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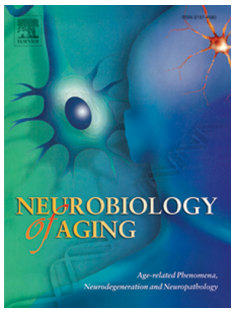

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Anomalies occurring in lipid profiles and protein distribution in frontal cortex lipid rafts in Dementia with Lewy bodies (DLB) disclose neurochemical traits partially shared by Alzheimer's and Parkinson´s diseases.

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Running title: Altered lipid rafts in DLB frontal cortex

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Abstract

Lipid rafts are highly dynamic membrane microdomains intimately associated with cell signaling. Compelling evidence has demonstrated that alterations in lipid rafts are associated with neurodegenerative diseases such Alzheimer's disease, but at present, whether alterations in lipid raft microdomains occurs in other types of dementia such Dementia with Lewy Bodies (DLB) remains unknown. Our analyses reveal that lipid rafts from DLB exhibit aberrant lipid profiles including low levels of n-3 long chain polyunsaturated fatty acids (mainly docosahexaenoic acid), plasmalogens and cholesterol, and reduced unsaturation and peroxidability indexes. As a consequence, lipid raft resident proteins holding principal factors of the β-amyloidogenic pathway, including β-amyloid precursor protein, presenilin 1, β-secretase and PrP, are redistributed between lipid rafts and non-raft domains in DLB frontal cortex. Meta-analysis discloses certain similarities in the altered composition of lipid rafts between DLB and Parkinson's disease which are in line with the spectrum of Lewy Body Diseases. In addition, redistribution of proteins linked to the β-amyloidogenic pathway in DLB can facilitate generation of β-amyloid, thus providing mechanistic clues to the intriguing convergence of Alzheimer's disease pathology, particularly β-amyloid deposition, in DLB.

Key words: Lipid rafts, docosahexaenoic acid, cholesterol, plasmalogens, β-secretase, amyloid precursor protein, amyloidogenic processing, human brain cortex, Lewy Body Diseases, Alzheimer's Disease, Parkinson's Disease

1. Introduction

Dementia with Lewy bodies (DLB) is the second most common neurodegenerative dementia in the elderly, clinically manifested by neuropsychiatric symptoms, abnormal behavior, fluctuating course, visual hallucinations, early cognitive impairment and dementia and moderate parkinsonian symptoms (McKeith et al., 2013). DLB is pathologically characterized by widespread α-synuclein deposition in Lewy bodies and Lewy neurites in the brainstem, limbic system and cortical areas (Ince, 2011; McKeith et al., 2013). Other changes are neuron loss, microvacuolation and Alzheimer-like pathology, particularly β-amyloid deposition, senile plaque formation and early changes in neurofibrillary tangle pathology (Ince, 2011). The main pathological change is the accumulation of abnormal α-synuclein, which is phosphorylated and nitrated, has abnormal solubility and forms different oligomeric species and eventually aggregates into fibrils (Kaplan et al., 2003; Mukaetova-Ladinska and McKeith, 2006). Synapses are primarily damaged in DLB (Dalfó et al., 2004; Kramer and Schulz-Schaeffer, 2007; Overk and Masliah, 2014; Schulz-Schaeffer, 2010) leading to alterations in neurotransmitter signaling (Dalfó et al., 2004).

Lipid rafts are membrane microdomains characterized by their high level of cholesterol, sphingolipids and saturated fatty acids, as well as by reduced amounts of polyunsaturated fatty acids (PUFA). These microdomains provide a highly saturated liquid-ordered microenvironment that promotes protein-lipid and protein-protein interactions (Brown and London, 2000; Pike, 2004). Lipid rafts comprise a highly dynamic clustering of proteins and lipids playing a central role in signal transduction and intercellular communication. Their alterations have been associated with impaired synaptic transmission, excitotoxicity, abnormal neurotransmitter signaling, neuronal apoptosis and neurodegeneration (Allen et al., 2007; Ferrer, 2009a; Marín et al., 2007; Ramírez et al., 2009). Lipid rafts have also been implicated in β-amyloid processing in Alzheimer's disease (AD) (Cordy et al., 2006; Hicks et al., 2012; Vetrivel and Thinakaran, 2010) and with α-synuclein-membrane interactions in Parkinson's disease (PD) (Fabelo et al., 2011; Ferrer, 2009b; Kubo et al., 2005; Park et al., 2009).

Our previous studies have shown marked alterations in the composition of lipid rafts in the frontal cortex in AD and neocortex and related models and in the frontal cortex

in PD at different stages of disease progression (Fabelo et al., 2011; Fabelo et al., 2014; Martín et al., 2010). Although modifications in the composition of lipid rafts exist in both groups of diseases, disease-specific changes occur in AD and PD.

For this reason, the present study is first focused on assessing the lipid composition and protein redistribution in lipid rafts in the frontal cortex of DLB, and second on performing a meta-analysis comparing observations in DLB, PD and AD. The latter point is sustained by the fact that the same methods and the same hands performed the studies in the different diseases, with the goal of identifying patterns that may contribute to revealing links between PD and DLB, the main diseases of the Lewy Body Disease (LBD) spectrum, and the high prevalence of AD in DLB related mainly to β-amyloid pathology.

2. Materials and Methods

2.1. Human brain tissue

Brain tissues were obtained from the Institute of Neuropathology Brain Bank (Hospital Universitari de Bellvitge, Barcelona, Spain) following the guidelines of the local ethics committee. Eight cases (average age 80.63 ± 1.69) had suffered from severe (Global deterioration scale) dementia of DLB type. Ten cases were neurologically normal and classified as control subjects (average age 72.80 ± 1.79). Post-mortem delay was between 2 h and 16 h 30 min. Frontal cortex tissue (cortex area 8) was used for the isolation of lipid rafts. This area was selected because frontal cortex is affected in DLB and this region has been also analyzed in other neurodegenerative diseases with abnormal protein aggregates causing dementia, which permits comparative assessment of disease-dependent modifications of lipid rafts. Cases with and without clinical neurological disease were processed in the same way following the same sampling and staining protocols. At autopsy, half of the brain was fixed in 4% buffered formalin for about one month while the other half was cut in coronal sections 1 cm thick, and 1-mm-thick sections of selected brain regions were dissected, kept on labeled plastic bags and immediately frozen and stored at -80ºC; the rest of the hemi-encephalon was frozen and stored at -80ºC. The neuropathological study was carried out on de-waxed 4 µm-thick paraffin sections from twenty-five brain regions which were stained with haematoxylin and eosin, Klüver Barrera and for immunohistochemistry to glial fibrillary acidic protein, CD68 and *Licopersicum esculentum* lectin for microglia, β-amyloid, phosphorylated tau (clone AT8), phosphorylation-specific tau Thr181, Ser202, Ser214, Ser262, Ser396 and Ser422, αB-crystallin, α-synuclein, ubiquitin, p62, TDP-43 and PrP. Neuropathological classification of the present cases was carried out following the guidelines of the BrainNet Europe Consortium (Alafuzoff et al., 2009). A summary of the cases is shown in Table 1.

2.2. Isolation of lipid rafts

Samples of frontal cortex area 8 grey matter were carefully dissected avoiding contamination with white matter. Lipid raft fractions were isolated following the protocols previously described for human brain (Fabelo et al., 2014; Martín et al., 2010). Briefly, 0.1g of frontal cortex was homogenized in homogenization buffer

(50mM Tris-HCl, pH 8.0, 10mM $MgCl_2$, 20mM NaF, 1mM Na_3VO_4, 5mM β-mercaptoethanol, 1mM phenylmethylsulfonyl fluoride (PMSF) and a cocktail of protease inhibitors (Roche Diagnostics, Barcelona, Spain) containing 1% Triton X-100 and 5% glycerol in a glass homogenizer grinder. Homogenates were then centrifuged at 500xg for 5 min and the supernatant was collected and mixed in an orbital rotor for 1 h. All steps were performed on ice or in a cold room at 4ºC. About 800µl of each sample was mixed with an equal volume of 80% sucrose in buffer A and then over-layered with 7.5 ml of 35% sucrose solution and 2.7 ml of 15% sucrose solution in homogenization buffer in 10 ml ultracentrifuge tubes (Ultraclear, Beckman, Brea, CA, USA). Sucrose gradients were centrifuged at 150,000xg for 18 h at 4ºC in a Beckman SW41Ti rotor. Two ml fractions were collected from the top to the bottom of the ultracentrifuge tubes, and the final pellet, corresponding to the precipitated detergent soluble fractions (i.e. non-raft fractions), was re-suspended in 200 µl of homogenization buffer and frozen at -80 ºC until analysis.

2.3. Immunoblotting

Protein analysis was performed by Western blotting. The different lipid raft fractions were resuspendend in loading buffer (625 mM Tris-HCl, 1% sodium dodecyl sulphate, 100% glycerol, 5% β-mercaptoethanol and 0.001 % bromophenol blue, pH 6.8), and boiled for 5 min. Protein electrophoresis was performed on 12.5% SDS-PAGE using Mini-Protean TGX Gels (456-1034 Bio-Rad), and transferred to PVDF membranes, using Trans-blot Turbo Transfer System (Bio-Rad), according to manufacturer's instructions. Membranes were treated with Blotting grade blocker diluted in Tris-buffered saline at 5% (BLOTTO), followed by incubation with the different primary antibodies used in the study diluted 1:1,000 in BLOTTO: mouse monoclonal anti-VDAC antibody (ab1474 Abcam), mouse monoclonal anti-PSEN1 antibody (ab15456 Abcam), rabbit polyclonal anti-BACE antibody (AP5940 Millipore), rabbit polyclonal α-synuclein (α-Syn) antibody (S3062 Sigma Aldrich), rabbit polyclonal anti caveolin 1 (N—20 Santa Cruz Biotechnology) and rabbit polyclonal anti-APP antibody (ab32136 Abcam). Mouse monoclonal antibodies against Flotilin-1 (SC-74566, Santa Cruz Biotechnology) and prion protein (PrPc) (SC-47730, Santa Cruz Biotechnology) were diluted 1:500. Incubations were overnight at 4ºC with gentle agitation. Primary antibody specific signals were revealed by incubation with horseradish peroxidase conjugated secondary antibodies (diluted 1:10,000 in

BLOTTO) for 2 h at room temperature with gentle agitation. Immunospecific signals were visualized by Clarity Western ECL substrate kit (Bio-Rad). ECL signals were processed using ChemiDoc MP System (Bio-Rad), and analyzed using Image Lab programme provided by the manufacturer.

2.4. Lipid analyses

Total lipids from lipid rafts fractions were extracted with chloroform/methanol (2:1 v/v) containing butylated hydroxytoluene (BHT, 0.01%) as antioxidant (Christie and Han, 2003). Lipid classes were separated from total lipids with HPTLC (one-dimensional double development high performance thin layer chromatography), and quantified by densitometry, as detailed elsewhere (Martín et al., 2006).

Lipids from lipid rafts were subjected to acid-catalyzed trans-methylation with 1% sulfuric acid (v/v) in methanol. The resultant fatty acid methyl esters (FAME) and dimethyl acetals (DMA) were purified with thin layer chromatography (TLC) and quantified using a TRACE GC Ultra (Thermo Fisher Scientific, Waltham, MA, USA) gas chromatograph equipped with a flame ionization detector. Helium was used as a carrier gas.

2.5. Statistical analyses

Comparison between Ctrl and DLB groups was assessed with Student's *t*-test or Mann-Whitney *U*-test depending on the presence of homoscedasticity and normality of experimental values. Results from bivariate statistics are expressed as mean ± SEM. Data from lipid assays were arcsine transformed (percent lipid content) in order to attain normality. Multivariate statistics was applied to metadata comparisons, and was performed using Principal Component Analyses (PCA) and Discriminant Function Analysis (DFA). Predictive variables in DFA were chosen according to the number of cases in each group to fulfill the assumptions of discriminant analysis (Huberty, 1994). Individual canonical scores for each case and centroids for each group were calculated and plotted to predict adscription to a particular group.

3. Results

3.1. Lipid profiles of lipid rafts from DLB frontal cortex

Lipid profiles of lipid rafts were significantly altered in DLB frontal cortex area 8. The most important changes in lipid classes were related to lower cholesterol (CHO) levels and higher levels of sterol esters (SE). Significant amounts of lyso-phosphatidylcholine (LPC), which was totally absent in age-matched control cases, were detected in DLB. Changes resulted in modifications in phospholipid-cholesterol ratio which was significantly increased in DLB (Table 2).

In contrast, only minor changes were detected in the analyses of fatty acids in lipid rafts, mainly in certain saturated and monoenoic fatty acids. Levels of saturated 15:0, 22:0 and 24:0, and monoenoic fatty acids, i.e. 15:1, 16:1, 17:1 and 18:1n-7, were all augmented in DLB lipid rafts. However, in spite of these changes, total levels of saturated and monoenoic fatty acids remained unaffected (Table 3).

The most important changes were observed in long-chain polyunsaturated fatty acids (LCPUFA). Thus, levels of docosahexaenoic acid (22:6n-3, DHA) and total n-3 LCPUFA were significantly reduced, by more than 30%. Further, levels of the main n-6 LCPUFA, arachidonic acid (20:4n-6, AA), were statistically lower than in control lipid rafts, yet the magnitude of reduction was only 13.8%. Paralleling these changes, unsaturation and peroxidability indexes were both dramatically reduced in DLB lipid rafts. These effects were mostly attributable to changes in n-3 LCPUFAs since only minor variations were detected for total fatty acids of the n-6 and n-9 series. Another important group of fatty acids severely affected in DLB are dimethylacetals (DMA), which specifically derive from plasmalogens. This lipid group was observed to be reduced by 35% in lipid rafts from DLB, with the main dimethylacetal in control lipid rafts, 18:0 DMA, the most affected in DLB.

Next, we performed factor analyses (principal component analyses, PCA) in order to identify what of sort lipid components were responsible for the divergence between controls and DLB (Figure 1). When applied to lipid classes we found that the two principal components explained 60% of total variance (Fig. 1B), with lyso-phosphatidylcholine (LPC), sterol esters, free fatty acids, phosphatidylserine (PS) and phosphatidylinositol (PI) positively related, and cholesterol negatively related, to PC1. Fatty acid levels also allowed a segregation based on the distribution of

variance, with 47.37% of total variance explained and 18:1n-7, 15:0 and 16:1 being negatively correlated, and 22:6n-3, 22:5n-6, 18:2n-6 and 18:0 DMA positively correlated, to PC1 (Fig. 1A). Plotting factor scores for each case showed that Control and DLB groups could be distinguished on the basis of their lipid raft fatty acid (Fig. 1C) and lipid class (Fig. 1D) composition.

3.2. Meta-analysis of lipid raft composition in frontal cortex of Controls, DLB, AD and PD

In order to perform a comparative meta-analysis of lipid rafts in the three neurodegenerative diseases, we used previously published data from our laboratories on PD and AD lipid rafts using the same analytical methodologies used in the present study (Fabelo et al., 2011; Martín et al., 2010). First, we used PCA to determine lipid groups mainly contributing to overall variance in controls, DLB and PD. We observed that using three principal components (64.43% of total variance explained), the three groups could be clearly separated (Fig. 2A), and that the main variables within fatty acids contributing to this segregation were DHA, AA, 18:0 DMA, 22:5n-6, 20:3n-6, 16:1n-7 within PC1, monoenes and saturates in PC2, and 18:2n-6, 20:2n-6 and 20:1n-9 within PC3. For lipid classes, using a similar procedure, segregation of groups was also evident (not shown), with the three principal components explaining 74.54% of total variance. The lipid classes mainly contributing to this variance were LPC, SE, cholesterol, PI and PS. Using these multivariate outcomes, we next performed discriminant function analyses to determine the quantitative influence of these lipid variables in discriminating PD, DLB and age-matched controls based only on the lipid fingerprint of lipid rafts. The results shown in Figure 2B reveal that the first canonical function accounted for the great majority of the variation between groups (90.9%) while the second canonical variable accounted for only 9.1%. The variables which showed the highest absolute correlation with respect to every discriminate function were 22:6n-3, 22:4n-6, 22:2n-6, cholesterol, sterol esters, and PI. The 1st discriminant function clearly separates PD and control groups from DLB, while the 2nd function separates CTRL and DLB groups (Fig. 2B). Sequential Chi-square test revealed that the 1st discriminant function contributes to the discrimination of the groups to a large extent (χ^2=67.24, p<0.001), and according to the structure coefficients it was mainly determined by 22:6n-3 variable. The second discriminant function contributes to the discrimination

of the groups to a lesser degree (χ^2=15.16, p<0.05), and it was mainly determined by cholesterol and SE variables.

We next performed similar multivariate analyses for Ctrl, AD and DLB. In these analyses, plasmalogens (as determined by their dimethylacetal content) were excluded from the analyses because they were not determined in the AD group (Martín et al., 2010). The results showed that a combination of three factors in fatty acids explained 59.94% of total variance and 72.51% in the case of lipid classes. According to component matrixes, the main lipid groups accounting for these variances were, for lipid classes, sulphatides, cerebrosides and phosphatydilinositol (PC1), sterol esters (PC2) and cholesterol (PC3), while for fatty acids they were 20:4n-6 and 16:1 (PC1), 22:6n-3 and 20:2n-6 (PC2) and 18:1n-7 and total saturates (PC3). Plotting factor scores for each case in the whole dataset revealed that the three groups could clearly be segregated (Fig. 2C). Discriminant function analyses applied to these groups indicated that the contribution to overall variance of the first and second canonical functions was 83.0% (χ^2=58.98, p<0.001) and 17.0% (χ^2=17.04.84, p>0.01), respectively. The 1st discriminant function clearly separates LBD group from control groups, while the 2nd function roughly separates C>60 and AD groups (Fig. 2D). Structure coefficients revealed that the LPC, sterol esters and total saturates determined the 1st discriminant functions, while the 2nd function was mainly defined by 22:6n-3, 22:2n-6 16:1, cholesterol and sulphatide levels.

3.3. Distribution of lipid raft proteins in DLB

We aimed to investigate whether the raft lipid alterations correlated with changes in the distribution of raft integrated proteins relevant to DLB pathology (Fig. 3). Proteins from F1-F2 raft and non-raft (F6-P) fractions were processed for immunoblotting with the different primary antibodies against Flotillin 1 (Flot 1) and Caveolin 1 (Cav 1) proteins, such as lipid raft scaffolding markers, amyloid precursor protein (APP), β-secretase (BACE) and Presenilin 1 (PSEN1) proteins involved in amyloid beta processing, voltage dependent anion channel 1 (VDAC1), which participates in amyloid beta toxicity, α-synuclein (α-Syn) protein as a main hallmark of PD progression, and Prion Protein (PrPc) (Fig. 3, central panel). The results demonstrated important differences in the distribution of some of these markers in

lipid raft (F1+F2) and non-raft fractions (F6+P). In particular, both VDAC and PrPc were highly abundant in lipid rafts from control samples, but were displaced outside lipid rafts to non-raft fractions in DLB brains as compared with healthy controls (Fig. 3, left panel). Interestingly, similar to previous data observed in cortical areas from AD brains, APP was observed to increase its presence in lipid raft fractions as a consequence of DLB pathology (Fig. 3, right panel). Furthermore, a dramatic change was observed for BACE, which was more abundant in non-raft fractions in control individuals compared to DLB, but considerably augmented in lipid rafts from DLB brains (Fig. 3, right panel). No alterations were detected for PSEN1. Similarly, α-Syn and scaffolding flotillin-1 and caveolin-1 markers remained unaltered (Fig. 3, left panel). These results indicate that lipid alterations in these microstructures are accompanied by alterations in the distribution of components of the amyloidogenic processing machinery and other relevant raft proteins which may be related not only to DLB progression but also to features overlapping with other neurodegenerative diseases.

4. Discussion

The present study provides the first evidence of anomalous lipid composition and protein distribution in lipid rafts in frontal cortex area 8 in DLB. Lipid rafts in DLB have significantly lower levels of cholesterol and higher levels of sterol esters. In addition, significant amounts of lyso-phosphatidylcholine, which is undetectable in brain lipid extracts and absent in lipid rafts from controls, are detected in DLB lipid rafts. This lipid species is generated, in part, by free radical-catalyzed oxidation of polyunsaturated phosphatidylcholines, and its presence is indicative of oxidative damage to membrane phospholipids (Choi et al., 2011). This finding is in line with increased oxidative damage in LBDs, including PD and DLB (Dalfó et al., 2005; Dexter et al., 1986; Dexter et al., 1989; Dexter et al., 1994; Ferrer, 2009; Gómez and Martínez et al., 2010; Kidd, 2000). A reduction in cholesterol with an increase in sterol esters and lyso-phosphatidylcholine is singular to DLB; these modifications are not observed in AD and PD using the same methods (Fabelo et al., 2011; Fabelo et al., 2014; Martín et al., 2010).

Lipid alterations in DLB also affect fatty acids from lipid raft phospholipidism, including saturates, monoenes, and dimethylacetals, and polyunsaturated and long-chain fatty acids. Moreover, total plasmalogens, total n-3 LCPUFA, unsaturation index, peroxidability indexes and n3/n6 ratio are all significantly reduced in DLB. Interestingly, some changes occur in the same direction in PD and DLB but to a variable, disease-dependent degree (Fabelo et al., 2011). However, levels of 18:0, 23:0, total saturates, 20:3n-6, 20:2n-6, 20:3n-6 and 20:4n-6 and 22:5n-3 are altered in frontal cortex in PD but not in the same area in DLB lipid rafts (Fabelo et al., 2011). In contrast, 15:0, 22:0, 24:0, 15:1, 22:1 and 18:1n-7 are altered in frontal cortex in DLB but not in frontal cortex in PD lipid rafts (Fabelo et al., 2011). Together, similar alterations in PD and DLB are consistent within the spectrum of LBDs. However, differences point to disease-specific changes that underlie more dramatic reductions in the unsaturation and peroxidability indexes in PD compared with DLB.

Reduction in the polyunsaturated n-3 and n-6 series in DLB lipid rafts likely alters the biophysical state of phospholipids, thereby affecting the physicochemical properties of lipid rafts. Modifications in the polyunsaturated lipid matrix of lipid rafts affect microdomain viscosity, lateral mobility and molecular interactions between lipids and proteins, eventually leading to abnormal distribution of proteins between raft and non-

raft domains (Chapkin et al., 2008; Díaz et al., 2012; Díaz et al. 2015; Fabelo et al., 2014; Shaikh et al., 2004; Stillwell and Wassall, 2003). In addition, the reduction in total plasmalogens would exacerbate this destabilizing effect, as plasmalogens form non-lamellar structures which allow the transition from lamellar gel to liquid-crystalline states (Brites et al., 2004), while vinyl ether bonds modulate the hydrophobic interactions of phospholipid aggregates within the membrane plane (Hermetter et al., 1989). These physical properties are critical for the dynamic formation and maintenance of lipid rafts. Based on these assumptions, the considerable reduction in plasmalogen levels in DLB strongly suggests altered physicochemical properties and protein-lipid dynamics in frontal cortex lipid rafts in DLB. Moreover, plasmalogens can act as storage depots for DHA and AA (Brites et al., 2004; Ford and Gross, 1989) which is line with the impoverishment of DHA and AA in DLB lipid rafts. Given that AA and DHA are precursors for eicosanoid and docosanoid formation in the brain (Schmitz and Ecker, 2008; Youdim et al., 2000) and neuroprotectin D1 (Kim et al., 2010; Lukiw et al., 2005), their reduction would have an impact on signal transduction and endogenous neuroprotection in frontal cortex. In line with this, n-3 (DHA) depletion was shown to reduce the p85α subunit of PI3K (Calom and Cole 2007; Oster and Pillot, 2010; Su, 2010). This signalling pathway critical for neuronal preservation since DHA (and its incorporation to phosphatidylserine) stimulates Akt (a downstream effector of the PI3K survival pathway) to translocate and dock at the neuronal membrane via PIP3 binding (Akbar et al., 2005), leading to activated phospho-Akt. If not efficiently phosphorylated (as in the case of DHA depletion or Aβ-exposure), Akt is unable to trigger neurotrophic and anti-apoptotic signalling pathways, and predominates the phosphorylation of PTEN which inhibits PIP3 formation by PI3K, leading to apoptosis (Calom and Cole 2007; Oster and Pillot, 2010; Su, 2009). In addition, DHA depletion also impacts synaptic protein expression and synaptogenesis. Thus, NMDA receptor subunits NR1, NR2A and NR2B are reduced, and LTP impaired, in the rat hippocampus from animals n-3 deficient diets (Cao et al., 2009). Likewise, presynaptic proteins synapsin-1 and syntaxin-3, and postsynaptic proteins drebrin, PSD-95 and GluR1 are reduced in the hippocampus of AD rodent models receiving n-3 polyunsaturated fatty acids deficient diets (Oster and Pillot, 2010; Su, 20010; He et al., 2009; Cao et al., 2009). Overall, these data indicate that, even in the absence of cell death, low levels of DHA, hampers normal synaptic function and neuronal communication. In agreement,

frontal cortex in DLB shows decreased numbers of neurons, slight increase in the number of astrocytes but relatively low activation of microglia; and vacuolization of the upper cortical layers is not uncommon (McKeith et al., 2004; Ince, 2011). This is accompanied by reduced number of synapses and decreased expression of synaptic proteins (Scott et al., 2010; Overk, Masliah, 2014).

Our present outcomes also revealed that the composition of fatty acids in lipid rafts in DLB largely differs from that seen in AD (Martín et al., 2010). First, none of the lipid classes altered in DLB lipid rafts is modified in AD. Second, levels of 18:1n-9 and total monoenes are reduced while 16:1 is increased in AD but not in DLB. Third, levels of 15:0, 17:1, 18:1n-7, 20:2n-9, 20:2n-6 are increased while 22:0, 18:2n-6, 20:4n-6, 20:5n-3 and 22:5n-6 are reduced in DLB but not in AD (Martín et al., 2010).

Present meta-analyses further show that differences among control, DLB, PD and AD lipid rafts are sufficient to differentiate the diseases, as it is feasible to segregate the different groups only on the basis of the lipid structure of lipid rafts, and that in spite of the overlapping characteristics among DLB, PD and AD, sufficient differences make lipid raft profiles in DLB singular.

Alteration in lipid composition in frontal cortex area 8 in DLB is accompanied by anomalous distribution of proteins in membrane microdomains in DLB. Thus, proteins that are prototypical lipid raft residents such as PrPc are partly displaced towards non-raft domains. Other protein that concentrates in lipid rafts and colocalizes with lipid raft markers (but not exclusively), such as VDAC (Herrera et al., 2011; Marín et al., 2008), are redistributed so that most of VDAC is found in non-raft domains in DLB. This a relevant finding since VDAC is a porin associated with cellular apoptosis, and amyloid beta toxicity (Fernández-Echevarría et al., 2014; Marín et al., 2007). Importantly, proteins involved in the amyloidogenic processing of β-amyloid precursor protein (APP), including APP itself, and BACE (β-secretase) (Nalieva and Turner, 2013; Reitz, 2012), accumulate in lipid rafts at the expense of reducing their presence in non-raft domains. The functional implications of these changes are important, as multiple lines of evidence implicate lipid rafts in the amyloidogenic processing of APP (Cordy et al., 2006; Hicks et al. 2012; Vetrivel and Thinakaran, 2010), and corralling BACE in lipid rafts that contain APP triggers the amyloidogenic cleavage of APP (Das et al., 2013). Redistribution of proteins facilitating the β-amyloidogenic pathway in cell membranes also occurs in AD (Abad-Rodríguez et al.,

2004; Díaz et al., 2012; Díaz et al., 2015 Fabelo et al., 2014; Kaether and Haass, 2004). Since lipid rafts were obtained from total grey matter, differences in the percentage of cell types, mainly neurons and synapses versus glial cells, might modify *per se* the profile of lipid rafts. However, the shift of PrP, but not of other proteins, from lipid rafts to non-lipid rafts domains may have pathogenic implications in α-synucleinopathies which deserve further study. Moreover, increased interaction of APP and BACE in DLB is similar to that observed in AD. As stated in the introduction, DLB is commonly combined with AD pathology mainly due to the increased numbers of β-amyloid plaques. Several hypotheses have been proposed to link these two diseases (Barrachina et al., 2005; Beyer et al., 2004; Sánchez-Mut et al., 2016; Winslow et al., 2014). The present findings support the idea that contributory factors may be found in the cell membranes due to the altered lipid composition leading to redistribution of proteins linked to the β-amyloidogenic pathway of lipid rafts in both diseases.

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Table 1: Summary of cases; M: male; F: female; p-m: post-mortem delay; NL: no lesions; AD: Alzheimer disease I, II, III and IV refer to Braak stages of neurofibrillary tangle pathology, AD 0, A: refers to Braak stages of senile plaques; LBD 5: Lewy Body Disease stage 5: AGD: Argyrophilic grain disease. Cases 1-10 had not suffered from neurological deficits. Cases 11-18 had suffered from dementia and parkinsonism consistent with Dementia with Lewy bodies (DLB) and showed LBD stage 5 often accompanied by AD and AGD.

	Gender	age	neuropathology	post-mortem delay
Controls				
5	F	73	AD I/0	5 h 30 min
6	F	82	AD I/A	11 h
7	F	75	ADI/A, status cribosus	3 h
8	F	69	AD I/0	2 h 30 min
9	F	66	NL	8 h
10	F	65	NL	4 h
1	M	78	NL	2 h 15 min
2	M	79	NL	7 h
3	M	70	AD I/A	13h
4	M	71	Status cribosus	12h
DLB				
12	F	84	LBD 5, AD III/B	4 h
15	F	78	LBD 5, AD III/C	7 h 45 min
18	F	85	LBD 5, AD III/C, AGD II	6 h 15 min
11	M	77	LBD 5, AD II/B	11 h
13	M	82	LBD 5, AD I/B	16 h 30 min
14	M	75	LBD 5, AD II/B	N/A
16	M	88	LBD 5, ADI/B, AGD II	2 h
17	M	76	LBD 5, AD IV/C	9 h 40 min

Table 2: Lipid class composition of lipid rafts from control and DLB cases. Results are expressed as mole % and represent means ± SEM; *: $p<0.05$ vs control, #: $p<0.1$ vs control.

lipid class	Controls	DLB	
Lyso-phosphatidylcholine	0.0 ± 0.0	0.5 ± 0.1	*
Sphingomyelin	11.0 ± 1.5	10.3 ± 0.1	
Phosphatidylcholine	5.7 ± 0.4	6.0 ± 0.8	
Phosphatidylserine	7.0 ± 0.5	7.1 ± 0.3	
Phosphatidylinositol	2.3 ± 0.2	2.7 ± 0.3	#
Phosphatidylglycerol	0.7 ± 0.1	1.0 ± 0.1	
Phosphatidylethanolamine	21.1 ± 0.6	21.2 ± 0.1	
Sulfatides	10.3 ± 0.7	11.0 ± 0.5	
Cerebrosides	5.1 ± 0.9	5.8 ± 0.8	
Diacylglycerols	0.0 ± 0.0	0.0 ± 0.7	
Cholesterol	32.7 ± 1.2	27.6 ± 0.0	*
Free fatty acids	2.3 ± 0.3	3.1 ± 1.0	
Triglycerides	0.0 ± 0.0	0.0 ± 0.3	
Sterol esters	1.9 ± 0.6	3.7 ± 0.0	*
Total			
Neutral Lipids	36,9 ± 1.4	34.4 ± 1.4	
Polar Lipids	63,1 ± 1.6	65.6 ± 1.4	
Ratio			
Phospholipid/cholesterol	1.5 ± 0.1	1.79 ± 0.08	*

Table 3: Fatty acid composition of lipid rafts from control and DLB. Results are expressed as mole % and represent means ± SEM. *: $p<0.05$ vs control; #: $p<0.1$ vs control.

Fatty acid	Controls	DLB	
14 : 0	0.54 ± 0.04	0.53 ± 0.02	
14 : 1	0.15 ± 0.11	0.17 ± 0.08	
15 : 0	0.92 ± 0.15	2.18 ± 0.14	*
15 : 1	0.11 ± 0.03	0.26 ± 0.07	*
16:0 DMA	1.75 ± 0.27	1.53 ± 0.21	
16 : 0	24.57 ± 1.35	26.95 ± 1.54	
16 : 1	1.11 ± 0.08	2.41 ± 0.23	*
16 : 2	0.19 ± 0.03	0.29 ± 0.04	
17 : 0	0.31 ± 0.01	0.34 ± 0.02	
17 : 1	0.06 ± 0.02	0.14 ± 0.02	*
18:0 DMA	3.44 ± 0.11	1.51 ± 0.21	*
18:1n-9 DMA	0.69 ± 0.12	0.96 ± 0.07	
18:1n-7 DMA	1.34 ± 0.39	0.85 ± 0.19	
18 : 0	22.19 ± 0.46	22.43 ± 0.39	
18:1 n-9	17.39 ± 1.18	16.79 ± 0.62	
18:1 n-7	4.96 ± 0.35	5.95 ± 0.25	*
18 : 2 n-6	0.88 ± 0.09	0.52 ± 0.06	*
20 : 1	0.96 ± 0.17	1.04 ± 0.17	
20 : 2 n-9	0.00 ± 0.00	0.42 ± 0.06	*
20 : 2 n-6	0.11 ± 0.04	0.18 ± 0.02	
20 : 3 n-6	0.53 ± 0.02	0.48 ± 0.03	
20 : 4 n-6	3.68 ± 0.20	3.18 ± 0.15	#
20 : 5 n-3	0.11 ± 0.04	0.00 ± 0.00	*
22: 0	0.26 ± 0.05	0.06 ± 0.03	*
22 : 1	0.36 ± 0.11	0.00 ± 0.00	*
22 : 2 n-6	0.67 ± 0.09	1.23 ± 0.24	*
23 : 0	2.36 ± 0.13	2.06 ± 0.12	
22 : 5 n-6	0.45 ± 0.06	0.31 ± 0.02	*
22 : 5 n-3	0.15 ± 0.03	0.11 ± 0.03	
24 : 0	0.29 ± 0.10	0.02 ± 0.02	*
22 : 6 n-3	7.02 ± 0.34	4.75 ± 0.45	*
24 : 1 n-9	1.11 ± 0.16	1.30 ± 0.27	
Total			
Saturates	54.46 ± 1.56	52.64 ± 1.48	
n-9	19.19 ± 1.31	18.50 ± 0.89	
n-6	6.32 ± 0.26	5.94 ± 0.21	
n-3 LCPUFA	7.28 ± 0.36	4.94 ± 0.43	*
Monoenes	28.26 ± 1.67	28.07 ± 1.18	

DMA	7.22 ± 0.69	4.85 ± 0.63	*
Ratios			
n-3/n-6	1.16 ± 0.04	0.83 ± 0.06	*
18:1/n-3 H	2.45 ± 0.21	3.62 ± 0.40	*
Saturates/n-3	7.64 ± 0.42	11.04 ± 0.64	*
Saturates/n-9	2.91 ± 0.26	2.91 ± 0.21	
Indexes			
Unsaturation Index	93.94 ± 2.54	79.59 ± 2.16	*
Peroxidability Index	73.88 ± 3.35	55.65 ± 3.84	*

1
2

Figure 1: Principal component analysis of fatty acids (A) and lipid classes (B) in lipid rafts from control and DLB lipid rafts; Factor analyses for fatty acids (C) and lipid classes (D). CHO: cholesterol, PE: phosphatidylethanolamine, PG: phosphatidylglycerol, PS: phosphatidylserine, PI: phosphatidylinositol, CER: cerebrosides, SUL: sulphatides, SM: sphingomyelin.

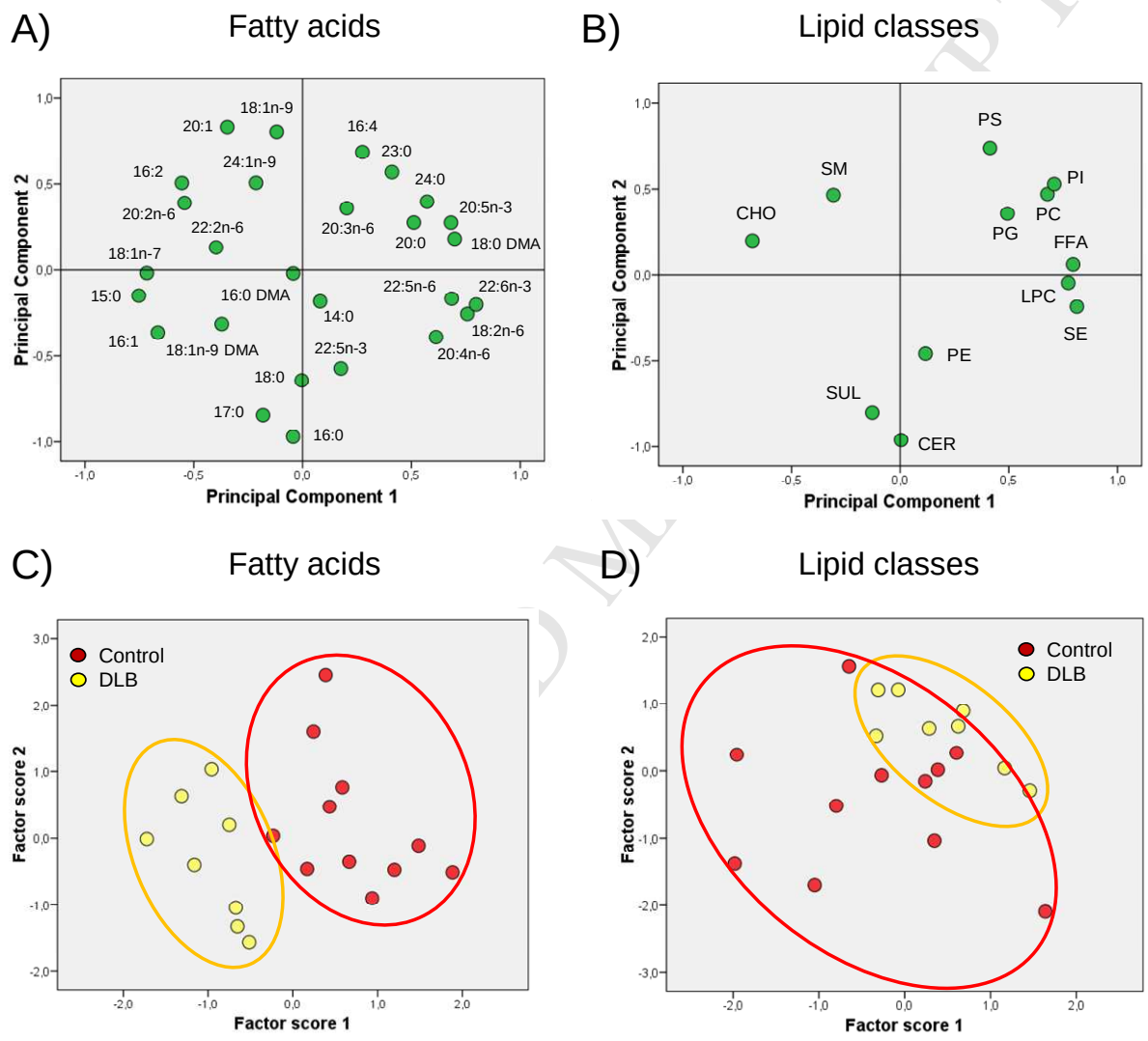


Figure 2: Multivariate factor analyses and discriminant function analyses of lipid rafts from control (CTRL), Parkinson's disease (PD) and Dementia with Lewy Bodies (DLB) (A, B) cases, and control (CTRL), Alzheimer's disease (AD) and DLB (C,D). Control cases were the same across different comparisons.

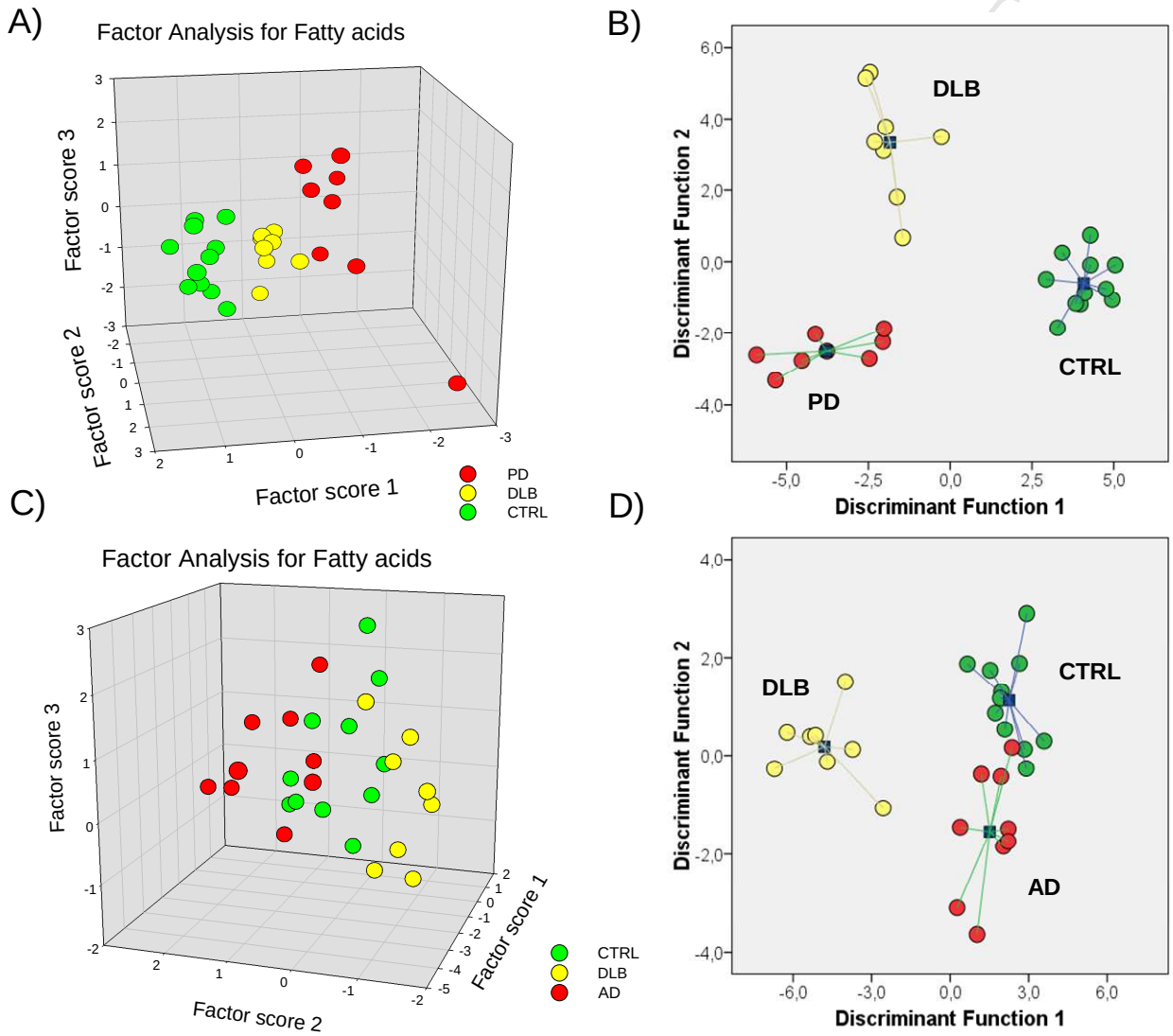
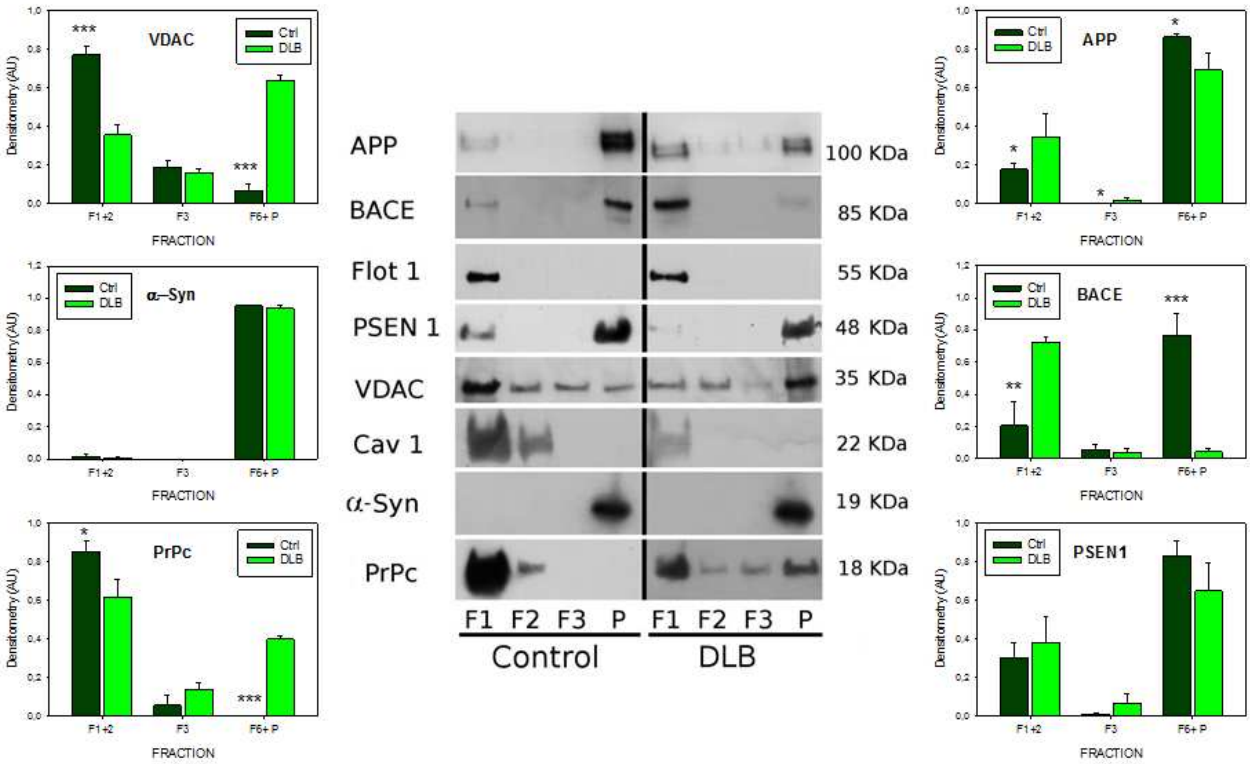


Figure 3: Protein distribution within different fractions of lipid raft isolation from control and Dementia with Lewy Bodies (DLB) as seen with western blotting. APP: β-amyloid precursor protein; BACE: β-secretase; Flot 1: flotilin 1; PSEN 1: presenilin 1; VDAC: voltage-dependent anion channel; Cav: caveolin; α-syn: α-synuclein; PrPc: prion protein. Results in bar graphs are expressed as means ± SEM; *: $p<0.05$; **: $p<0.01$; ***: $p<0.005$.



Highlights:

- Lipid rafts from DLB exhibit anomalous lipid contents and protein distribution
- Lipid rafts from DLB contain reduced levels of DHA, plasmalogens and cholesterol
- Lipid alterations in DLB partly overlap those observed in Alzheimer's disease
- Lipid alterations correlate with increased accumulation of amyloidogenic proteins
- Some lipid alterations in lipid rafts in DLB are common to Parkinson's disease