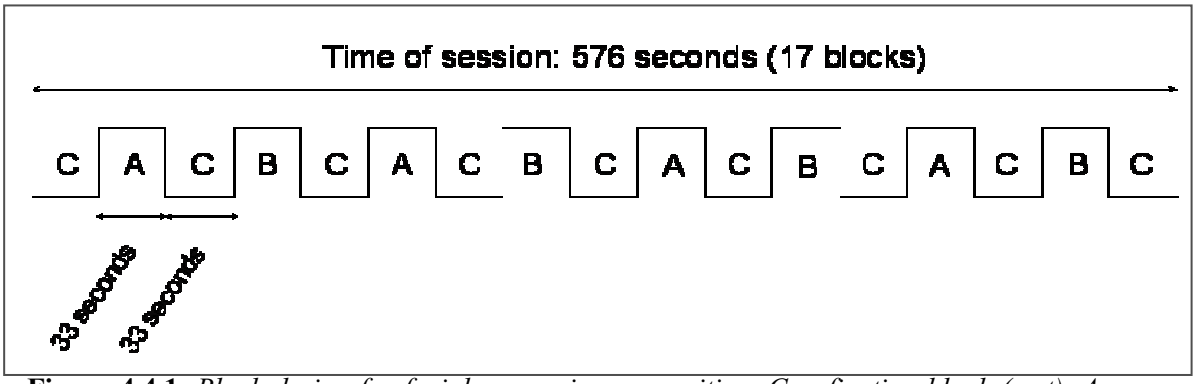

Figure 4.4.1. *Block design for facial expression recognition. C = fixation block (rest), A = fear fixation block, and B = happy fixation block.*

a tilted mirror attached to the head coil of the scanner. Because external characteristics of faces were previously removed, discrimination of the gender could be difficult for the subjects. Subjects' performance of the discrimination task was not recorded.

Image acquisition and analysis

Functional images were acquired in an axial orientation, covering 40 slices (thickness of 3 mm), beginning from the base of the temporal lobes upward, using a 1.5-T MRI system (Siemens, MAGNETOM Sonata, Germany) equipped with single shot EPI (TR = 3.0 s, TE = 50 ms, flip angle = 90º, matrix = 124*124*35, 3 mm isotropic voxels) which was used to measure blood-oxygen level-dependent (BOLD) contrast. High-resolution 3D T1-weighted FLASH structural images were also acquired (TR = 5.6 ms, flip angle = 19º, 1mm isotropic voxels, coronal). This was used for coregistering scans from different individuals to a common standard.

fMRI analysis was carried out by means of FEAT (FMRI Expert Analysis Tool) Version 5.00, (part of FMRIB´s Software Library, www.fmrib.ox.ac.uk/fsl). The following pre-statistics processing was applied: motion correction using MCFLIRT (Jenkinson, 2002); non-brain removal using BET (Smith, 2002); spatial smoothing using a Gaussian kernel of FWHM 5mm; mean-based intensity normalisation of all volumes by the same factor; highpass temporal filtering (Gaussian-weighted LSF straight line fitting, with cut off of 100s). Time-series statistical analysis was carried out using FILM (FMRIB´s Improved Linear Model) with local autocorrelation correction (Woolrich, 2001). *Z* statistic images were threshold using clusters determined by $Z > 2.0$ and a (corrected) cluster significance threshold of $P = 0.01$ (Worsley, 1992; Friston, 1994; Forman, 1995). Regitration to high resolution and standard space images was carried out using FLIRT (Jenkinson, 2001, 2002). In the first-level analysis, individual activation maps were produced comparing the response to the fear and happy stimuli compared to a fixation baseline (2 contrasts). A mixed-effects group cluster analysis (a second-level analysis) was carried to establish group effects for the same contrasts.

To obtain percentage of signal change in the amygdala a region of interest (ROI) was performed. ROI's for the left and the right amygdala were drawn manually on the high resolution image for each subject. Predetermined condition effects at each voxel were calculated using a t-statistic, producing a statistical image for two contrasts of the emotions (fear and happy) versus rest for each subject. These individual contrast images were then used in second-level random effects models, that account for both scan-to-scan and subject-to-subject variability, to determine task-specific regional responses at the group-level with one-sample (main effects of task) and paired t-tests (direct comparisons). A statistical threshold of $p < 0.05$, with a small volume correction for multiple comparisons, was used to identify significant responses for all comparisons.

Comparisons between groups for mood scales were carried out by means of t-tests. A repeated-measures ANOVA for every session was carried out with "group" (high N subjects versus low N subjects) as the main between-subjects factor, and "emotion" (fear faces versus happy faces) and lateralization (left versus right) as the within-subjects factors. A Huynh-Feldt correction was used where the assumption of sphericity was violated (uncorrected *df* reported). Significant interactions for group and for emotion were further analysed using t-tests. The study was conducted at the University of Oxford Centre for Clinical Magnetic Resonance Research (OCMR) John Radcliffe Hospital, Oxford.

4.4.3. Results

Subjective state

High N subjects tended to rate themselves significantly more sad ($t = 3.43$, $p = 0.03$), frightened ($t = 3.11$, $p = 0.05$) and anxious ($t = 3.14$, $p = 0.01$) than low N at the scanning time. All other mood ratings measured with the visual analogue scales were not significantly different between groups. As it could be expected, BDI, STAI-S and STAI-T scores (Table 4.4.1) were significantly higher in high N subjects ($t = 3.78$, $p = 0.001$; $t = 4.18$, $p = 0.001$; $t = 8.5$, $p < 0.001$, respectively).

	High N group		*Low N group*	
	Mean	SD	Mean	SD
BDI	9.82	7.00	2	2.34
STAI-State	39.73	11.05	24.83	4.88
STAI-Trait	50.18	10.27	25.25	4.22

Table 4.4.1. *Subjective state ratings at scanning time. BDI = Beck´s Depression Inventory, STAI = Spielberger State and Trait Anxiety Inventory.*

Functional imaging results

The repeated-measures ANOVA showed no group by emotion interaction. There were no main effects of lateralization. Further analysis of the imaging data revealed a similar bilateral BOLD response in the amygdala during the task for both groups (see Figure 4.4.2).

Provided that the subjective state of both groups was significantly different, a repeated-measures ANCOVA was carried out, controlling for BDI and STAI-State scores. No significant main effect came up for emotion by group by lateralization (F =0.41, df = 1, 22, p = 0.53), nor for emotion by lateralization (F = 0.23, df = 1, 22, p = 0.64).

Whole brain analysis. To report unbiased results for non-hypothesized regions and to provide data that might generate further hypotheses regarding regions important in conscious affect processing, we also conducted a whole-brain FSL analysis with the voxel-wise height threshold set at uncorrected p<0.01. Table 4.4.2 summarizes the regions of activation seen in the high and low N volunteers. For individual unmasked affect conditions, fear faces were associated with a significant cluster of activation within the left insula, bilateral thalamus, and anterior and medial cingulate gyrus in the high N volunteers compared to the low N volunteers (see Figure 4.4.3). On the other hand, response to the presentation of happy faces in the right thalamus was affected by neuroticism (see Figure 4.4.4). At this level of correction, no regions survived direct paired *t*-test comparisons between the happy and fear conditions.

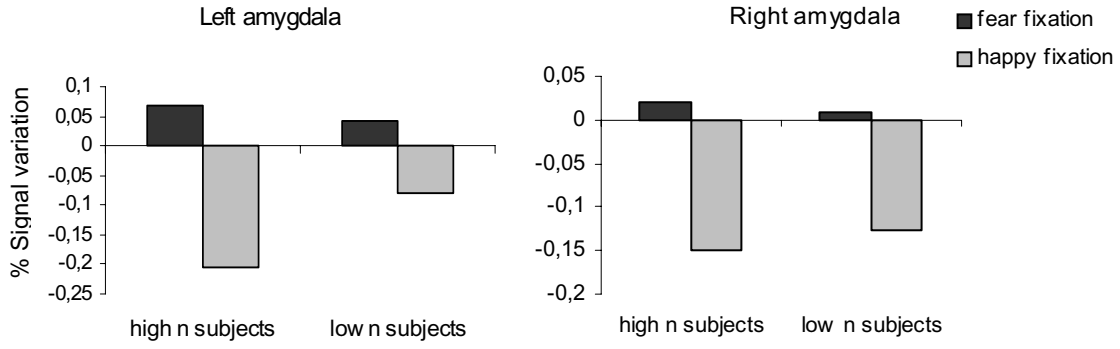

Figure 4.4.2. *Percent change in normalised magnetic resonance signal intensity in each amygdala for the comparison fear and happy fixation on the task.*

Brain Areas	*Coordinates*		
	x	*y*	*Z*
Fear fixation (high n > low n)			
Insula (left)	–42	–21	–4
	–40	–19	–4
	–42	–18	–4
Thalamus (left)	–13	–11	5
Thalamus (right)	18	–21	7
	11	–12	11
	23	–21	9
Cingulate gyrus (anterior, right)	3	34	11
Cingulate gyrus (right)	14	1	41
Happy fixation (high n > low n)			
Right thalamus	7	–18	1

Table 4.4.2. *Group effect on the neural response to emotional facial expressions. Talairach coordinates (x, y, z) refer to the center of activation (uncorrected p < 0.01) within each region.*

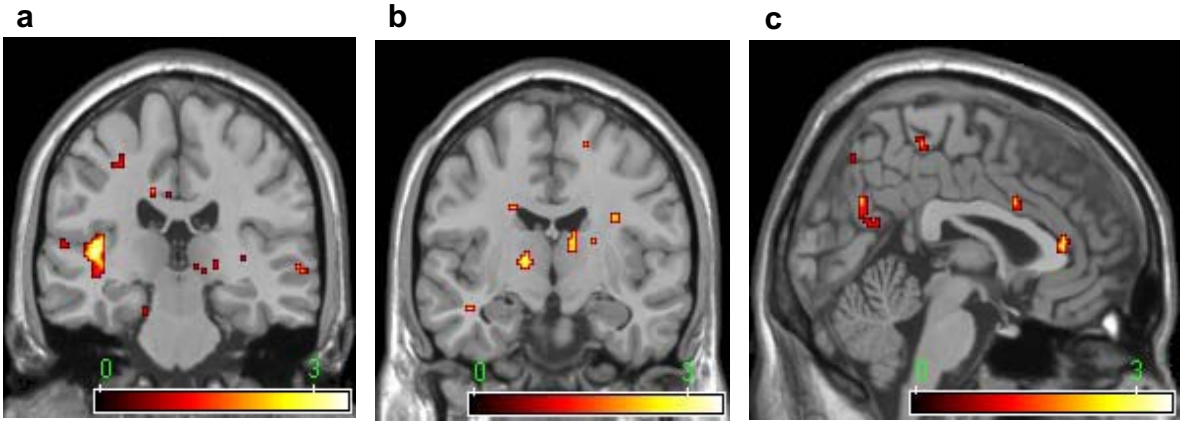

Figure 4.4.3. *Response to the presentation of fearful faces in the contrast of fear fixation compared between high N and low N groups. An uncorrected threshold of p < 0.01 was used to display the contrasts.* **a***, activation in the region of left insula (coronal slice y = – 24mm).* **b***, activation of bilateral thalamus (coronal slice y = – 10mm).* **c***, activation in the region of anterior cingulate gyrus (sagittal slice = 3 mm).*

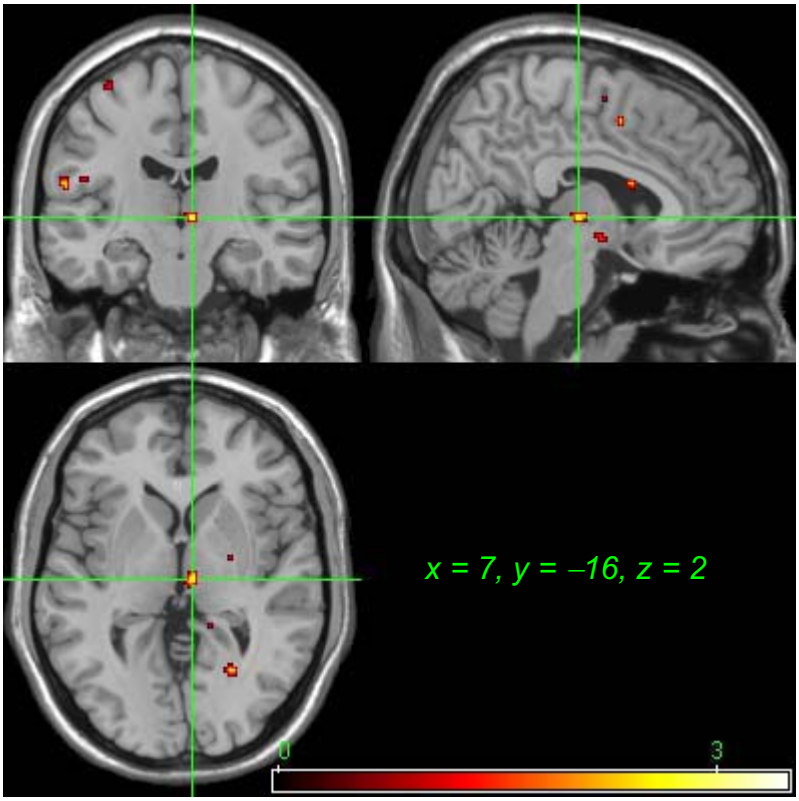

Figure 4.4.4. *Response to the presentation of happy faces in the contrast of happy fixation compared between high N and low N groups. Right thalamus activation.*

4.4.4. Discussion

Subjects were presented with photographs of happy and fearful faces in a manner that minimized or eliminated characteristics other than the presented affect. Emotional stimuli yielded specific patterns of activation within the amygdala: on one side, activation in all subjects within left amygdala for conscious fear, and deactivation of bilateral amygdali for conscious happy stimuli, which may resemble normal responses of amygdala. Recent studies by Morris *et al.* (1998, 1999) reported only left amygdala activation to consciously processed stimuli. This difference in the findings may be attributable to the requirement to engage in a gender discrimination task which may attenuate the activation of the amygdala relative to paradigms requiring only passive viewing of stimuli (Lange et al, 2003). The results indicate that amygdala functioning is not related to N for indirect and conscious processing of facial expressions. Similarly, Canli *et al.* (2001) did not found a correlation between neuroticism and responses within the amygdaloid complex. Further, we did not find an effect of state anxiety or depressive levels on the amygdala reactivity to emotional information, contrary to what Somerville *et al.* (2004) found.

Among structures that commonly activate across a wide variety of affective tasks are: amygdala, insula, medial frontal cortex (including medial prefrontal cortex) and anterior cingulate cortex, we therefore included a whole-brain analysis, to allow presentation of non-hypothesized regions involved in conscious affect processing. High N volunteers appear to process the fearful facial expression differently from low N: they engage the insula, the cingulate and the thalamus to a greater extent. By examining findings across studies in a meta-analysis, one of the conclusions yielded by Phan *et al.* (2002) was that emotional tasks with cognitive demand also involved the anterior cingulate and insula.

Activations within bilateral insula and medial frontal cortex have been observed during aversive pictures blocks (relative to neutral blocks) with real-time analysis (Phan *et al.*, 2004). Our findings might be consistent with this evidence. Insula activations have been reported during specific perception of faces with expressions of disgust (Phillips *et al.*, 1997), but more generally, this structure may be responsible for monitoring and responding to internal emotional states (feelings) as a limbic integration cortex, similarly to the anterior cingulate cortex. Our results of greater activation of the insula and cingulate gyrus to fearful faces in high N subjects may give support to the notion that these regions play an important role in emotion: they may interface emotion with cognition and exert top-down control over the emotional responses that are driven by other systems or structures (Dalgleish, 2004).

Along with the engagement of the insula and cingulate cortex, we have observed greater bilateral thalami activation to fearful faces and right thalamus activation to happy faces in the high N group. Increased blood flow within the thalamus (Drevets *et al.*, 1992) was reported in patients with major depressive disorder, together with other limbic structures. Keightley *et al.* (2003) found that indirect processing of faces were characterised by bilateral amygdala, left insula and right thalamus activation. Indeed, compatible findings in depressed patients after treatment have reported reduced metabolism in the thalamus, ventral striatum, and insula (Mayberg *et al.*, 1999; Nobler *et al.*, 1994), regions important for the generation of emotional states.

Considered as a whole, the results show that high N subjects recruit a number of limbic areas when processing emotional stimuli, compared to subjects with very low scores on N. The amygadala hyperactivation is not observed in this study. However, these volunteers were past the high risk age for depression and anxiety and may represent an unusual subset of highly resilient neurotic volunteers. Further studies are required to assess whether amygdala hyperactivity may be observed in younger high N volunteers.

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4.5. MEDIAL TEMPORAL LOBE ABNORMALITIES IN NEUROTICISM

(Submitted by Portella *et al*. *Biol Psychiatry*)

4.5.1. Introduction

Neuroticism (N) is associated with depressive disorders and has been suggested be a predisposing factor to affective disorders (Schmitz *et al.,* 2003; Roberts and Kendler, 1999; Duggan *et al.,* 1995), and with the genetic risk for the illness (Sen *et al.,* 2004). However, few studies have addressed the specific role of N on developing affective disorders, presumably because there is an important overlap between neurotic symptoms and major depressive disorders.

Depressive disorders are associated with cognitive deficits, which affect memory, executive functioning and attention (Austin *et al.,* 2001; Landro *et al.,* 2001). Particularly, mnemonic dysfunction, which is consistent across most studies, has been associated with a reduction of the hippocampal volume (Sheline *et al.*, 1996; Mervaala *et al.*, 2000). A recent meta-analysis (Videbech and Ravnkilde, 2004) revealed an average reduction of hippocampal volume of 8% on the left side and 10% on the right side across 12 different studies.

Abnormalities within the amygdala haven also been reported. Structural differences are less clear but separate literature suggests that depressed individuals have smaller core amygdala nuclei than never-depressed individuals (Drevets, 2000; Sheline *et al.*, 1998). Siegle *et al.* (2003) showed that amygdala activity during emotional information processing is related to amygdala volume in depression due to a potential hypercortisolaemia effect of the amygdala hyperactivity observed in MDD. However, other studies have found higher amygdala volumes in patients with MDD compared to healthy controls (Frodl *et al.*, 2002; 2003; Bremner *et al.*, 2000). Also, recent studies revealed enhanced amygdala responses to emotional stimuli. Sheline *et al.* (2001) reported that hemodynamic responses in the left amygdala were exaggerated in MDD subjects exposed to fearful or smiling faces that were displayed during 40 ms. The duration of the amygdala response to emotionally valenced stimuli is also abnormal in MDD. Siegle *et al.* (2002) reported that the elevation in hemodynamic activity occurring in the amygdala during exposure to sadly valenced words persisted for an abnormally long time in depressives relative to controls. These neural changes have been related to negative biases of information processing (Drevets, 2003).

This study aims to explore whether similar abnormalities are apparent in healthy volunteers at risk for developing depressive disorder who had never suffered from depression, identified through score on the neuroticism scale of the EPQ. Therefore, subjects were assessed on three main measures of medial temporal lobes function: the amygdala response to masked presentations of fearful facial expressions was assessed using fMRI and volumetric comparisons of the hippocampus and amygdala were also made between the two groups by means of quantitative MRI. A behavioural measure of amygdala function, the emotion-potentiated startle (EPS), was

also assessed. It was hypothesized that highly neurotic volunteers would show similar medial temporal abnormalities as the ones observed in subjects suffering from depressive disorders: enhanced amygdali activation, smaller bilateral hippocampi and amygdali, and enhanced startle responses with aversive stimuli.

4.5.2. Method

Sample characteristics

Forty-six healthy volunteers (aged 21-58 years old) were selected on the basis of their score in N in the Eysenck Personality Questionnaire (EPQ). Individuals were chosen form a cohort of 20,427 families collected as part of an investigation into the genetic basis of personality (Fullerton *et al.*, 2003). For the present study we contacted unrelated individuals form the selected extremes. Therefore, twenty-two subjects with extremely high scores in N –high N– (mean=21, SD=1.3, range 19-23) and twenty-four subjects with extremely low scores –low N– (mean=1.6, SD=1.7, range 0-4) were included in the analysis. Both groups were matched in terms of age in years (high N group: mean=38.6, SD=12.7: low N group: mean=37.9, SD=14.2), gender (12 males in each group) and IQ (National Adult Reading Test Score: mean=115.5, SD=6.9; mean=117.4, SD=6.4, high and low respectively).

On the basis of the structured clinical interview for DSM-IV (SCID-I) subjects were determined to be free of past or current axis I disorder. They had no current physical illness and had been free of medication for at least one month. To assess the impact of subjective state on emotional processing, State and Trait Anxiety Inventory (STAI; Spielberger *et al.,* 1970) and Beck Depression Inventory (BDI; Beck *et al.,* 1974) were collected. All subjects gave their written consent to participate in the study, which was approved by the local psychiatric ethical committee.

Twenty-four individuals of the sample gave their consent to be scanned which was needed for experiments 1 and 2. This sub sample did not differ in any of the controlled variables –$p>0.05$–: age (high N: mean=39.8, SD=13.2; low N: mean=40.6, SD=12.8), NART (high N: mean=114, SD=7.7; low N: mean=117.8, SD=7.9), N score (high N: mean=21, SD=1.4; low N: mean=1.4, SD=1.3).

Experiment 1

Stimulus and task design

The study was designed to compare amygdala activation (evoked unconscious neural responses to fear and happy facial expressions) between the two groups of subjects in a block design. The

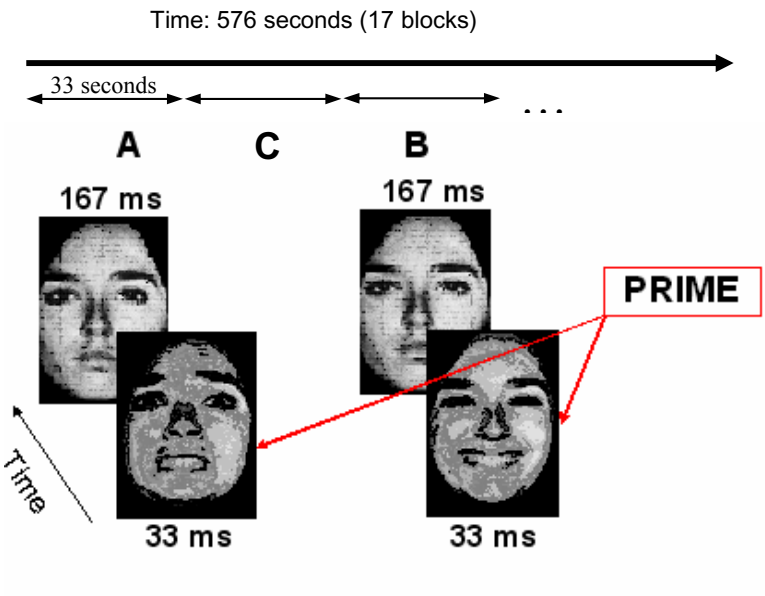

Figure 4.5.1. *A representation of the fearful and happy faces (emotional primes) presented just before the neutral face. Ten faces from different actors were presented in each block.*

experiment consisted of one session which was composed of 18 blocks (see subheading "Stimulus and task design" in Chapter 4.4.2 and Figure 4.4.1), where faces were also presented one by one, but a prime of 33-msec duration (fear face or happy face alternated for each block) was presented just before the presentation of the neutral face (which lasted 167 msec) (Figure 4.5.1). The subjects could not consciously identify the primes in this procedure.

In order to maintain their attention on the faces form the task, subjects were instructed to discriminate the gender of faces by pressing two keys (one for male and one for female) on a pad. Stimuli were generated on a personal computer and projected onto a half transparent screen by a projector. Subjects observed the stimuli through a tilted mirror attached to the head coil of the scanner. Because external characteristics of faces were previously removed, discrimination of the gender could be difficult for the subjects.

Image acquisition and analysis

Functional images acquisition is fully explained elsewhere (Chapter 4.4.2).

A repeated measures ANOVA was carried out with "group" (high N subjects versus low N subjects) as the main between-subjects factor, and "emotion" (fear faces versus happy faces) and "lateralization" (right and left amygdali) as the within-subjects factors. A Huynh-Feldt correction was used where the assumption of sphericity was violated (uncorrected *df* reported). Significant interactions were further analysed using *t*-tests.

To obtain percentage of signal change in the region of interest (i.e. bilateral amygdale) voxel-wise signal intensities were ratio normalized to the whole-brain global mean. Predetermined condition effects at each voxel were calculated using a *t*-statistic, producing a

statistical image for four contrasts of the emotions (fear minus rest, happy minus rest, fear minus happy and happy minus fear) for each subject. These individual contrast images were then used in second-level random effects models, that account for both scan-to-scan and subject-to-subject variability, to determine task-specific regional responses at the group-level with one-sample (main effects of the task) and paired t-tests (direct comparisons). A statistical threshold of $p<0.05$, with a small volume correction for multiple comparisons, was used to identify significant responses for all comparisons. This experiment was conducted at the University of Oxford Centre for Clinical Magnetic Resonance Research (OCMR) John Radcliffe Hospital, Oxford.

Experiment 2

Volumetric analysis

T1-weighted anatomical images were acquired by means of a 1.5-T MRI system (Siemens, MAGNETOM Sonata, Germany) (a complete description of imaging acquisition can be found in heading "Image acquisition and analysis" in Chapter 4.4.2).

Amygdali were outlined manually using a mouse-driven cursor on these high-resolution structural images (Figure 4.5.2). Limits were determined following MacKay *et al.* (2000) and Honeycutt *et al.* (1998): the amygdala was first measured in coronal slices with the anterior boundary set at the last section on which its boundary could be clearly identified in respect of the adjacent white matter of the temporal lobe. The amygdale lies anterior and superior to the neighbouring hippocampus, separated by the alveus and typically additionally by a region of CSF superior to the alveus on the most posterior sections containing both structures (Mackay *et al.*, 2000). The posterior limit was the slice on which the grey matter superior to the hippocampus could be distinguished. The dorsal boundary was set at the temporal horn of the lateral ventricle. The lateral boundary was set at the most medial white matter protruding into amygdaloid grey matter. The medial and ventral boundary was set at the uncus. After measuring in coronal slices, sagittal and axial slices were consulted; any grey matter medial to the uncal notch was erased.

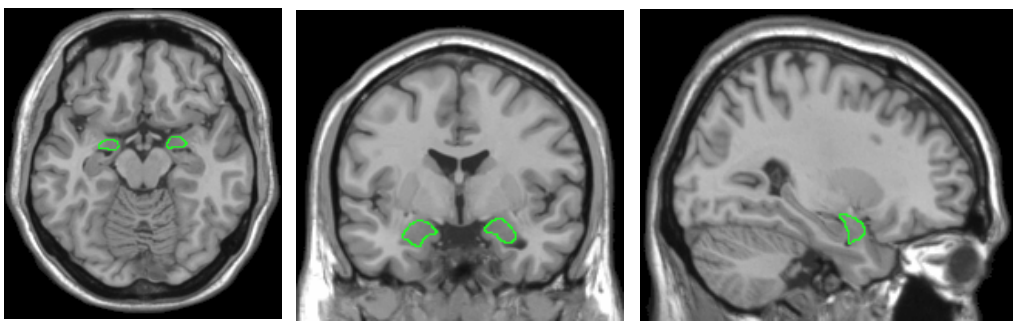

Figure 4.5.2. *T1-weighted MRI showing boundaries for volumetric measures of amygdala (in order, axial view z =–15, coronal view y = –2, and sagittal view x = 29).*

Hippocampi were outlined by using the same means as for amygdali drawings (Figure 4.5.3). Limits were here determined following Pruessner *et al.* (2000). The coronal view was used as default. References to sagittal or axial orientations were made whenever these were felt to be clearer for identification of structure boundaries. The tail of the hippocampus was defined as including the dentate gyrus, the cornu ammonis (CA) regions, the part of the fasciolar gyrus that is adjacent to the CA regions and the alveus. The Andreas–Retzius gyrus (ARG), the part of the fasciolar gyrus (FG) that is adjacent to this gyrus, and the crus of the fornix were omitted from the tail. The most posterior part of the tail was found in the slice where an ovoid mass of grey matter started to appear inferiomedially to the trigone of the lateral ventricle where its horn served as a lateral boundary. Medially, the border of the tail is easy to identify by white matter. An arbitrary border was defined for the superior border of tail. The inferior border of the tail is easy to identify by the transition of grey to white matter. Moving further anteriorly, the grey matter of the tail descended in the coronal slices. Next, the body of the hippocampus is reached, whose superior and inferior borders are clearly perceptible in the sagittal orientation. In coronal orientation, body and entorhinal cortex (EC) consist of several parts that fold onto each other to form an S-shaped structure (left body) or an inverted S-shaped structure respectively (right body). At this point, the following landmarks of the body were employed for labeling. First, the most visible inferior-lateral layer of grey matter was excluded, assuming that it actually represents parahippocampal gyrus. The sagittal view of the MRI images offers the best visualization of this superior border of the body. Finally, the dentate gyrus, located in between the four CA regions in the hippocampal formation, together with the CA regions themselves and the subiculum, were included. In cases where the subiculum was more detached from entorhinal cortex, a line of white matter was visible between the two structures, and this line was employed as border for the body. The lateral border of the body was identified by the inferior horn of the lateral ventricle or the caudally adjacent white matter. Moving further anteriorly, one reaches the head of the hippocampus. The first coronal slice showing the head can be identified by the emergence of the uncal recess of the head in the superomedial region of the hippocampus. The sagittal view was employed for identification of the anterior border, since the alveus could often

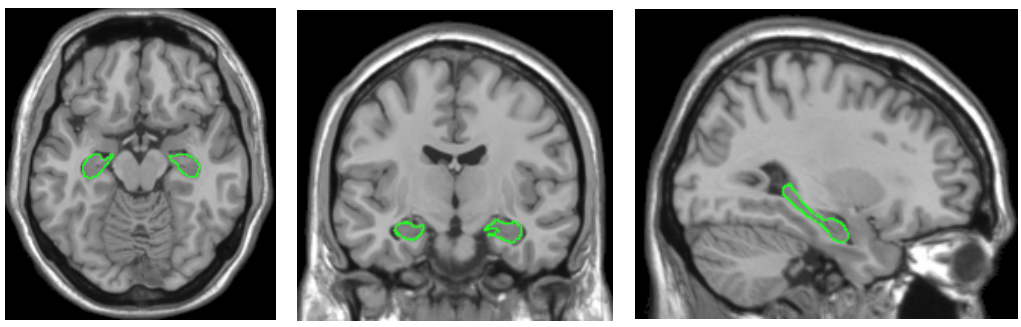

Figure 4.5.3. *T1-weighted MRI showing boundaries for volumetric measures of hippocampus (in order, axial view z = –15, coronal view y = –12, and sagittal view x = 30).*

be better identified in this plane. The axial view was consistently employed in addition to the coronal and sagittal views for demarcation of the anterior and medial borders of the hippocampus. The medial and inferior borders were identified including subiculum, the four CA regions and the dentate gyrus. In the superior-medial part, the head often forms a distinct protuberance, which can be best identified in the coronal plane. Also, the uncal cleft often served as an excellent marker of the inferior border of the head of the hippocampus.

Amygdali and hippocampi were traced for each volunteer on both sides and they were drawn by the same trained researcher (MJP) who was blind for drawings. Intraclass reliability coefficient was 0.93. A two-way repeated measures ANOVA was carried out for the volumes of hippocampi and amygdale, with "lateralization" (left versus right) as the within-subjects factor, and "group" (high N versus low N) and "gender" as between-subjects factors. Significant interactions were further analysed using t-tests. All amygdali and hippocampi volumes were divided by whole brain volume for each subject.

Experiment 3

Emotion Potentiated Startle

Individuals were seated in a comfortable chair positioned approximately ½ m from a 17-in. monitor upon which pictures were displayed. Prior to the picture presentation, electrodes for recording startle responses were placed around the eye and impedances checked. In order to familiarize the participants with the procedure and habituate them to the acoustic startle probe, they then viewed an introductory set of nine neutral pictures, during seven of which startle probes were presented.

Pictures were presented in two blocks of 21 pictures, with seven pictures of three different valences (pleasant, unpleasant and neutral) included in each block. The presentation of the pictures and acoustic startle probes were controlled by in-house software on a PC. Pictures were presented in a quasi-random order, with the constraint that no more than two stimuli of a given valence were presented consecutively. Pictures were presented for five seconds each. Immediately after each picture, a blank screen was presented for fifteen seconds.

The acoustic startle probe was a 50-ms burst of white noise at 95dB with a nearly instantaneous rise time. Startle probes were delivered binaurally through headphones. Raw and integrated electromyography (EMG) was collected using three Sensormedics electrodes placed on the inferior left orbitocular muscle. Electrodes were placed around the left eye.

Peak magnitude was scored in a window between 20 and 150 ms following probe onset by subtracting EMG activity at reflex onset from peak amplitude. Some eye blink reflexes were excluded when reflex onset was prior to 20 ms following probe onset or due to an unstable baseline. Trials with no perceptible eye blink reflex were assigned a magnitude of zero and included in the analysis. Peak magnitude was z-transformed within subject. After emotional potentiated startle, subjects were asked to rate the pictures in terms of arousing characteristics

(highly arousing versus lowly arousing) and valence (positive versus negative) using a scale form 1 (negative) to 10 (positive).

A repeated-measures ANOVA was performed for the task, with group and gender as between-group factors and pictures' valence (pleasant, unpleasant and neutral) as within-subject factor. A Huynh-Feldt correction was used where the assumption of sphericity was violated (uncorrected df reported). Significant interactions were further analyzed by using simple main effect analyses. We conducted a repeated-measures ANCOVA, with scores on BDI and STAI-T as covariates.

4.5.3. Results

Experiment 1: amygdala activation to unconscious fearful and happy faces

The repeated-measures ANOVA did not show any significant main effect in group by emotion by lateralization interaction (F = 1.41, df = 3, 66, p = 0.25). All subjects showed a similar activation in left amygdala for fear and happy fixation (Figure 4.5.4). Since the subjective state of both groups was significantly different (see Table 4.4.1), a repeated-measures ANCOVA was carried out, controlling for BDI and STAI-State scores. No significant main effect came up for emotion by group by lateralization (F = 1.33, df = 1, 22, p = 0.26), or for emotion by lateralization (F = 0.07, df= 1, 22, p = 0.79). In an exploratory analysis, there was a significant difference in the right amygdala between fear and happy fixation (t = –2.17, df = 11, p =0.04) in the high N group.

Experiment 2: volumetric results

There was a significant interaction in group by gender by side for amygdala volumes: F = 13.96, df = 1, 20, p = 0.001. Post hoc analyses showed a group effect in right amygdala (F = 9.25, df = 1, 22, p = 0.006), which was smaller in high N subjects. A significant asymmetry between amygdali was observed in low N subjects (t = –4.03, df = 11, p = 0.002), but not in the high N group (Figure 4.5.5). Regarding hippocampi volumes, there was a significant group effect (F = 4.7, df = 1, 20, p = 0.04), in which high N subjects had bigger bilateral hippocampi (Figure 4.5.6).

Experiment 3: emotion potentiated startle responses

The potentiated startle repeated measures ANOVA showed a significant interaction for emotion (pleasant, unpleasant and neutral) by group (F = 3.45, df = 2, 84, p = 0.05). Post-hoc analysis showed reduced emotion potentiated startle in high N subjects compared to low N subjects, with greater relative response to neutral versus emotional pictures (see Figure 4.5.7). ANCOVA revealed that the interaction for emotion by group remained significant, after controlling for the effects of anxiety and depressive symptoms (F = 3.49, df = 2, 84, p = 0.03). Ratings of pictures in terms of arousal or valence did not show any difference between groups.

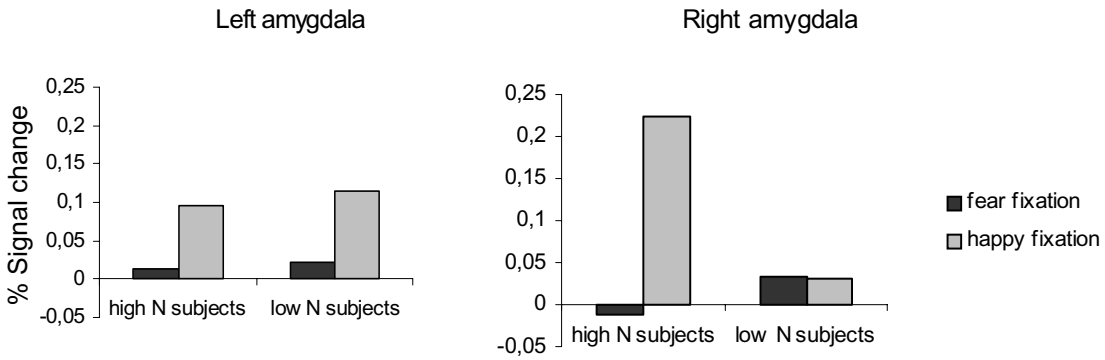

Figure 4.5.4. *Percent change in normalised magnetic resonance signal intensity in each amygdala for the comparison fear and happy fixation on the task.*

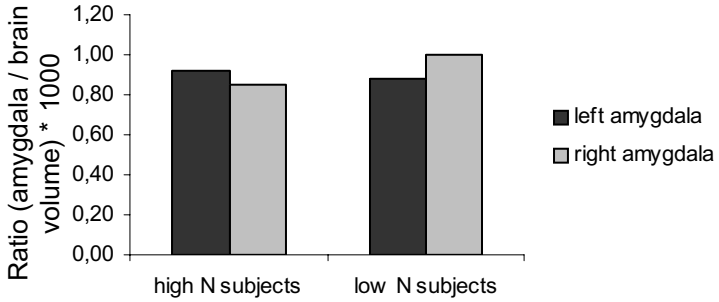

Figure 4.5.5. *Bilateral relative amygdali (amygdala/total brain) mean volumes in both groups.*

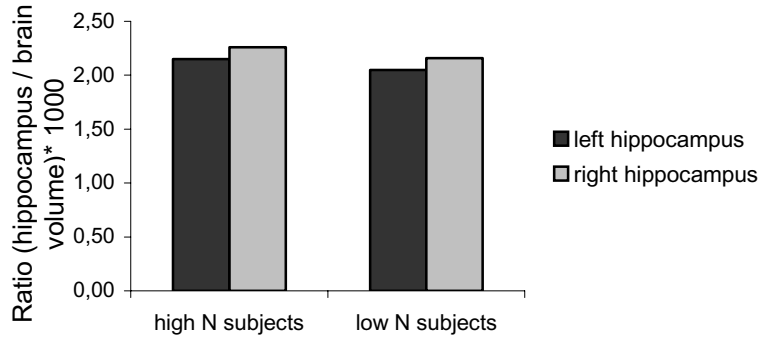

Figure 4.5.6. *Bilateral relative hippocampi (hippocampus/total brain) mean volumes in both groups.*

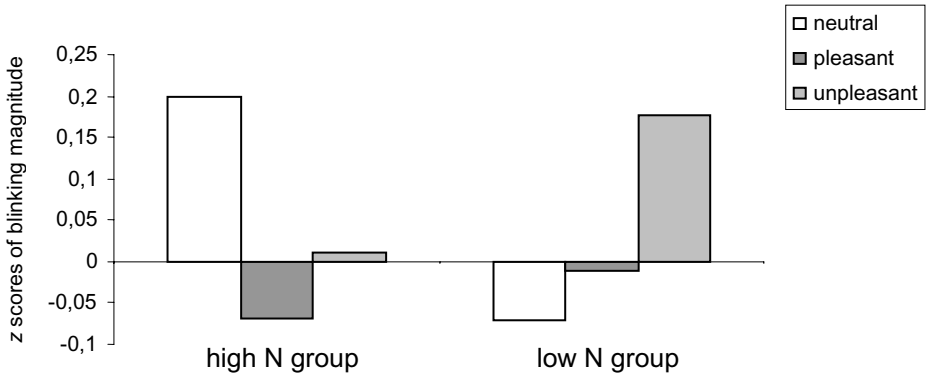

Figure 4.5.7. *Startle potentiation by group. The values represent average of z-scores for neutral, pleasant and unpleasant probe pictures.*

4.5.4. Discussion

Experiment 1

The principal aim of this experiment was to examine activity within the amygdala in response to fearful and happy facial expressions presented out of conscious awareness. Our results show that high N subjects activate left amygdala similarly to low N subjects. This activation is greater for happy faces in both groups, although interaction does not reach significance. High N volunteers show a greater activation for happy versus fearful faces (in an exploratory analysis).

Although the neuroimaging and neuropsychological literature on the topic of emotional facial expressions recognition has tended to emphasise roles for the amygdala in perception of negative facial expressions (Adolphs *et al.*, 2002; Hariri *et al.*, 2003; Morris *et al.*, 1996), a more considered view suggest that the amygdala responds to stimuli of motivational significance, independent of their emotional valence (Winston *et al.*, 2003). Accordingly, Williams *et al.* (2004) found that amygdala activity was significantly increased for happy versus neutral faces when the face was suppressed from consciousness.

Regarding amygdala activation studies using masked fearful facial expressions, the results of such fMRI studies are mixed. Increases in amygdala activation to the presentation of masked (unseen) fearful facial expressions have been demonstrated in neurologically normal individuals by Whalen *et al.* (1998) and Öhman (2002). By contrast, Phillips *et al.*, (2004), who used a similar masked fear fMRI block design study as ours, observed no amygdalar activation in response to the covert fear stimulus compared to neutral stimulus, concluding that in a covert fear condition the amygdala was not activated. Overall, these findings suggest a broader role for the amygdala in modulating the vigilance level during the perception of both negative and positive facial emotions, and distinct neural correlates of conscious and unconscious emotion perception.

In depressive disorders, left amygdala hyperarousal is observed, even when processing stimuli outside conscious awareness (Sheline *et al.*, 2001). Moreover, these authors find that increased amygdala activation normalises with antidepressant treatment. Another study (Siegle *et al.*, 2002) suggests that depressed individuals display sustained amygdala processing in response to negative information. Our results do not resemble the pattern of hyperactivation observed in depressive disorders. This could be related to the fact that these subjects had never suffered from depression in the past, and were past the high risk age for a first depressive episode, at the time of the study. However, high N individuals show increased amygdala activation (specially the right amygdala) for unconscious happy facial expressions, which could suggest amygdala hyperarousal in N for positive stimuli and some kind of habituation for aversive stimuli to avoid harmful consequences in the system.

Experiment 2

The main finding of this experiment is that some volumetric differences in the amygdala and the hippocampus are related to N. Highly neurotic individuals show a lack of asymmetry between amygdali, smaller right amygdala volumes when compared to low N subjects' volumes, and bilaterally bigger hippocampi.

Up to now, the pathophysiologic background of amygdala volumes in MDD has remained unclear. One study suggests enlarged amygdala volumes in first-episode patients (Frodl *et al.*, 2002). These authors, in a subsequent study (Frodl *et al.*, 2003), find that the disease progression with stress-related excitotoxic processes during recurrent depressive episodes results in decreased amygdala volumes. It is difficult to elucidate whether differences in amygdala volumes result in a predisposition or vulnerability for depression from our results. However, our results show some kind of abnormality related to high scores on N: we observed similar bilateral amygdala volumes in high N subjects, whereas the low N group showed amygdala volumetric asymmetry (with bigger right amygdala). A recent study (Pedraza *et al.*, 2004) reports asymmetry of the amygdala in normal adults, with larger right amygdala volumes. We observed smaller volume together with greater activation for masked happy faces in the high N group's right amygdala. Greater activation of the amygdala could contribute to the decreased volume, potentially due to excitotoxic effects of glutamate.

The stress toxicity model, together with the BDNF hypothesis relates brain volume loss in depression, and suggests that hypercortisolaemia in patients with acute depression can affect the hippocampal neurons via glutamate excitation (Salposky, 2000). Campbell *et al.* (2004) in a meta-analysis conclude that hippocampal volume is lower in patients with depression. We observed bigger hippocampal volume in highly neurotic subjects, and the same pattern of asymmetry when compared to low N subjects. These results would go in the opposite direction of the stress-related excitotoxic processes. We previously reported that high N subjects show elevated waking salivary cortisol levels (Portella *et al.* in press), and these levels were then normalised during the day. Thus, it could be possible that some kind of neuroprotective system would control the excess of cortisol as well as its toxic effects on the hippocampus.

Overall, this experiment has some limitations. First, volumetric analyses were conducted using edge manual tracing. As has been pointed out, edge tracing does not offer the precision that unbiased stereological volumetric determination allows. Second, regarding the boundaries of the structures examined in this study, differentiation between the amygdala and the anterior hippocampus is difficult because the posterior part of the amygdala is partly overlapping the anterior part of the hippocampal head. However, a three-dimensional protocol for manual tracing has been used (Pruessner *et al.*, 2000), so the overlap between these two structures can be disclosed using simultaneous sagittal and coronal views.

Experiment 3

Abnormalities in the emotion potentiated startle task are seen in the high N volunteers. Both groups rated the affective stimuli comparably in terms of valence and arousal. However, high N volunteers showed reduced startle responses when viewing aversive compared to neutral stimuli. A reduction of aversive startle responses has also been reported in patients suffering from panic disorder and post-traumatic stress disorder (Cuthbert et al. 2003). Similarly, major depression has been associated with larger eye-blink responses while viewing positive compared to aversive stimuli (Allen et al. 1999). The current results suggest that emotion potentiated startle may be affected in volunteers vulnerable to depression in the absence of history or current symptoms of mood or anxiety disorders. There are a number of possible explanations for this paradoxical down-regulation of startle responses to aversive images in depression, anxiety and neuroticism. First, the modulating effects of aversive stimuli on startle responses may follow an inverted U-shaped function, with increasing anxiety leading to greater startle responses up to asymptote, after which further increases in anxiety lead to relatively reduced responses. This could be tested by examining startle responses with less traumatic aversive stimuli or in volunteers lower in neuroticism. Second, this reduced response may reflect top-down gating of aversive information in highly anxious or neurotic individuals, which might avoid over-stimulation. Since the paradigms used to examine EPS vary quite considerable (Grillon et al. 2003), further research is required to assess the impact of anxiety and mood disorders and the role of risk factors, using standard protocols, stimuli and analysis.

To our knowledge, this is the first study that addresses the personality characteristics involvement in the pathogenesis of depressive disorders in terms of medial temporal function and structure. The present findings show temporo-limbic abnormalities in highly neurotic subjects: i) hyperactivation of right amygdala for happy facial expressions, but not for fearful stimuli, when compared to subjects with low scores on N; ii) decreased amygdala volumes, especially in the right amygdala, and bigger bilateral hippocampi, which casts doubt on the hypercortisolaemia hypothesis in N; iii) startle responses when viewing aversive compared to neutral stimuli. These results do not fully confirm our initial hypothesis that highly neurotic volunteers would show similar medial temporal abnormalities as the observed in subjects suffering from depressive disorder. However, it should be pointed out that the high N subjects were mainly in their forties and maybe they had passed the age of risk for a first episode (Burke et al. 1990). Therefore, these abnormalities observed in this study may reflect top-down gating of aversive information in highly neurotic individuals, potentially operating as a protective mechanism to prevent over-stimulation of the limbic areas. Such actions could act as a protective mechanism against the development of depression. As such this raises the question of what we are assessing in high N volunteers who have not become depressed by their late 30's to 40's. In particular, we may be assessing a particularly resilient sub-group of this personality type.

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5. CONCLUSIONS OF THE THESIS

In this thesis we have discussed different trait–state factors of depressive disorders and neuroticism -as a marker of psychobiological vulnerability-. Next, we present a list of the main conclusions reached throughout the work:

- Cognitive impairment of visuoperception, processing speed, and memory functions can be considered a trait characteristic of elderly major depression, since no significant improvement was observed regardless of the successful response to treatment. The residual cognitive impairment may reflect non–progressive temporal lobe dysfunctions rather than progressive dementia.

  The lack of executive dysfunction in elderly major depression found in our study could be due to a ceiling effect of the Tower of London task, which can probably be amended by changing the correction and the scoring system of the test, since this change provides a significantly wider variance, which could overcome the ceiling effect.

- Cognitive and emotional processing abnormalities in extremely high-neurotic healthy subjects are found, when compared with extremely low-neurotic subjects. Our findings show that high scores on neuroticism are associated with abnormalities of cognitive functioning without past or current illness. Thus, neuropsychological impairments in planning and attentional domains may in part mediate trait vulnerability to depression. The emotional bias in high neurotic subjects is similar to that observed in anxiety and depressive disorders, but is partly related to current anxious-depressive symptomatology. So we cannot assure that the results prove that there is a mechanism for trait-related risk factors for major depressive disorders. In any case, a new challenge is to unpack the likely neuropsychological mechanisms mediating the vulnerability of high neurotic subjects to depression.

- Elevated morning salivary cortisol levels can exist in the absence of major depression, as they are observed in subjects with high N scores who have never experienced depression. The salivary cortisol response to waking represents a single aspect of Hypothalamic-Pituitary-Adrenal axis function; thus we cannot conclude that neuroticism is associated with generally increased HPA axis activity, but the results are consistent with a greater adrenocorticotrophic hormone response to waking or increased sensitivity of the adrenal gland to ACTH stimulation in highly neurotic subjects.

- High neurotic volunteers recruit a number of limbic areas such as the insula, the cingulate gyrus, and the thalamus, when processing emotional stimuli. Faces best activate these regions associated with emotion when the emotional content is not the focus. The greater activation observed in high neurotic subjects in limbic regions, but not the amygdala, leads to consider that neuroticism itself does not fully resemble the abnormalities of depressive disorders. Nevertheless, neuroticism would exert an influence on the emotional responses in a higher level of processing.

- Highly neurotic volunteers show medial temporal lobe abnormalities, but these are different to the observed in subjects suffering from depressive disorders. However, it should be pointed out that the high N subjects were mainly in their forties and might have passed the age of risk for a first episode. These abnormalities observed in this study may reflect top–down gating of aversive information in highly neurotic individuals, potentially operating as a protective mechanism to prevent over-stimulation of the limbic areas, and possibly to become definitely depressed.

We bring this thesis to an end by facing up future work directions through the following open issues:

a. The involvement of temporolimbic brain structures in elderly major depression, focusing in trait-state factors of the illness.

b. Replication of the experiments presented in this dissertation in younger high N subjects. At present, studies are being carried out to examine cognitive task performance in samples of younger neurotic volunteers to assess whether similar effects are found in a sample still at high prospective risk for anxiety and depression.

c. Replication of these experiments in high N volunteers who have suffered from depression. We are carrying out new studies to examine the protective mechanism discussed in this thesis.

ANNEX A. 'THE TOWER OF LONDON': MENTAL PLANNING, VALIDITY AND THE CEILING EFFECT

(Translation of the published article by Portella *et al.* 2003. *Rev Neurol* 37: 210-213)

A.1. INTRODUCTION

The Tower of London (TOL) is a neuropsychological test developed by Shallice (1982) to identify the deterioration in planning processes associated with frontal lobe dysfunctions. The planning component of the TOL involves making an analysis of means and ends in order to solve a set of problems that increase in difficulty. Planning processes are considered to be executive behaviours controlled by the frontal lobes (Stuss, 1992; Estévez *et al.*, 2000). The TOL is an adaptation and simplification of the type of problem presented in the Tower of Hanoi, and allows gradual changes in the difficulty of the problem.

There are various systems of administration and correction, ranging from the classical version with wooden pegs to the computerized version (Sahakian and Owen, 1992). In recent years the use of the TOL as a test of planning ability has increased, both in subjects with frontal lobe lesions and in those with mental disorders (Baker *et al.*, 1996; Dehaene and Changeux, 1997; Purcell *et al.*, 1997; Schnirman *et al.*, 1998; Phillips *et al.*, 1999; Phillips *et al.*, 2001; Welsh and Pennington, 1988).

With the classical version (Shallice, 1982) the results of the studies have been inconsistent. The observation of a ceiling effect, especially in younger subjects, raised doubts about the test's validity and reliability (Baker *et al.*, 2001; Welsh *et al.*, 2000). In the only study performed in normal subjects, Kafer and Hunter (1997) concluded that there are still many unanswered questions on the meaning of the latent construct of planning/problem-solving and the psychometric structure of the TOL.

So the main aim of this study was to find a system of administration and correction for the TOL that would overcome some of the limitations of the classical version. First we propose a set of instructions that increase the cognitive demand of the planning task, and second, we present a new scoring system that broadens the range of scores and thus avoids the ceiling effect observed in normal and young subjects.

A.2. METHODS

Design

A correlational study with two independent, counterbalanced groups administered two versions of the TOL. One group was administered the version proposed by Krikorian *et al.* (1994), and the other a new procedure specially devised for this study. All subjects were also administered the Porteus Mazes (Porteus, 1965) as a correlation variable in each group.

We selected a sample of 39 healthy subjects, age range 18 - 50, controlling the variables sex and years of schooling, who were assigned randomly to two groups. Mean ages were 31.40 years (SD = 8.77) in group 1 and 31.95 years (SD = 9.63) in group 2. Mean years of schooling in group 1 were11.90 (SD = 3.65 years) in group 1 and 12.16 (SD = 3.66) in group 2.

Procedure

Each group was administered the Porteus Mazes and one of the two versions of the TOL. Both versions were based on the original task devised by Shallice, comprising a base with three equidistant pegs of different lengths and three beads of the same size, one red, one blue and one green (Figure A.1). The subject had to move the three beads on the pegs to reproduce the various final positions shown in Figure A.2 in a particular number of moves.

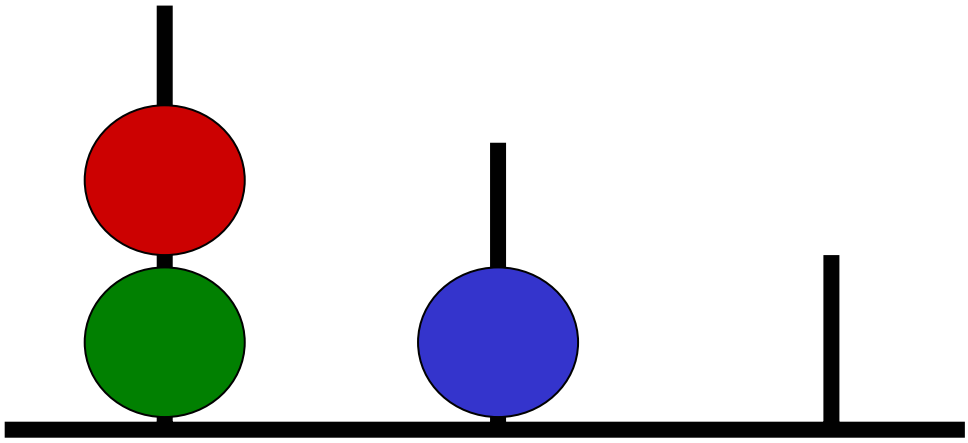

Figure A.1. *Shallice's Tower of London..*

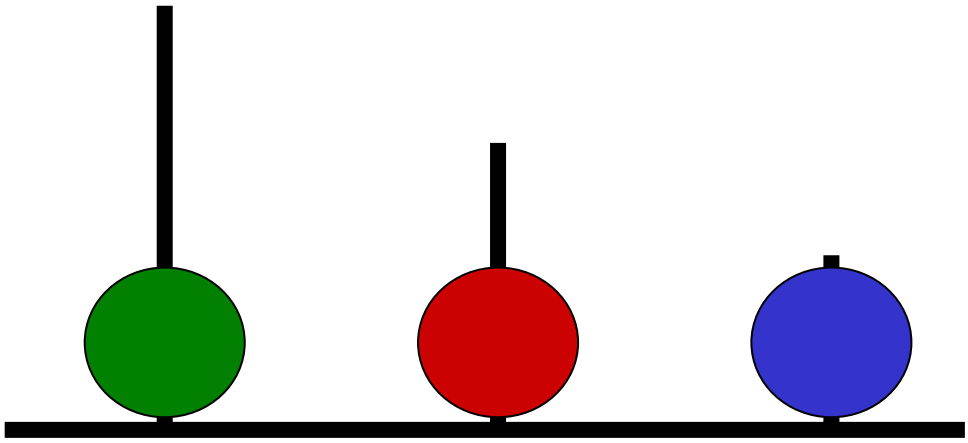

Figure A.2. *Example of one of the models shown to subjects.*

In Krikorian's version (Krikorian *et al.*, 1994) there are 12 problems (final positions) and a series of rules that must be observed. Subjects are told to move the beads from their initial position (which is the same for each problem) to a final position in a specific number of moves, and observing a set of rules. In this version subjects have three goes for each problem, and the score depends on whether they manage to reach the final position using the number of moves and within the number of attempts allowed. The highest possible score is 36 (Krikorian *et al.*, 1994).

The new version proposed for the present study was based on Krikorian's version of Shallice's original model. The physical characteristics of the test and the number of problems were the same, but we added a new rule: students were explicitly asked to think carefully beforehand about the moves they would make. Another modification was that we did not allow three attempts at each problem. Finally, the score depends on the number of moves made for each problem. The highest possible score is 46. These modifications were introduced in order to increase the test's mental resolution, above all the elimination of the number of attempts: subjects who do not plan in advance the moves they will make are unlikely to solve the problems with more moves and thus obtain a high score on the task.

For the correlational study in each group, the Porteus mazes were administered. This test provides information on higher areas of mental functioning that involve planning (Lezak, 1995). The extended series for adults was used (Porteus, 1965).

Statistical analysis

Data were analysed using the SPSS 10.0 statistical package for Windows. The level of significance was set at $\alpha = 0.05$ (bilateral), with a power of $\alpha = 0.72$.

To see the effects of a change in the instructions and the correction of the TOL, we obtained the correlation coefficients for each group (group 1 was administered Krikorian's procedure and the Porteus mazes, and group 2 the new procedure and the Porteus mazes). The dispersion linearity of the two variables to be related was controlled beforehand.

To compare the two correlation coefficients a statistical contrast was calculated, and the coefficients were transformed into Fisher '*Z*' scores.

To check the effect of the changes in the scoring system proposed in this study, we obtained the coefficients of variation (CV) of the TOL in each group to analyse the variability, since the ranges of scores for each version were different.

A.3. RESULTS

The correlation coefficients in both groups showed a direct and statistically significant relation (for group 1, $r = 0.56$, $p = 0.01$; for group 2, $r = 0.55$, $p = 0.01$). Though direct, significant relations were found, the explained variance was 31.3% for group 1 and 30.2% for group 2.

There was no statistically significant difference between the two correlation coefficients, (Z = –0.041; p = 0.967). The values of the CV were 9.06 and 19.32 for groups 1 and 2 respectively and the variances of both groups for this variable were significantly different ($F(19.18) = 5.81$; $p < 0.001$).

A.4. DISCUSSION

Two conclusions can be drawn from the study. On the one hand, the two versions used seem to be measuring the same mental planning ability, when they are related to another test that measures this ability. Similarly, the change in the instructions to introduce a higher cognitive demand does not seem to have any effect on the task, since very similar correlation coefficients were found in both groups. This finding is in agreement with other studies which analysed the effect on the validity of the TOL of using different versions of this neuropsychological task. Though the studies do not present a clear pattern, one of them reports major variations in the results when using different versions (Welsh *et al.*, 2000); other studies found different versions of both the TOL and the Tower of Hanoi to be highly consistent (Baker *et al.*, 2001; Mataix and Bartres-Faz, 2002).

This study also tried to modify the scoring system of the classical version of the TOL in order to overcome the ceiling effect. This effect, observed in Krikorian's version (Krikorian*et al.*, 1994), may be due to the fact that three attempts were allowed per problem or because subjects obtained the same score on each of the problems, regardless of difficulty. In the present study the task could not be solved using trial and error, and only the problems solved at the first attempt were scored; in addition, attempts in which a greater number of moves were required to reach the final position were awarded higher scores. The results show that with this correction, based on the difficulty of the problem (that is, according to the number of moves to be made in each problem) a much wider CV is found, which appears to avoid the ceiling effect found in the classical version of the TOL. So the new scoring system proposed in this study, as well as avoiding high scores, also rules out the possibility of solving problems by trial and error, and improves the apparent validity of the planning test. It has been clearly shown in one of the computerized versions (Sahakian and Owen, 1992; Robbins *et al.*, 1998) of the TOL that correction systems in which the second and third attempts are eliminated are better tests of planning.

In conclusion, the increase in cognitive demand introduced in the instruction conditions does not appear to alter the test significantly, since similar correlations are found with Krikorian's method. However, the test's apparent validity does seem to improve; furthermore, the change in the correction of the test and in the scoring system provides a significantly wider variance which overcomes the ceiling effect.

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