

# Lipid Classes and Fatty Acid Patterns are Altered in the Brain of $\gamma$ -Synuclein Null Mutant Mice

Irina Guschina · Steve Millership ·  
Valerie O'Donnell · Natalia Ninkina ·  
John Harwood · Vladimir Buchman

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**Abstract** The well-documented link between  $\alpha$ -synuclein and the pathology of common human neurodegenerative diseases has increased attention to the synuclein protein family. The involvement of  $\alpha$ -synuclein in lipid metabolism in both normal and diseased nervous system has been shown by many research groups. However, the possible involvement of  $\gamma$ -synuclein, a closely-related member of the synuclein family, in these processes has hardly been addressed. In this study, the effect of  $\gamma$ -synuclein deficiency on the lipid composition and fatty acid patterns of individual lipids from two brain regions has been studied using a mouse model. The level of phosphatidylserine (PtdSer) was increased in the midbrain whereas no changes in the relative proportions of membrane polar lipids were observed in the cortex of  $\gamma$ -synuclein-deficient compared to wild-type (WT) mice. In addition, higher levels of docosahexaenoic acid were found in PtdSer and phosphatidylethanolamine (PtdEtn) from the cerebral cortex of  $\gamma$ -synuclein null mutant mice. These findings show that  $\gamma$ -synuclein deficiency leads to alterations in the lipid profile in brain tissues and suggest that this protein, like  $\alpha$ -synuclein, might affect neuronal function via modulation of lipid metabolism.

**Keywords** Lipid composition · Synuclein · Midbrain · Cortex · Phosphatidylserine · Docosahexaenoic acid ·  $\gamma$ -Synuclein null mutant mice

## Abbreviations

ARA	Arachidonic acid
AD	Alzheimer's disease
alphaKO	$\alpha$ -Synuclein null mutant
Ptd <sub>2</sub> Gro	Cardiolipin (diphosphatidylglycerol)
CNS	Central nervous system
DHA	Docosahexaenoic acid
DLB	Dementia with Lewy bodies
DMA	Dimethylacetal
ESI-MS-MS	Electrospray ionisation tandem mass spectrometry
FAME	Fatty acid methyl ester(s)
gammaKO	$\gamma$ -Synuclein null mutant
LIT	Linear ion trap
LnA	$\alpha$ -Linolenic acid
PD	Parkinson's disease
PtdCho	Phosphatidylcholine
PtdEtn	Phosphatidylethanolamine
PtdGro	Phosphatidylglycerol
PtdIns	Phosphatidylinositol
PNS	Peripheral nervous system
PtdSer	Phosphatidylserine
PUFA	Polyunsaturated fatty acid(s)
CerPCho	Sphingomyelin
TAG	Triacylglycerol(s)
WT	Wild-type

I. Guschina (✉) · S. Millership · N. Ninkina · J. Harwood · V. Buchman  
School of Biosciences, Cardiff University, Museum Avenue,  
Cardiff CF10 3AX, UK  
e-mail: GuschinaIA@cf.ac.uk

V. O'Donnell  
School of Medicine, Cardiff University, Cardiff CF14 4XN, UK

## Introduction

The synuclein family comprises three small, closely-related, natively unfolded proteins,  $\alpha$ -,  $\beta$ - and  $\gamma$ -synuclein, that are expressed predominantly in neural tissues. Alpha- and  $\beta$ -synucleins are abundant in the neurons of the central

nervous system (CNS) and concentrated within presynaptic terminals where they are loosely associated with synaptic vesicles.  $\gamma$ -Synuclein is mainly cytosolic and expressed in the neurons of the peripheral nervous system (PNS) and certain populations of CNS neurons.  $\gamma$ -Synuclein is also expressed in some malignant tumours and may be involved in tumorigenesis and metastasis [1].

Aggregated and fibrillated forms of  $\alpha$ -synuclein are major components of Lewy bodies, histological hallmarks of hereditary and idiopathic forms of Parkinson's disease (PD). Moreover, several mutations in SNCA, a gene encoding  $\alpha$ -synuclein, are associated with early onset autosomal dominant PD [2–7] and polymorphisms of this gene are risk factors for PD and other diseases associated with  $\alpha$ -synuclein aggregation [8–12]. The accumulation of aggregation intermediates, i.e. partially or fully soluble oligomeric forms of  $\alpha$ -synuclein, is believed to be the principle pathogenic event responsible for neuronal dysfunction [13–16] and interactions with lipids play an important role in oligomerisation of  $\alpha$ -synuclein [17–19]. Interestingly,  $\beta$ - and  $\gamma$ -synucleins may act to suppress  $\alpha$ -synuclein aggregation and toxicity [20].

In immortalised cell lines and primary neuronal cultures, both WT and PD mutant  $\alpha$ -synuclein could be found associated with the phospholipid surface layer of lipid droplets [21] and are enriched in lipid rafts [22]. In the rafts,  $\alpha$ -synuclein was associated with specific PtdSer species containing polyunsaturated fatty acids (PUFA) [23] or with gangliosides [24, 25]. Moreover, polyunsaturated fatty acyl groups were shown to promote multimerisation of  $\alpha$ -,  $\beta$ -, and  $\gamma$ -synucleins [26] and lipid-associated oligomers of  $\alpha$ -synuclein have been detected in the brain of patients with  $\alpha$ -synucleinopathies [27, 28]. It has been suggested that  $\alpha$ -synuclein—PUFA interactions can reciprocally regulate neuronal PUFA levels and the oligomerisation stage of  $\alpha$ -synuclein in normal and disease nervous system [27, 28].

The importance of PUFA in brain structure and functions is well established [29]. These fatty acids, especially arachidonic acid (C20:4n-6, ARA) and docosahexaenoic acid (C22:6n-3, DHA), are enriched in certain brain phospholipids, for example, in PtdIns and PtdEtn, respectively [30]. In animal models, an inadequate supply of n-3 fatty acids during prenatal and early postnatal development decreases DHA levels in the brain. As a result, adult animals develop learning and memory deficits, which can be improved by dietary supplementation of DHA or other n-3 PUFA, e.g.  $\alpha$ -linolenic acid (C18:3n-3, LnA) [31–33]. In humans, mental development may be improved by dietary DHA supplementation during infancy [34]. Conversely, low levels of DHA in human brains are associated with the risk of developing neurological diseases such as general-

ised peroxisomal disorders [35] and Alzheimer's disease (AD) [36, 37]. In contrast, elevated levels of DHA, docosahexaenoic acid and linoleic acid have been shown in those brain areas of PD patients that contain  $\alpha$ -synuclein inclusions [28].

Although the precise mechanism of DHA-enriched phospholipid action on cognitive function is still poorly understood, possible effects on the blood–brain barrier, membrane fluidity, activity of certain enzymes, neural signalling, ionic channels, and control of nerve growth factor have all been suggested [38]. Recently, a neuroprotective action of the n-3 PUFA, DHA and LnA, in PD has been also demonstrated using rat and mice models of this disease [39, 40]. Interestingly, expression of genes encoding  $\alpha$ -synuclein and  $\gamma$ -synuclein increased in brains of rats fed high n-3 PUFA diets [38]. In turn, an important role of  $\alpha$ -synuclein in brain lipid metabolism as well as for fatty acid uptake and metabolism has been documented [41–43].

In contrast to the well-studied role of  $\alpha$ -synuclein in lipid metabolism in the normal and diseased nervous systems, very little is known about the involvement of  $\gamma$ -synuclein in these processes. Nevertheless, some recent studies demonstrated that  $\gamma$ -synuclein is directly involved in lipid metabolism in mature adipocytes ([44, 45] and our unpublished observations). Therefore it was logical to investigate a possible link between  $\gamma$ -synuclein and lipids in the nervous system.

Here we analysed lipid composition and fatty acid patterns of individual lipids from brain regions of  $\gamma$ -synuclein null mutant (gammaKO) mice. We found that the level of PtdSer is increased in the midbrain whereas no changes in the relative proportions of membrane lipid classes were observed in the cerebral cortex of these animals. In addition, higher percentages of DHA were found in both PtdSer and PtdEtn from the cerebral cortex of gammaKO mice. These data suggest a role for  $\gamma$ -synuclein in lipid metabolism in the nervous system and are discussed in relation to what is known about alterations of lipid metabolism in  $\alpha$ -synuclein-deficient animals.

## Experimental Procedures

### Materials

FA standards were obtained from Nu-Chek-Pre. Inc. (Elysian, MN) and silica gel G plates were from Merck KGaA (Darmstadt, Germany). Lipid standards were from Sigma (Poole, UK). Other reagents were of the best available grades and were from Fisher Scientific (Loughborough, UK).

## Experimental Animals

All animal work was carried in accordance with the United Kingdom Animals (Scientific Procedures) Act (1986). Production of  $\gamma$ -synuclein null mutant mice on a pure (C57Bl6J, Charles River) genetic background and the method of animal genotyping by PCR have been described previously [46, 47]. For this study, male  $\gamma$ -synuclein null mutant mice and their WT littermates were kept in individual cages from the age of 9 weeks with access to water and food ad libitum. Animals were fed special diet DOI 58Y2 with 10% energy from fats (LabDiets). No significant differences in animal weight and food uptake were observed between groups of mutant and WT animals throughout the experimental period. In order to evaluate the effect of  $\gamma$ -synuclein deficiency on the lipid composition in the fully-developed nervous system, tissues of young but fully mature (21 week-old) adult mice were analysed.

## Lipid Analysis

At the age of 21 weeks animals were fasted for 4 h before killing by lethal injection of phenobarbital. Lipids were extracted immediately from dissected brain regions by a modified Folch method [48]. In this procedure [originally developed for mitochondrial lipids such as cardiolipin (Ptd<sub>2</sub>Gro)] efficient extraction even of highly polar compounds is ensured.

Polar lipids were separated by two-dimensional TLC on 10 × 10 cm 1.2% boric acid-impregnated silica gel G plates using chloroform: methanol: ammonium hydroxide (65:25:4; v/v/v) in the first dimension and then *n*-butanol: acetic acid: water (90:20: 20; v/v/v) in the second. Plates were sprayed with 0.05% (wt/vol) 8-anilino-4-naphthosulphonic acid in methanol and viewed under UV light to reveal lipids. Preliminary identification was made by reference to authentic standards and confirmed using specific colour reagents [49]. Additionally, the structural identification of lipids was confirmed by mass spectrometry (see next paragraph for details). Non-polar lipids were separated using 1-dimensional TLC on 10 × 10 cm silica gel G plates with double development using toluene: hexane: formic acid (140:60:1, v/v/v) for the entire plate followed by hexane: diethyl ether: formic acid (60:40:1, v/v/v) to half height. Individual lipids were scraped from the TLC plates and their fatty acid compositions and contents were determined by gas chromatography using an internal standard of pentadecanoate (for details, see “Fatty Acid Analysis” section below) [49].

Mass spectrometry was performed on an Applied Biosystems 4000 Q-Trap. Lipid extracts were diluted in methanol and introduced at 10  $\mu$ l/min into the electrospray

source operating in the negative ion mode. All scans were obtained using an ionspray voltage of  $-4,500$  V and a declustering potential of  $-140$  V. Q1 scans were performed scanning a mass range of 600–1,000 amu over 4 s, with 10 scans acquired and averaged. MS/MS scans using the ion trap mode of the Q-Trap were run at a scan rate of 1,000 amu/s with collision energies of  $-50$  and  $-60$  V, and a linear ion trap (LIT) fill time of 150 ms with Q0 trapping enabled. Again 10 scans were acquired and averaged and the data analysed using the software Analyst 1.4.1.

## Fatty Acid Analysis

Fatty acid methyl esters (FAME) were prepared by trans-methylation with 2.5% H<sub>2</sub>SO<sub>4</sub> in dry methanol/toluene (2:1, by vol.). FAME were separated using a Clarus 500 gas chromatograph (Perkin-Elmer, Norwalk, Connecticut) fitted with a 30 m × 0.25 mm i.d. capillary column (Elite 225, Perkin Elmer). The oven temperature was programmed: 170 °C for 3 min, heated to 220 °C at 4 °C/min, held at 220 °C for 15 min. FAME were identified routinely by comparing retention times with fatty acid standards (Nu-Chek Prep. Inc., Elysian, USA) but confirmation of the structure of the major fatty acids had also been made by MS. Quantification was made with an internal standard of pentadecanoate.

## Statistics

Statistical significance between groups was assessed by Student's *t* test.

## Results

### Fatty Acid Composition of Diet

The fatty acid composition of the diet used is presented in Table 1. It contained oleic acid as a major compound (35% of total diet FA) followed by linoleic (25%), palmitic (20%) and stearic (13%) acids. The relative amount of  $\alpha$ -linolenic (an essential n-3 fatty acid) was low and did not exceed 2.5% of total FA. Thus, the ratio of n-6/n-3 fatty acids was high giving a value of 13 whereas a balanced human diet is believed to range from 1:1 to 1:4 [50]. Lipids accounted for 10% of total energy in this diet.

### Fatty Acid Composition of Plasma

Fatty acid composition of plasma from WT and  $\gamma$ -synuclein null mutant mice (gammaKO) is present in Table 1. Palmitic, stearic, oleic, linoleic and arachidonic acids were the

**Table 1** Fatty acid composition (% total acids) of the diet and the plasma from wild-type (WT) and  $\gamma$ -synuclein null mutant (gammaKO) mice

Fatty acid	Diet	Plasma	
		WT	gammaKO
C14:0	0.5 ± 0.1	tr.	tr.
C16:0	20.3 ± 2.6	17.8 ± 4.7	19.8 ± 2.6
C16:1 (n-7)	1.0 ± 0.2	2.0 ± 0.7	3.1 ± 0.8
C18:0	12.6 ± 2.1	13.0 ± 2.2	13.0 ± 4.8
C18:1 (n-9)	35.0 ± 1.3	16.0 ± 1.9	20.5 ± 2.8*
C18:1 (n-7)	2.0 ± 0.1	2.2 ± 0.2	3.0 ± 0.8
C18:2 (n-6)	24.9 ± 6.0	17.3 ± 1.8	15.1 ± 1.8
C18:3 (n-3)	2.2 ± 0.6	0.5 ± 0.2	0.5 ± 0.3
C20:1 (n-9)	0.6 ± 0.2	tr.	tr.
C20:3 (n-6)	n.d.	1.6 ± 0.8	2.0 ± 0.7
C20:4 (n-6)	n.d.	20.3 ± 4.5	15.9 ± 4.4
C22:6 (n-3)	n.d.	7.3 ± 2.4	5.2 ± 1.9

Data as means ± SD ( $n = 3$  for diet and  $n = 5$  for plasma)

Fatty acids are indicated with the number before colon showing the number of carbon atoms, the figure afterwards denoting the number of double bonds. The position of the first double bond is shown in brackets. Only the major fatty acids ( $\geq 0.5\%$ ) are listed

*n.d.* none detected; *tr* trace  $<0.5\%$

The asterisk (\*) indicates a significant effect of  $\gamma$ -synuclein deficiency when compared with WT ( $p < 0.05$ )

major fatty acids in plasma together with a moderate amount of DHA in both, WT and gammaKO mice. The relative amount of oleic acid was increased significantly in the plasma of gammaKO mice compared to WT (Table 1).

#### Cortex and Midbrain Fatty Acid Composition of the Total Polar Lipids in WT and $\gamma$ -Synuclein Null Mutant Mice

Table 2 shows the FA profile of the total polar lipid fractions from cortex or midbrain in WT and gammaKO mice. Palmitic and stearic acids were dominant FA in cortex followed by DHA, oleic acid and ARA. In midbrain, stearic and oleic acids were the major compounds followed by palmitic, DHA and ARA. The percentages of palmitic, arachidonic and DHA in total polar lipids were significantly higher in the cortex region compared to midbrain in both WT and gammaKO mice, whereas proportions of oleic and nervonic (C24:1) acids were higher in this lipid fraction from midbrain. There were no statistically significant differences in the relative proportions of fatty acids from the total polar lipid fraction between WT and gammaKO mice in either brain region.

**Table 2** Fatty acid composition (% of total fatty acids) of the total polar lipid fraction from cortex or midbrain of wild-type (WT) and  $\gamma$ -synuclein null mutant (gammaKO) mice

FA	CORTEX		MIDBRAIN	
	WT	gammaKO	WT	gammaKO
C16:0	22.2 ± 0.5	22.6 ± 0.8	16.8 ± 0.2 <sup>#</sup>	17.5 ± 0.8
C16:1 (n-7)	1.3 ± 0.2	1.4 ± 0.2	0.1 ± 0.0 <sup>#</sup>	0.2 ± 0.0
C18:0	22.6 ± 0.4	22.8 ± 0.4	21.8 ± 2.3	21.6 ± 2.4
C18:1 (n-9)	15.9 ± 0.2	15.6 ± 0.3	21.7 ± 0.6 <sup>#</sup>	21.1 ± 1.1
C18:1 (n-7)	3.8 ± 0.4	4.0 ± 0.3	4.7 ± 1.3	5.0 ± 0.5
C18:2 (n-6)	0.9 ± 0.1	0.8 ± 0.1	2.8 ± 0.9 <sup>#</sup>	2.8 ± 0.8
C18:3 (n-3)	10.4 ± 0.5	10.5 ± 0.3	7.7 ± 0.9 <sup>#</sup>	7.4 ± 1.2
C20:3 (n-6)	2.4 ± 0.1	2.4 ± 0.1	3.0 ± 0.9	3.7 ± 2.0
C20:4 (n-6)	16.5 ± 2.0	16.0 ± 0.6	12.1 ± 1.7 <sup>#</sup>	11.5 ± 1.0
C22:6 (n-3)	0.8 ± 0.2	0.8 ± 0.2	2.5 ± 0.6 <sup>#</sup>	2.6 ± 0.6

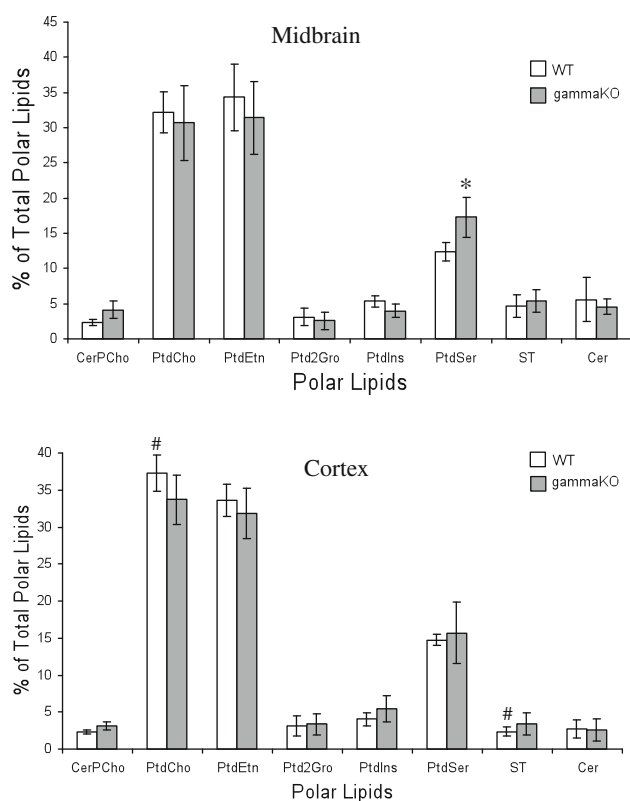
Data as means ± SD ( $n = 6-8$ )

See legend to Table 1 for other details

The hash (#) indicates significant differences between midbrain and cortex in WT animals ( $p < 0.05$ )

#### Polar Lipid Composition of Cortex and Midbrain Regions from WT and gammaKO Mice

The relative proportions of different polar lipids in the two brain regions are shown in Fig. 1. Three phospholipids, namely phosphatidylcholine (PtdCho), ethanolamine phospholipids and PtdSer, were the major polar lipids in both brain regions (accounting for about 70–80% of the total polar lipids). Phosphatidylinositol (PtdIns), sphingomyelin (CerPCho), Ptd<sub>2</sub>Gro as well as sulfatide and cerebroside were present in smaller proportions and were each less than 5% of the total polar lipids. Sulfatides and cerebroside were identified by electrospray ionisation tandem mass spectrometry (ESI-MS-MS) [51, 52]. The relative amounts of polar lipids did not vary much between midbrain and cortex samples from WT animals, although the levels of sulfatides and PtdCho were higher and lower, respectively, in the midbrain region (Fig. 1). In this brain region,  $\gamma$ -synuclein deficiency resulted in a statistically significant (~40%) increase in the relative proportion of PtdSer compared to WT animals, whereas the proportions of other lipids were not altered significantly (Fig. 1). No differences in the polar lipid composition were found in the cortex of WT compared to gammaKO mice (Fig. 1). Also, no differences in the concentrations of total polar lipids and triacylglycerols (TAG) were observed for these brain regions as a result of  $\gamma$ -synuclein deficiency (data not shown).

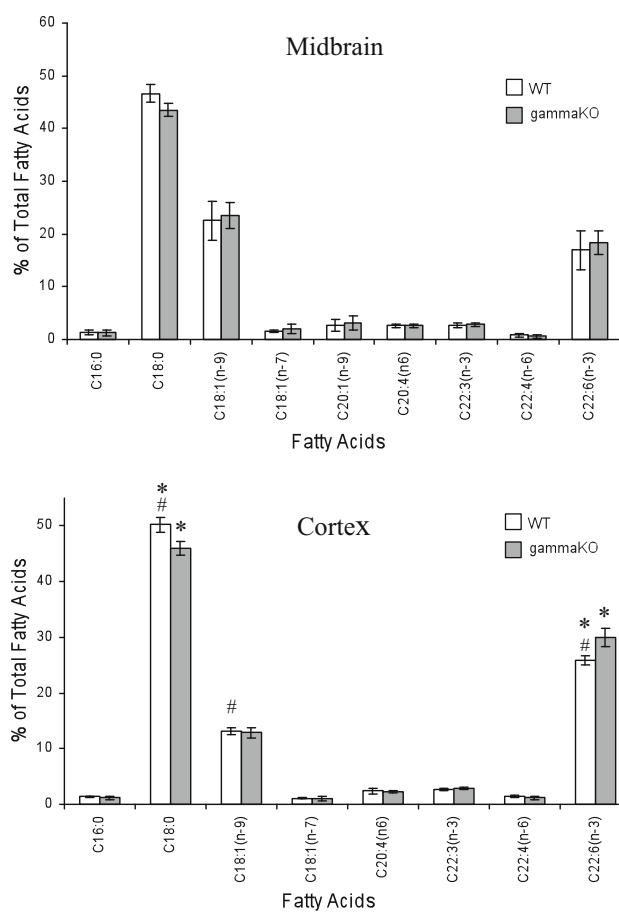


**Fig. 1** Midbrain and cortex polar lipid composition (% of total polar lipids) from wild-type (WT) and  $\gamma$ -synuclein null mutant (gammaKO) mice. Values represent mean  $\pm$  SD,  $n = 5$ . The asterisk (\*) indicates a significant effect of  $\gamma$ -synuclein deficiency when compared with WT, and the hash (#) indicates significant differences between midbrain (top panel) and cortex (bottom panel) in WT animals ( $p < 0.05$  for both). CerPCho sphingomyelin, PtdCho phosphatidylcholine, PtdEtn phosphatidylethanolamine, Ptd2Gro cardiolipin, PtdIns phosphatidylinositol, PtdSer phosphatidylserine, ST sulfatide, Cer cerebroside

#### Effect of $\gamma$ -Synuclein Deficiency on the Fatty Acid Composition of Individual Polar Lipid Classes in Cortex and Midbrain

Figures 2, 3 and Table 3 show data on the fatty acid composition for the major polar lipids in cortex and midbrain from WT and gammaKO mice. These data show a fatty acid distribution typical of that for murine brain tissues.

PtdCho in both cortex and midbrain is characterised by a domination of palmitate (about 48 and 40% in cortex and midbrain, respectively), stearate (14 and 16%) and oleate (21 and 24%) with much lower levels of the two major brain long-chain polyunsaturated fatty acids, ARA and DHA (Table 3). In the cortex, the relative concentrations of ARA and DHA in PtdCho were about 6 and 3%, respectively. In the midbrain, about 4% of each of ARA and DHA was found in PtdCho. The levels of all above mentioned fatty acids were significantly different between the two

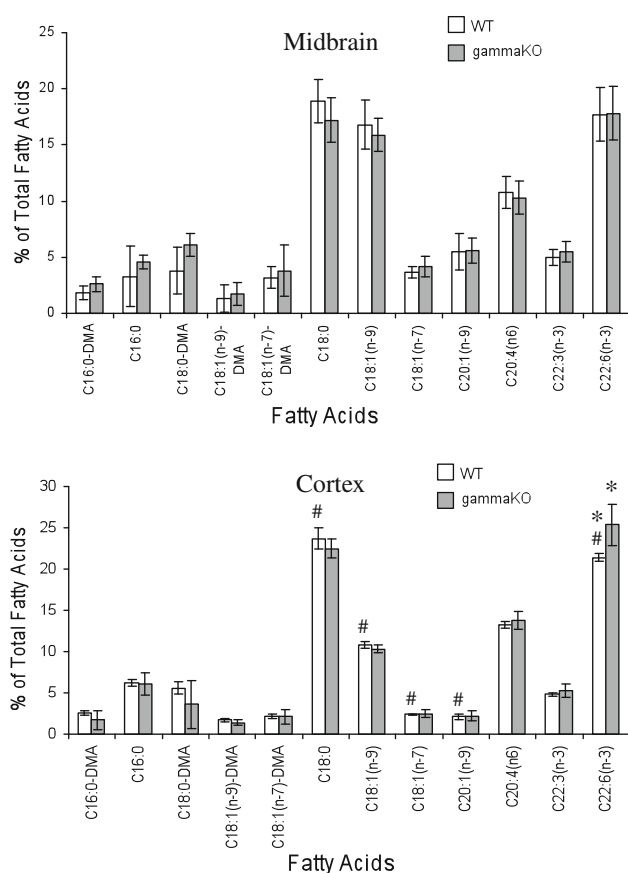


**Fig. 2** Fatty acid composition of phosphatidylserine from midbrain or cortex in wild-type (WT) and  $\gamma$ -synuclein null mutant (gammaKO) mice. Values represent mean  $\pm$  SD,  $n = 5$ . The asterisk (\*) indicates a significant effect of  $\gamma$ -synuclein deficiency when compared with WT, and the hash (#) indicates significant differences between midbrain (top panel) and cortex (bottom panel) in WT animals ( $p < 0.05$  for both). See legend to Table 1 for identification of fatty acids

brain regions. In contrast, there were no significant differences in these parameters between WT and gammaKO mice.

PtdIns is enriched with two fatty acids, stearic (around 44% in both cortex and midbrain) and ARA (37 and 34% in cortex and midbrain, respectively). DHA is a minor component in PtdIns and its relative concentration was about 2% in cortex and 4% in midbrain, these being significantly different.  $\gamma$ -Synuclein deficiency resulted in an increased level of ARA in PtdIns in cortex but did not affect the fatty acid profiles of this lipid in midbrain (Table 3).

Oleic and ARA were the major acids found in brain Ptd2Gro. In this lipid, another C18:1 isomer, *cis*-vaccenic acid, was also present in appreciable amounts especially in the midbrain (around 11 vs. 7% in cortex). The relative concentration of ARA was higher in Ptd2Gro from the cortex than in Ptd2Gro from the midbrain (18 and 13%,



**Fig. 3** Fatty acid and dimethylacetate composition of ethanolamine phospholipids from midbrain or cortex in wild-type (WT) and  $\gamma$ -synuclein null mutant (gammaKO) mice. Values represent mean  $\pm$  SD,  $n = 5$ . The asterisk (\*) indicates a significant effect of  $\gamma$ -synuclein deficiency when compared with WT, and the hash (#) indicates significant differences between midbrain (top panel) and cortex (bottom panel) in WT animals ( $p < 0.05$  for both)

respectively). In comparison to other polar lipids isolated from the brain, Ptd<sub>2</sub>Gro contains higher levels of C16 and C18 monoenoic acids, namely C16:1n-7 (up to 5%), C18:1n-9 (up to 38%) and C18:1n-7 (up to 15%). The proportion of the latter was significantly higher in the midbrain than in cortex at the expense of arachidonic acid (Table 3). No statistically significant changes were found when comparing Ptd<sub>2</sub>Gro fatty acid profiles in the cortex between WT and gammaKO animals, whereas in midbrain the proportion of C18:1n-7 was increased in gammaKO mice compared to WT (Table 3).

CerPCho from both cortex and midbrain contains stearic acid as its main fatty acid (up to 81% of total FAs in cortex, and up to 65% of that in midbrain). The presence of two very long chain acids, lignoceric (C24:0) and nervonic (C24:1n-6), is also characteristic for this lipid. Their levels were higher in midbrain than cortex. CerPCho fatty acids were unchanged as a response to  $\gamma$ -synuclein deficiency in

**Table 3** Fatty acid composition (% of total fatty acids) in individual polar lipid classes from cortex or midbrain of wild-type (WT) and  $\gamma$ -synuclein null mutant (gammaKO) mice

FA	CORTEX		MIDBRAIN	
	WT	gammaKO	WT	gammaKO
<b>Phosphatidylcholine</b>				
C16:0	48.0 $\pm$ 2.7	45.2 $\pm$ 1.9	39.2 $\pm$ 3.5 <sup>#</sup>	39.2 $\pm$ 2.6
C16:1 (n-7)	0.7 $\pm$ 0.1	0.8 $\pm$ 0.3	0.7 $\pm$ 0.2	0.9 $\pm$ 0.2
C18:0	3.7 $\pm$ 0.7	13.0 $\pm$ 0.7	16.5 $\pm$ 1.2 <sup>#</sup>	15.1 $\pm$ 1.6
C18:1 (n-9)	20.6 $\pm$ 0.8	20.9 $\pm$ 1.1	24.1 $\pm$ 1.6 <sup>#</sup>	23.9 $\pm$ 1.1
C18:1 (n-7)	5.1 $\pm$ 2.0	6.8 $\pm$ 0.9	7.2 $\pm$ 0.6	8.1 $\pm$ 1.6
C20:1 (n-9)	0.7 $\pm$ 0.1	0.7 $\pm$ 0.1	1.7 $\pm$ 0.5 <sup>#</sup>	1.6 $\pm$ 0.3
C20:4 (n-6)	5.7 $\pm$ 0.6	6.4 $\pm$ 0.5	3.9 $\pm$ 0.7 <sup>#</sup>	4.1 $\pm$ 0.6
C22:6 (n-3)	3.2 $\pm$ 0.5	3.8 $\pm$ 0.4	3.7 $\pm$ 0.6	4.3 $\pm$ 0.6
<b>Phosphatidylinositol</b>				
C16:0	8.2 $\pm$ 1.8	6.7 $\pm$ 1.9	7.3 $\pm$ 1.8	9.4 $\pm$ 2.1
C18:0	44.1 $\pm$ 3.2	40.7 $\pm$ 1.1	44.9 $\pm$ 3.2	40.7 $\pm$ 2.1
C18:1 (n-9)	5.1 $\pm$ 0.8	5.1 $\pm$ 0.6	5.5 $\pm$ 0.5	8.4 $\pm$ 2.4*
C18:1 (n-7)	2.2 $\pm$ 0.2	2.5 $\pm$ 0.4	2.5 $\pm$ 0.4	3.4 $\pm$ 0.7
C20:4 (n-6)	36.8 $\pm$ 3.5	41.8 $\pm$ 2.4*	33.5 $\pm$ 3.6	32.4 $\pm$ 1.1
C22:6 (n-3)	2.0 $\pm$ 0.8	2.2 $\pm$ 0.5	4.6 $\pm$ 1.7 <sup>#</sup>	4.2 $\pm$ 1.3
<b>Cardiolipin</b>				
C16:0	6.7 $\pm$ 2.2	6.1 $\pm$ 2.2	7.8 $\pm$ 1.3	8.5 $\pm$ 1.9
C16:1 (n-7)	5.3 $\pm$ 1.1	5.1 $\pm$ 1.1	4.1 $\pm$ 2.6	4.8 $\pm$ 1.6
C18:0	8.1 $\pm$ 3.4	8.2 $\pm$ 3.5	10.6 $\pm$ 3.3	6.8 $\pm$ 2.9
C18:1 (n-9)	38.1 $\pm$ 2.2	36.4 $\pm$ 2.2	36.8 $\pm$ 2.3	33.9 $\pm$ 3.2
C18:1 (n-7)	6.9 $\pm$ 0.7	5.8 $\pm$ 0.9	10.5 $\pm$ 2.3 <sup>#</sup>	15.4 $\pm$ 3.0*
C18:2 (n-6)	5.7 $\pm$ 2.1	4.8 $\pm$ 0.4	3.9 $\pm$ 0.6	4.2 $\pm$ 0.5
C20:3 (n-6)	1.9 $\pm$ 0.2	2.3 $\pm$ 0.4	1.7 $\pm$ 0.2	1.8 $\pm$ 0.2
C20:4 (n-6)	17.7 $\pm$ 2.3	19.4 $\pm$ 2.1	13.1 $\pm$ 2.2 <sup>#</sup>	13.4 $\pm$ 0.8
C22:6 (n-3)	9.4 $\pm$ 2.3	11.5 $\pm$ 3.3	10.4 $\pm$ 2.9	10.7 $\pm$ 1.4
<b>Sphingomyelin</b>				
C16:0	4.8 $\pm$ 2.1	5.2 $\pm$ 2.0	5.2 $\pm$ 2.3	6.9 $\pm$ 4.0
C16:1 (n-7)	0.8 $\pm$ 0.2	0.7 $\pm$ 0.4	1.2 $\pm$ 0.6	1.6 $\pm$ 0.6
C18:0	80.6 $\pm$ 3.4	77.8 $\pm$ 1.7	65.0 $\pm$ 8.4 <sup>#</sup>	55.1 $\pm$ 5.8*
C18:1 (n-9)	0.5 $\pm$ 0.2	1.2 $\pm$ 0.5	0.7 $\pm$ 0.3	1.7 $\pm$ 0.8
C20:0	2.3 $\pm$ 0.3	2.3 $\pm$ 0.3	2.8 $\pm$ 0.4	2.4 $\pm$ 0.2
C22:0	2.7 $\pm$ 0.5	2.4 $\pm$ 0.5	5.0 $\pm$ 2.1	4.6 $\pm$ 1.6
C24:0	2.1 $\pm$ 0.4	1.7 $\pm$ 0.4	4.5 $\pm$ 2.5	3.9 $\pm$ 1.0
C24:1 (n-6)	4.1 $\pm$ 2.1	7.3 $\pm$ 1.9	11.1 $\pm$ 5.7	19.9 $\pm$ 9.3
<b>Sulfatide</b>				
C16:0	6.9 $\pm$ 3.1	8.6 $\pm$ 1.8	7.9 $\pm$ 2.4	8.4 $\pm$ 2.4
C16:1 (n-7)	1.7 $\pm$ 0.2	1.8 $\pm$ 0.5	1.7 $\pm$ 1.0	1.6 $\pm$ 0.9
C18:0	20.7 $\pm$ 2.9	23.6 $\pm$ 2.4	19.0 $\pm$ 2.8	16.5 $\pm$ 3.5
C18:1 (n-9)	11.5 $\pm$ 1.7	13.2 $\pm$ 3.6	13.3 $\pm$ 1.4	14.1 $\pm$ 2.8
C18:1 (n-7)	1.4 $\pm$ 0.4	1.7 $\pm$ 0.4	2.2 $\pm$ 0.7	1.5 $\pm$ 0.9
C20:0	1.6 $\pm$ 0.2	1.5 $\pm$ 0.3	1.5 $\pm$ 0.4	1.3 $\pm$ 0.2
C20:1 (n-9)	1.6 $\pm$ 0.5	1.5 $\pm$ 0.7	2.2 $\pm$ 0.7	1.9 $\pm$ 0.6
C20:4 (n-6)	2.1 $\pm$ 1.1	2.3 $\pm$ 1.3	2.0 $\pm$ 0.5	2.2 $\pm$ 0.6
C22:0	6.6 $\pm$ 1.1	5.8 $\pm$ 0.8	6.0 $\pm$ 0.8	5.4 $\pm$ 0.8

**Table 3** continued

FA	CORTEX		MIDBRAIN	
	WT	gammaKO	WT	gammaKO
C24:0	14.4 ± 2.0	11.5 ± 2.1*	12.9 ± 2.6	11.7 ± 1.2
C24:1 (n-6)	28.8 ± 4.9	27.7 ± 4.3	29.2 ± 3.7	34.5 ± 5.2
Cerebroside				
C16:0	3.1 ± 0.7	5.3 ± 2.7	3.7 ± 1.3	5.0 ± 2.5
C16:1 (n-7)	1.7 ± 0.2	1.7 ± 0.8	1.4 ± 0.7	0.8 ± 0.1
C18:0	11.4 ± 4.2	13.2 ± 4.4	12.1 ± 2.9	9.1 ± 3.0
C18:1 (n-9)	2.8 ± 1.1	4.6 ± 2.8	4.0 ± 0.7	7.0 ± 3.1
C18:1 (n-7)	0.4 ± 0.1	0.4 ± 0.2	0.8 ± 0.5	1.2 ± 0.5
C20:0	2.5 ± 0.1	2.0 ± 0.4	1.8 ± 0.5	1.6 ± 0.7
C20:1 (n-9)	0.4 ± 0.2	0.6 ± 0.3	0.8 ± 0.3	1.4 ± 0.5
C20:4 (n-6)	1.5 ± 0.9	2.3 ± 1.0	1.7 ± 1.0	2.2 ± 0.4
C22:0	12.0 ± 1.6	9.1 ± 1.7	10.2 ± 1.6	7.5 ± 0.8*
C22:1	1.9 ± 0.8	2.5 ± 0.2	2.0 ± 0.2	2.3 ± 0.3
C24:0	22.8 ± 4.0	15.7 ± 3.7*	21.4 ± 4.3	15.2 ± 2.8
C24:1 (n-6)	39.6 ± 6.5	42.4 ± 5.5	40.2 ± 6.3	46.9 ± 4.7

Data as means ± SD ( $n = 6-7$ )

See legend to Table 1 for other details

The asterisk (\*) indicates a significant effect of  $\gamma$ -synuclein deficiency when compared with WT ( $p < 0.05$ ). The hash (#) indicates significant differences between midbrain and cortex in WT animals ( $p < 0.05$ )

the cortex whereas in the midbrain decreased percentages of C18:0 was found.

Fatty acids from both sulfatides and cerebrosides did not show any differences between cortex and midbrain.  $\gamma$ -Synuclein deficiency resulted in a decreased proportion of lignoceric acid in sulfatides and cerebrosides in the cortex (Table 3). In midbrain region, a reduced relative proportion of behenic acid (C22:0) was found in cerebrosides in gammaKO mice (Table 3).

The fatty acid composition of PtdSer and PtdEtn which, in brain tissues, represent two lipid classes significantly enriched with the n-3 PUFA, DHA, are shown in Figs. 2 and 3, respectively. For PtdSer, the levels of both C18:0 and DHA were higher in cortex compared to midbrain while oleate was reduced (Fig. 2). Moreover, the level of DHA in PtdSer was significantly increased in the cortex of gammaKO mice compared to WT animals (30.0 and 25.8% of total FA, respectively) with a concomitant decrease in the proportion of stearate (C18:0) (Fig. 2). PtdEtn contained both ARA and DHA as major fatty acids and, similar to PtdSer, the proportion of DHA was enhanced significantly in cortex tissue (25.4%) in gammaKO mice as compared to control (21.4%). The proportions of ARA were unaffected by  $\gamma$ -synuclein deficiency in PtdEtn from both cortex and midbrain tissues (Fig. 3). In addition to fatty acids from the diacyl form of PtdEtn, we also

analysed the profile of dimethylacetal (DMA) derivatives that represent aliphatic chains from ether derivatives (mainly plasmalogens) of PtdEtn (Fig. 3). Four DMA were identified with a domination of C18:0-DMA, but no significant changes in the relative proportion of these compounds in cortex and midbrain were found between WT and gammaKO mice.

In addition to the polar lipid classes described above, a C2-ceramide, *N*-acetylsphingosine, was present in lipid extracts from both cortex and midbrain in small but significant amounts [53]. The structure of this ceramide was elucidated by using ESI-MS (data not shown).

## Discussion

The importance of lipids in neural tissue physiology and cell signalling has been demonstrated by the association of lipid imbalances and/or deregulated lipid metabolism with the development of various CNS disorders, including Alzheimer's disease, Parkinson's disease, Niemann-Pick disease, multiple sclerosis, Huntington's disease, amyotrophic lateral sclerosis, schizophrenia, bipolar disorders and epilepsy [54]. Recently, relationships between glucosylsphingosine accumulation due to mutations in the glucocerebrosidase gene (*GBA*) and Parkinsonism as well as dementia with Lewy bodies, have been reported [55, 56]. Because alterations in brain lipid biochemistry have been previously linked to the  $\alpha$ -synuclein deficiency [41, 57] and since  $\gamma$ -synuclein and  $\alpha$ -synuclein are closely-related proteins, whose functions are potentially redundant [47, 58, 59], it was clearly important to evaluate if  $\gamma$ -synuclein might also be involved in brain lipid homeostasis. In our study we examined the lipid composition of two brain regions, the midbrain that exhibits relatively high level of  $\gamma$ -synuclein expression, and the cerebral cortex, where expression level of this protein is substantially lower [46, 60].

No effect of  $\gamma$ -synuclein deficiency on the total polar lipid content and TAG accumulation in the cerebral cortex and midbrain was revealed in our work (data not shown). This contrasts to studies with  $\alpha$ -synuclein deficient mice where an increase in TAG content of the whole brain has been demonstrated [41].

The mitochondria-specific phospholipid, Ptd<sub>2</sub>Gro, which was found in both regions studied (4% of the total polar lipids), was not affected by  $\gamma$ -synuclein deficiency (Fig. 1). The fatty acid profile of Ptd<sub>2</sub>Gro was also almost the same (Table 3). This was different from  $\alpha$ -synuclein deficient mice that had a reduced total brain Ptd<sub>2</sub>Gro content with a strongly altered acyl chain composition, a mitochondrial lipid abnormality possibly associated with electron transport chain impairment in the brain of PD patients [42].

Therefore, it is unlikely that the  $\gamma$ -synuclein deficiency affects mitochondrial function in the nervous system.

Similar to  $\alpha$ -synuclein deficiency [41],  $\gamma$ -synuclein deficiency did not change the level of ethanolamine phospholipids (Fig. 1). The plasmalogen species of PtdEtn are important phospholipid components of most electroactive cellular membranes, such as cardiac sarcolemma and neuronal cell membranes. Between one-half and two-thirds of the ethanolamine phospholipids in the whole brain are in plasmalogen form, and 11–12% of myelin phospholipids are plasmalogens [61]. A deficiency of ethanolamine plasmalogens has been shown to be associated with aging and some degenerative diseases, especially those associated with peroxisomal disorders [62–64]. The absence of ethanolamine plasmalogen alterations is consistent with only mild alterations in normal neural function in both  $\alpha$ -synuclein and  $\gamma$ -synuclein deficient mice. However, further comparative studies of aging mice would be important, due to various effects of aging on their nervous systems [65].

Among polar lipids, only the relative proportion of PtdSer changed in gammaKO in comparison to WT mice (Fig. 1). This change was evident only in the midbrain region where expression of  $\gamma$ -synuclein is much higher than in the cerebral cortex [46]. Although this increase was relatively minor in the whole midbrain tissue, the changes in PtdSer content may be much more pronounced in specific neuronal populations since  $\gamma$ -synuclein has been shown to be expressed only in a subset of midbrain neurons [46]. Previously, increases in PtdSer have been noted in plasma membrane phospholipids from affected regions of AD brains [63] where they may induce formation of amyloid fibers [66]. It is also of note, that PtdSer has roles in apoptosis, in the regulation of many enzymes and in control of the channel function of the acetylcholine receptor [62, 67, 68]. Thus, alteration in PtdSer may have implications for neuronal cell functions. However, the changes observed were not sufficient for triggering pathological alterations in the nervous system of  $\gamma$ -synuclein deficient mice [46, 47, 59].

Three lipid classes in brain contain high levels of PUFA. Whereas PtdSer and PtdEtn are enriched in DHA, PtdIns contains substantial amounts of ARA. When comparing midbrain and cortex regions, the latter was enriched in ARA but depleted in DHA (Table 2). These differences may be partly explained by higher content of PtdCho (Fig. 1), which possesses elevated levels of ARA, in the cerebral cortex (Table 3). Interestingly, statistically significant changes were found in the relative amount of PtdSer in the midbrain region of  $\gamma$ -synuclein null mutant mice (Fig. 1) and in the DHA content of both PtdSer (Fig. 2) and ethanolamine phospholipids (Fig. 3) in the cerebral cortex. Because only a limited number of cortical

neurons normally express  $\gamma$ -synuclein, changes of DHA levels in these cells might be much more profound than those revealed by analysis of total cortex phospholipids. It is noteworthy that although  $\alpha$ -synuclein null mutant mice had slightly decreased levels of DHA in the whole brain PtdEtn and PtdSer, an increased uptake of this fatty acid into brain phospholipids has also been reported [69]. It is well known that DHA is essential to perinatal neurological development during which it increases in the CNS. The high demand for DHA in the brain is maintained either by dietary supply or by biosynthesis from  $\alpha$ -linolenate within the liver [33]. Since no very long-chain PUFA were present in the diet, and no changes in the liver (data not shown) or plasma PUFA profiles (Table 1) were found, we suggest that the differences in DHA levels are most likely related to possible effects of  $\gamma$ -synuclein deficiency on DHA metabolism in the developing brain. Alternatively, complete absence of  $\gamma$ -synuclein might trigger systemic changes, including alterations in adipose and other tissues normally expressing this protein, that activate compensatory mechanisms during brain development.

There is a growing body of evidence about the importance of PUFA in brain function where its deficiency is associated with cognitive decline during aging and with neurodegenerative diseases [70]. The beneficial neurophysiological role of DHA most probably relates to metabolites such as eicosanoids and other autacoids which are important as modulators of membrane microdomain composition, receptor signalling and gene expression [71]. Recent studies demonstrated a role for neuroprotectin D1 (NPD1) in the homeostatic regulation of brain cell survival and repair involving neurotrophic, anti-apoptotic and anti-inflammatory signalling in AD [70]. Unfortunately, there is no information about the possible involvement of such DHA metabolites in PD. But the gammaKO mutants, which exhibit higher levels of DHA accumulation in certain brain regions, may be a useful model for future research in this area.

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