Brain Research Bulletin 111 (2015) 69–75

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Impaired corticostriatal LTP and depotentiation following iPLA2 inhibition is restored following acute application of DHA

David Mazzocchi-Jones*

School of Life Sciences, Faculty of Natural Sciences, Keele University, Keele, Staffordshire ST5 5BG, United Kingdom

ARTICLE INFO

Article history:
Received 30 October 2014
Received in revised form
22 December 2014
Accepted 23 December 2014
Available online 3 January 2015

Keywords:
Docosahexaenoic acid
Synaptic plasticity
Striatum
Depotentiation
Omega-3

ABSTRACT

Docosahexaenoic acid (DHA) is a 22 carbon, six cis-double bonded (22:6, w3) omega-3 polyunsaturated acid (PUFA), found highly enriched with neuronal membranes, and believed to play a critical role in synaptic plasticity and cognitive correlates of learning and memory. DHA is released from the neuronal membrane via the action of the cytostolic calcium-independent phospholipase A2 (iPLA2) enzyme. Previous studies have demonstrated that inhibition of iPLA2 by bromoenol lactone (BEL), results in inhibition of CA1 long-term potentiation (LTP), restored following acute application of DHA. In the present study, we investigated the effect of selective iPLA2 inhibition and acute application of DHA on corticostriatal synaptic plasticity. We demonstrate that acute application of 30 μM DHA facilitates cotricostriatal LTP, whilst long-term depression (LTD), basal transmission, and paired-pulse facilitation (PPF) are unaffected. Conversely, selective inhibition of iPLA2, via acute application of 10 μM BEL, inhibits the expression of corticostriatal LTP, with no effect on LTD. Furthermore, we show that 10 μM BEL inhibition of LTP is reversed following acute application of 30 μM DHA. Finally, we demonstrate that 10 μM BEL inhibits depotentiation of corticostriatal LTP, which is restored following acute application of 30 μM DHA. Our findings indicate that appropriate release of DHA is a critical facet of corticostriatal LTP and depotentiation, and thus provides an exciting cellular target for the positive facilitation of cognitive function observed following DHA dietary supplementation.

1. Introduction

Docosahexaenoic acid (DHA) is a 22 carbon, six cis-double bonded (22:6, w3) omega-3 polyunsaturated acid (PUFA); considered an essential fatty acid, due to its inability to be synthesised by mammalian organisms, and thus must be obtained from dietary sources. DHA is obtained from dietary sources either directly, via consumption of highly enriched DHA food sources, such as most marine organisms, or indirectly via desaturation of its long chain PUFA precursor alpha-linoleic acid (LNA, 18:3, w3), enriched in most flax and seed food sources. Since epidemiological studies first demonstrated that consumption of high levels of DHA in the diet correlates with significantly reduced incidence of ischaemic heart disease (Bang et al., 1976), interest in the potential therapeutic effects of DHA has increased.

DHA is ubiquitously enriched across nearly all mammalian cellular membranes, though most significantly enriched within the membranes of neuronal cells, in particular within synaptosomal membranes, synaptic vesicles, and growth cones (Scott and Bazan, 1989). Within neuronal membranes DHA is predominantly found on the sn-2 position of phosphatidylethanolamine (PtdEtn), plasmenylethanolamine (PlsEtn) and phosphatidylserine (PtdSer). DHA is readily cleaved from the neuronal membrane via deacylation (Farooqui et al., 2000), where approximately 2–8% of the total cerebral DHA content is deacylated and replaced on a daily basis (Rapoport et al., 2001).

Phospholipase A2 (PLA2) is a diverse family of enzymes ubiquitously expressed across most mammalian cells, along with the being a major constituent of vertebrate and invertebrate venoms (Scott et al., 1990). The major function of PLA2 is to catalyse the cleavage of fatty acids from the sn-2 position of membrane phospholipids, leading to an increase in intracellular free fatty acid levels. A range of distinct sub-types of PLA2 have been identified, and classified into three main subgroups based on their modality, and calcium regulation: secreted calcium-dependent PLA2 (sPLA2); cytostolic calcium-dependent (cPLA2); and cytostolic calcium-independent PLA2 (iPLA2). sPLA2 is located within a predominantly extracellular location, has a calcium regulated activity, and is

* Tel.: +44 01782 733051; fax: +44 01782 733516.
E-mail address: d.mazzocchi-jones@keele.ac.uk

http://dx.doi.org/10.1016/j.brainresbull.2014.12.010
0361-9230/

considered to play a predominantly inflammatory role through the arachidonic acid (AA) mediated prostaglandin and leukotriene signalling pathways (Murakami et al., 1998). cPLA2 and iPLA2 are located within a predominantly intracellular location, and are considered responsible for the majority of signalling based PLA2 activity (Exton, 1994). cPLA2 is considered activity-dependent, via calcium dependent mechanisms, where increases in intracellular calcium levels leads to almost selective cleavage of AA from the phospholipid membrane (Murakami et al., 1998). iPLA2 is considered to mediate the majority of basal PLA2 activity, and has been shown to favour DHA selective cleavage, over AA, from the phospholipid membrane (Kudo and Murakami, 2002).

Little is known regarding the functional significance of PLA2 enzymes in neuronal transmission and synaptic plasticity. Since it's discovery four decades ago long-term potentiation (LTP) is still commonly regarded as the most likely model underlying cellular mechanisms of learning and memory in the central nervous system (Bliss and Collingridge, 1993). It has been shown that following LTP induction, PLA2 activity is increased, indicating a potential indicative link between LTP and PLA2 activity (Chabot et al., 1998). Critically, inhibition of PLA2 prevents LTP induction in the CA1 region of the hippocampus (Chabot et al., 1998; Fujita et al., 2001); impairs learning on a passive avoidance task (Hölscher and Rose, 1994); impairs spatial learning (Hölscher et al., 1995); impairs spatial working memory (Hölscher et al., 1995), and also impairs performance on the step-down inhibitory avoidance task (Hölscher and Rose, 1994). A number of studies have aimed to identify potential cellular mechanisms for the actions of PLA2 on LTP. Increased expression of PLA2 post-LTP induction in the rodent hippocampus has been shown to correlate with increased levels of AA (Massicotte et al., 1990). Further, abolition of CA1 LTP, seen following PLA2 inhibition is restored following AA treatment (Fujita et al., 2001). The role of AA in LTP is a matter for debate. However, numerous studies link AA activity with mechanisms including: retrograde increased transmitter release (Oregan et al., 1995); inhibition of transmitter re-uptake (Diamond, 2001); or direct modulation of NMDA receptor currents (Diamond, 2001). Further, PLA2 signalling has been implicated in membrane trafficking, where PLA2 inhibition impairs translocation of proteins from the endoplasmic reticulum to the cell membrane (Brown et al., 2003). Critically, PLA2 inhibition has been shown to impair AMPA receptor translocation to synaptic membranes (Mashimo et al., 2008), a key component to the expression of LTP. Finally, PLA2 activity increases the release of DHA from the neuronal membrane, resulting in an increase in intracellular DHA concentration (Strokin et al., 2003). DHA has been shown to facilitate LTP via IP3 mediated increased intracellular calcium concentration (Aires et al., 2007); DAG driven PKC activation (Denys et al., 2004); and retinoid mediated gene transcription (de Urquiza et al., 2000). Notably, DHA treatment has been shown to rescue ablated CA1 LTP following PLA2 inhibition (Fujita et al., 2001).

In this study we demonstrate that application of DHA facilitates LTP within corticostriatal synapses, whilst having no effect on LTD, or paired-pulse facilitation (PPF). Further, selective iPLA2 inhibition ablates the expression of LTP in corticostriatal synapses, where selective cPLA2 inhibition has no effect. Furthermore, we demonstrate that the effects of selective iPLA2 inhibition are reversed following the application of DHA though not following AA application. Finally, we demonstrate that selective iPLA2 inhibition prevents the corticostriatal synapse from undergoing depotentiation, which is restored following application of DHA. Therefore suggesting that DHA may influence the expression of corticostriatal synaptic plasticity through facilitation of LTP, and regulation of depotentiation.

2. Methods

2.1. Slice preparation and treatment

Experiments were undertaken in accordance with the UK Animals (Scientific Procedures) Act 1986. Corticostriatal slices were obtained from adult (6 month) CD-1 mice. Mice were killed by cervical dislocation and decapitated. Brains were quickly removed, hemisected and placed into ice-cold artificial cerebrospinal fluid (aCSF), composition 126 mM NaCl, 2.5 mM KCl, 2.5 mM $CaCl_2$, 1.3 mM $MgSO_4$, 1 mM $NaHPO_4$, 26 mM $NaHCO_3$ and 11 mM D-glucose (Sigma–Aldrich, UK) which was constantly bubbled with $95\%O_2/5\%CO_2$ to maintain the pH at 7.4. During dissection and subsequent slicing this solution also contained 1 mM kynurenic acid (Sigma–Aldrich, UK), a broad-spectrum glutamate antagonist, to protect against excitotoxicity. Sagittal sections of 400 μm thickness were cut on a vibratome (WPI, UK), and transferred to a holding chamber containing aCSF at room temperature, and incubated for 1 h prior to recording. To perform extracellular recording, slices were transferred to a submersion-recording chamber, during the equilibration period and remainder of the experiment, slices were perfused with aCSF (same composition as incubation) at 4 ml min^{-1} at a temperature of 37 ± 0.5 °C. Slices were left for a further 5–10 min in the chamber to equilibrate before electrode placement.

2.2. Electrode placement

For all experiments slices were viewed under 20× magnification using a stereoscope (Carl Zeiss, UK). Stainless steel monopolar stimulating electrodes, with a tip resistance of 6 MΩ (Intracel, UK), were placed in the cortex. Glass recording electrodes were produced from borosilicate glass capillaries (Harvard Apparatus, UK), with an external diameter of 1.5 mm and an internal diameter of 0.86 mm, and pulled using a motorised micropipette puller (Sutter Instrument Co., USA), then filled with 1 M KCl. Extracellular electrodes were placed within the dorsal–lateral striatum in a ventral location to the stimulating electrode.

2.3. Stimuli and recording

Stimulus patterns were digitally generated via WinLTP software (WinLTP, UK) and a stimulus isolator (Digitimer, UK). Responses were amplified using a differential amplifier (Warner Instrument Corp., USA), monitored and stored for off-line analysis using WinLTP software (Anderson and Collingridge, 2007).

In all recordings, population spike (PS) amplitude was used as the measure of synaptic strength. Once a response had been gained, the slice was submitted to a 30 min rest period, where the slice was stimulated every 5–10 min, until the PS amplitude had stabilised. Initially, an input/output (I/O) curve was generated, where the PS amplitude, was measured across a stimulus intensity of 1–10 mA, in steps of 1 mA. Slices were subjected to a 5 min rest period, before a stimulus intensity that evoked 60% of the maximal response size was selected. To assess paired-pulse facilitation (PPF), paired-pulses were delivered at a range of inter-stimulus interval (ISI: 10, 20, 40, 100, 200, 500 ms), at a stimulus intensity of 40% of response saturation.

Stimuli were delivered to the slice once every 20 s (square wave pulses, 0.1 ms duration) until a 20 min period of response stability was seen. Response stability was defined as a <5% response size change over the 20 min period. This period is considered as 'baseline', since it reflects the PS amplitude prior to any changes brought about by high frequency stimulation (HFS).

Synaptic plasticity was induced via HFS comprised of three trains consisting of 100 pulses at 100 Hz delivered every 10 s, was

administered using twice the intensity used during baseline recording. For the expression of LTD, under physiologically normal aCSF conditions (aCSF contains magnesium), HFS resulted in the expression of LTD. For the expression of LTP, where magnesium was omitted from the aCSF, HFS resulted in the expression of LTP, as reported previously by ourselves, and other groups (Calabresi et al., 2000, 1992; Mazzocchi-Jones et al., 2011, 2009). Following HFS, recordings were resumed at the baseline intensity and recording continued for a further 65 min. Successful expression of synaptic plasticity was defined as a greater than 10% change in response size, lasting at least 60 min post HFS.

For depotentiation experiments, recordings were potentiated via HFS, with the subsequent LTP depotentiated using a low-frequency stimulation (LFS) protocol of 10 min at 2 Hz stimulation as previously reported (Picconi et al., 2003).

2.4. Pharmacology

The following pharmacological compounds were used: docosahexaenoic acid (DHA), arachidonic acid (AA: Tocris, UK), Bromoenol lactone (BEL), arachidonyl trifluoromethyl ketone ($AACOCF_3$: Sigma–Aldrich, UK). All pharmacological compounds were dissolved in a 0.1% DMSO (Vehicle: final aCSF volume) and bubbled with 100% N_2 prior to use. During control experiments, slices received treatment with vehicle alone. Unless otherwise indicated, pharmacological compounds were introduced prior to recording, and remained throughout the entire experiment.

2.5. Data analysis and statistics

Data was analysed off-line. All data were normalised to a baseline level, which was defined as the average response size over the 20 min of baseline stimulation. These normalised values were expressed as a percentage of the baseline response (Baseline = 100%) and averaged into 1 min bins. Results are presented as the mean result gained ± the standard error of the mean (SEM).

Multi-factorial analyses of variance were conducted on the data using the SPSS statistical package (IBM, UK), with post hoc tests correcting for multiple comparisons as appropriate.

3. Results

3.1. Effect of DHA on corticostriatal basal transmission and synaptic plasticity

Previous studies have demonstrated that application of 30 μM DHA facilitates CA1 LTP in the hippocampus, whilst having no discernable effect on basal transmission (Fujita et al., 2001). Application of 30 μM DHA had no significant effect on corticostriatal basal transmission (Fig. 1a: $F_{(2,19)} = 2.511$, $P = 0.13$, N.S.), or on input/output curves (Fig. 1b: $F_{(2,9)} = 0.542$, $P \geq 0.05$, N.S.). However, application of 30 μM DHA results in a significant facilitation of LTP (Fig. 2a: $F_{(2,19)} = 7.607$, $P = 0.013$), whilst having no significant effect on LTD (Fig. 2b: $F_{(2,19)} = 0.083$, $P = 0.776$, N.S.). To ascertain whether application of exogenous DHA had a pre- or postsynaptic affect we observed the effect of 30 μM DHA on paired pulse facilitation (PPF). Application of 30 μM DHA had no significant effect on PPF (Fig. 1c: $F_{(2,15)} = 0.894$, $P \geq 0.05$ N.S>), therefore indicating a post-synaptic mechanism of action.

3.2. Effect of cPLA2 and iPLA2 inhibition on corticostriatal synaptic plasticity

To investigate the effects of PLA2 inhibition on corticostriatal synaptic plasticity we used the selective cPLA2 inhibitor $AACOCF_3$,

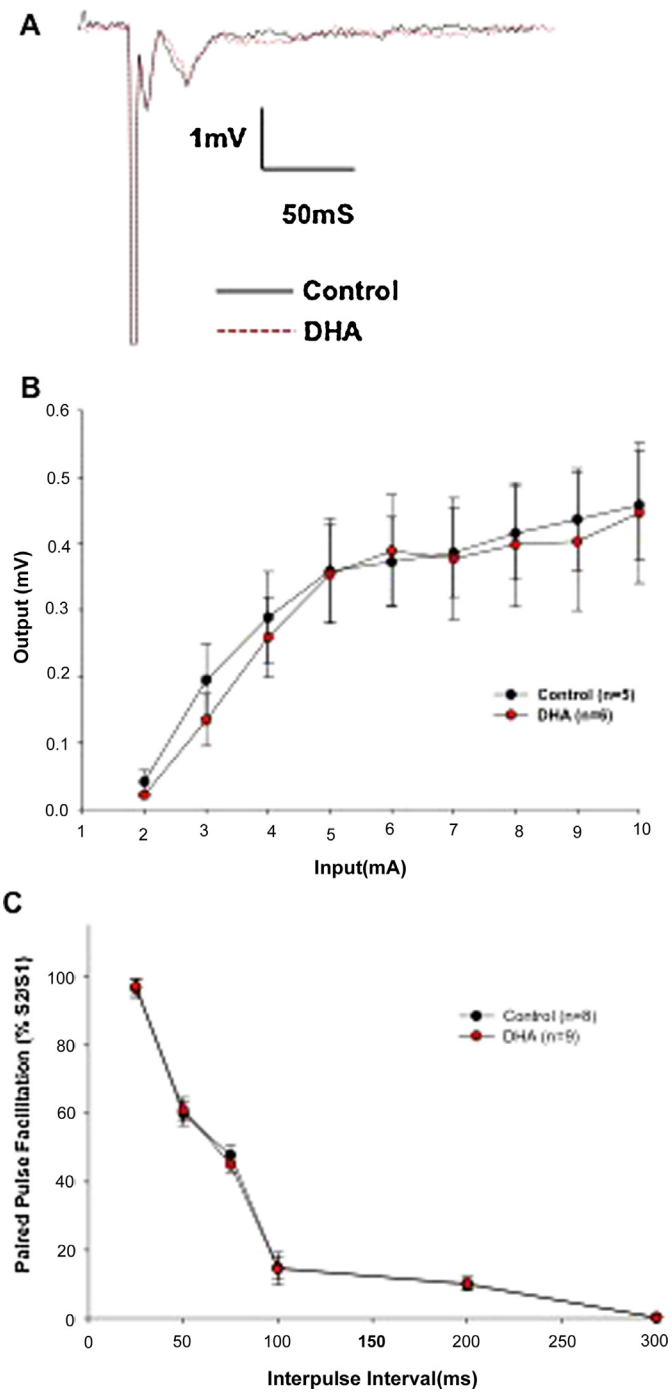

Fig. 1. The effect of DHA on basal synaptic transmission. (A) Representative fEPSP trace from control and 30 μm DHA recordings; bold line = control recording; dashed Line = DHA recording. (B) Input/output curve generated during control and 30 μm DHA recordings. (C) Paired-pulse facilitation (PPF) in control and DHA recordings. fEPSP = field excitatory post synaptic potential, PS = population spike, DHA = docosahexaenoic acid.

and selective iPLA2 inhibitor BEL. Previous studies have shown that 10 μM BEL impairs the expression of LTP from the CA1 region of the hippocampus (Fujita et al., 2001). Application of 10 μM BEL significantly impaired the expression of corticostriatal LTP (Fig. 3a: $F_{(2,14)} = 5.123$, $P = 0.039$), with no significant difference between levels of LTD observed in both control and BEL treated slices (Fig. 3b: $F_{(2,11)} = 19.361$, $P = 0.989$, N.S.). Application of 10 μM $AACOCF_3$ had no significant effect on levels of LTD observed in

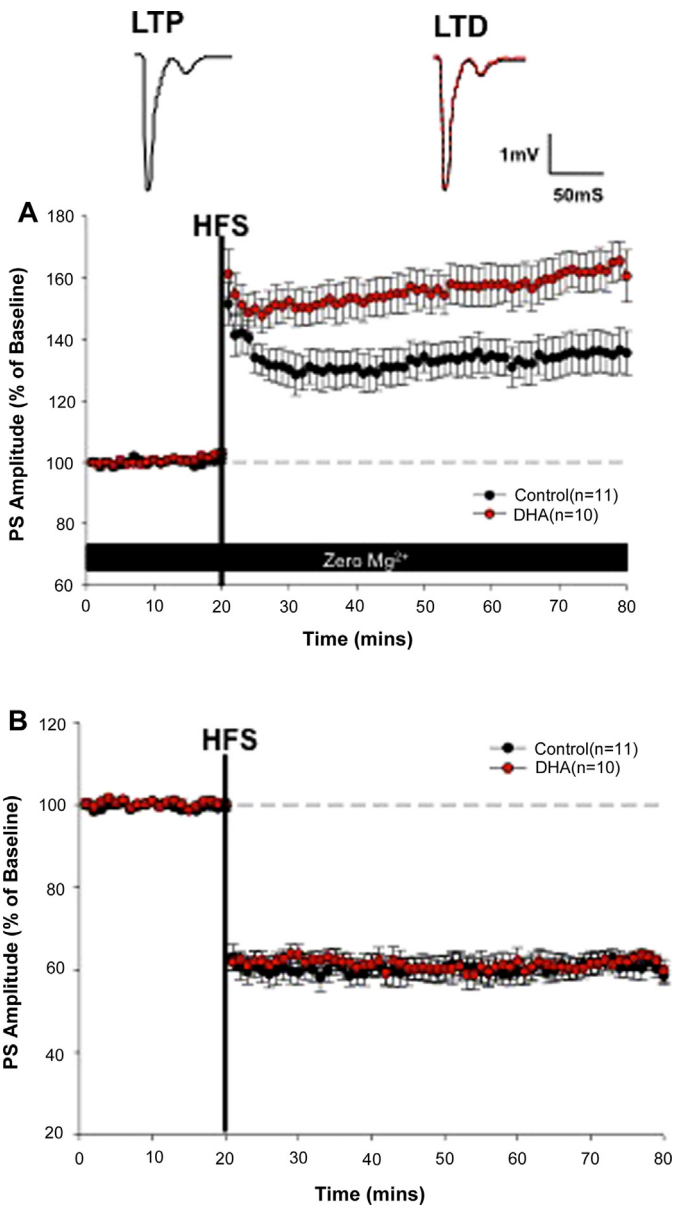

Fig. 2. The Effect of DHA on cortiostriatal synaptic plasticity. (A) Expression of LTP in control and 30 μm DHA recordings. (B) Expression of LTD in control and 30 μm DHA recordings. PS = population spike, HFS = high frequency stimulation, LTP = long-term potentiation, LTD = long-term depression.

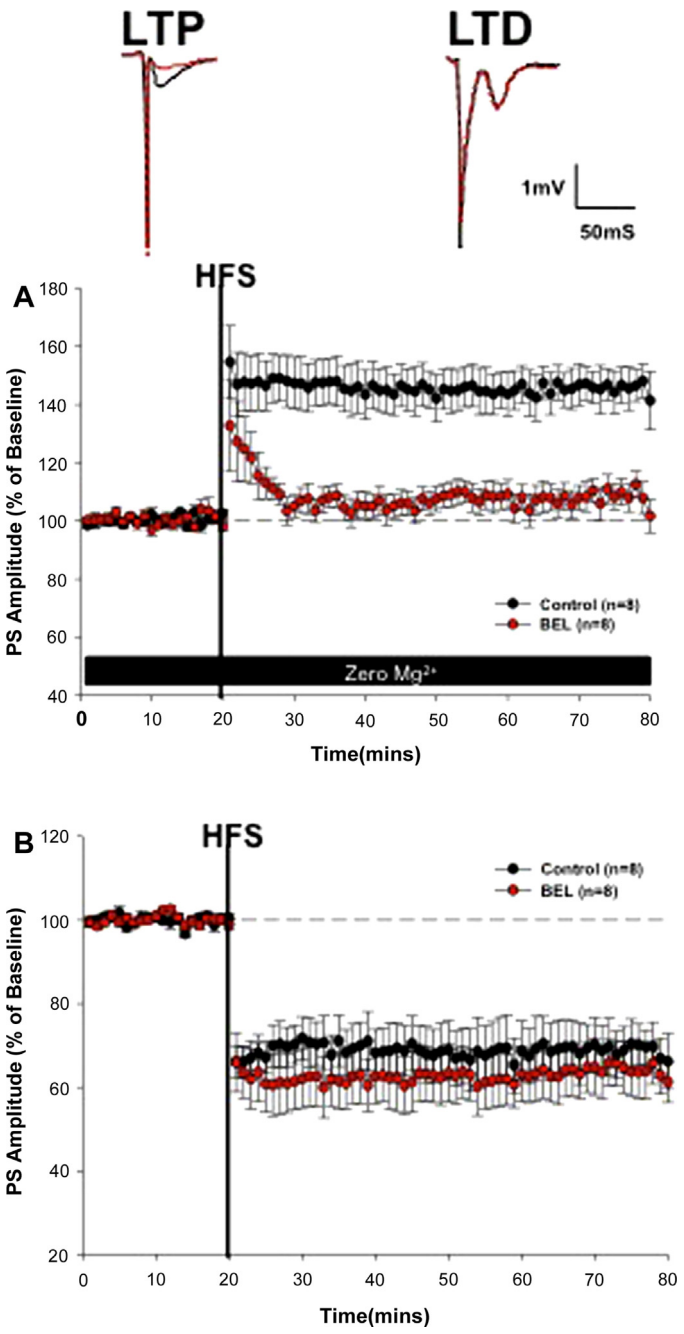

Fig. 3. The effect of iPLA2 inhibition on corticostriatal synaptic plasticity. (A) The effect of selective iPLA2 inhibition by 10 μm BEL on LTP. (B) The effect of selective iPLA2 inhibition by 10 μm BEL on LTD. PS = population spike, HFS = high frequency stimulation, LTP = long-term potentiation, LTD = long-term depression, BEL = bromoenol lactone.

control and $AACOCF_3$ treated slices (Fig. 4a: $F_{(2,11)} = 0.972$, $P = 0.611$, N.S.). Unlike BEL, application of $AACOCF_3$ failed to eliminate the expression of corticostriatal LTP, with no significant difference between control and $AACOCF_3$ treated slices (Fig. 4b: $F_{(2,12)} = 0.610$, $P = 0.776$, N.S.). Whilst these results confirm previous findings within the CA1 region of the hippocampus, this is the first demonstration within the corticostriatal synapse. To investigate the role of PLA2 inhibitors on pre-synaptic mechanisms we compared PPF in the presence of $AACOCF_3$ and BEL. As with previous findings in the CA1 region (Fujita et al., 2001), slices treated with BEL display similar levels of PPF to control slices (Fig. 5a). However, slices treated with $AACOCF_3$ display significantly lower levels of PPF at 10–100 ms interpulse intervals (Fig. 5b: $F_{(2,14)} = 19.23$, $P = <0.005$), indicating that cPLA2 activity is critical for pre-synaptic mechanism of synaptic plasticity within the corticostriatal synapse.

3.3. *Effect of AA and DHA on cPLA2 and iPLA2 inhibition in corticostriatal LTP*

Studies have demonstrated that cPLA2 and iPLA2 have differing targets for cleavage from the phospholipid membrane (Kudo and Murakami, 2002; Murakami et al., 1998). With an increased selectivity for AA by cPLA2 and conversely an increased selectivity for DHA by iPLA2 (Kudo and Murakami, 2002; Murakami et al., 1998). We earlier show that treatment of corticostriatal slices with 10 μM BEL eliminates the expression of LTP. Application of 30 μM DHA restores the expression of LTP to BEL treated slices, where application of 30 μM AA fails to restore LTP to 10 μM BEL treated slices,

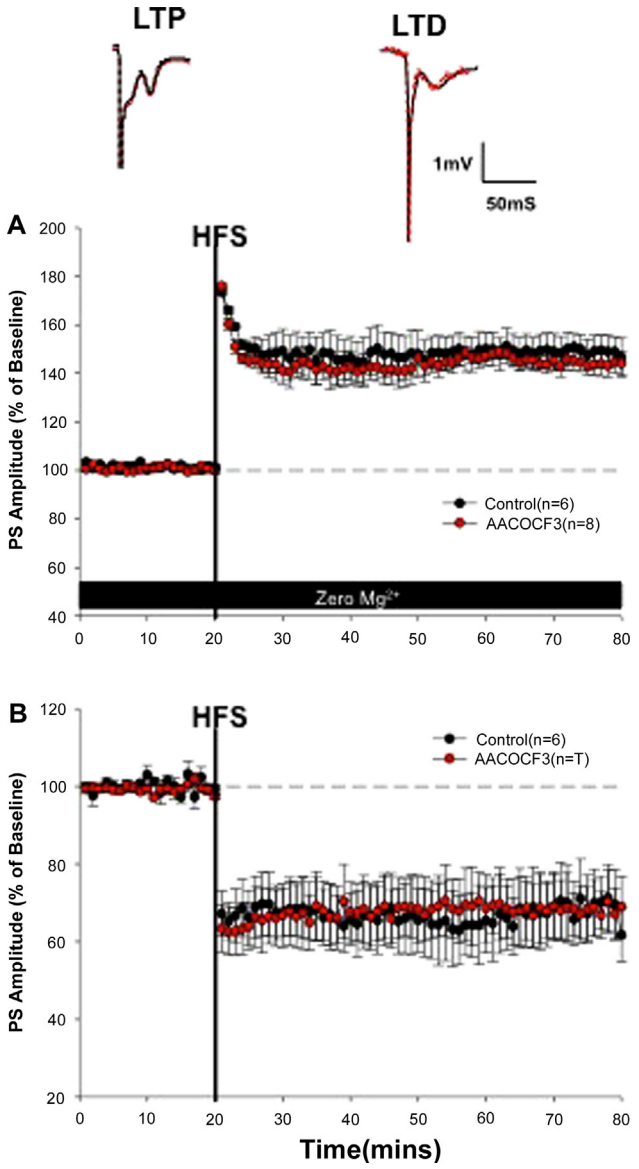

Fig. 4. The effect of cPLA2 inhibition on corticostriatal synaptic plasticity. (A) The effect of selective cPLA2 inhibition by 10 μm AACOCF3 on LTP. (B) The effect of selective cPLA2 inhibition by 10 μm AACOCF3 on LTD. PS = population spike, HFS = high frequency stimulation, LTP = long-term potentiation, LTD = long-term depression, AACOCF3 = arachidonyl trifluoromethyl ketone.

with a significant difference between the levels of LTP observed in BEL and DHA & BEL treated slices (Fig. 6: $F_{(2,10)} = 1.283$, $P = 0.014$).

3.4. Depotentiation

To investigate the role of DHA in depotentiation we subjected slices to a HFS followed by LFS protocol, as previously described (Picconi et al., 2003). To enable expression of LTP in 10 μM BEL treated slices 10 μM BEL was applied 5 min post-HFS induction. Following LFS for induction of depotentiation 10 μM BEL treated slices failed to depotentiate to baseline levels, with a significant difference in the level of depotentiation observed between control and 10 μM BEL treated slices (Fig. 7: $F_{(2,15)} = 10.248$, $P = 0.021$). Application of 30 μM DHA restores the expression of depotentiation to 10 μM BEL treated slices, with no significant difference between the level of depotentiation observed in control and DHA & BEL treated slices (Fig. 7: $F_{(2,13)} = 3.807$, $P = 0.381$, N.S.).

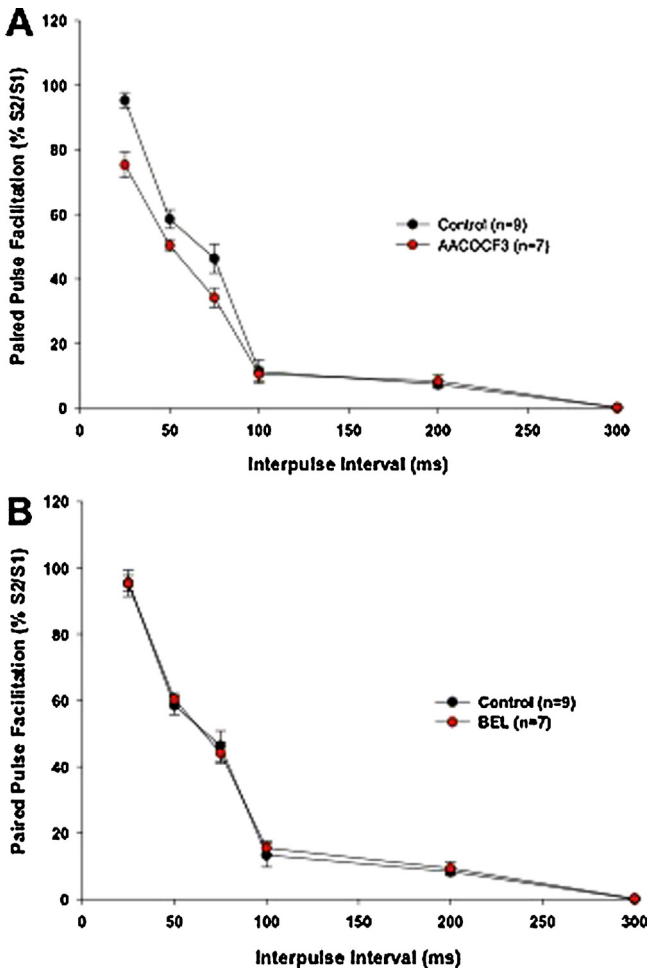

Fig. 5. The effect of iPLA2 and cPLA2 inhibition on paired-pulse facilitation. (A) The effect of selective cPLA2 inhibition by 10 μm AACOCF3 on PPF. (B) The effect of selective iPLA2 inhibition by 10 μm BEL on PPF. fEPSP = field excitatory post synaptic potential, PPF = paired-pulse facilitation, BEL = bromoenol lactone, AACOCF3 = arachidonyl trifluoromethyl ketone.

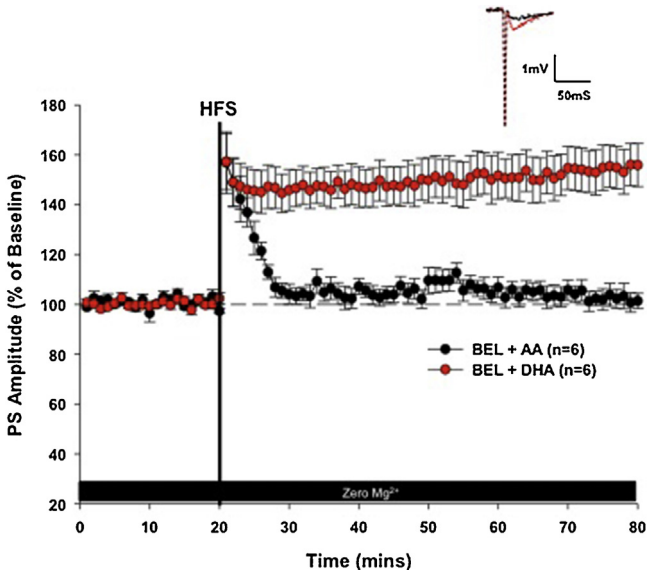

Fig. 6. The effect of DHA and AA following iPLA2 inhibition on corticostriatal LTP. The effect of 30 μm DHA and 30 μm AA following selective iPLA2 inhibition via 10 μm BEL on corticostriatal LTP. PS = population spike, HFS = high frequency stimulation, LTP = long-term potentiation, BEL = bromoenol lactone, DHA = docosahexaenoic acid, AA = arachidonic acid.

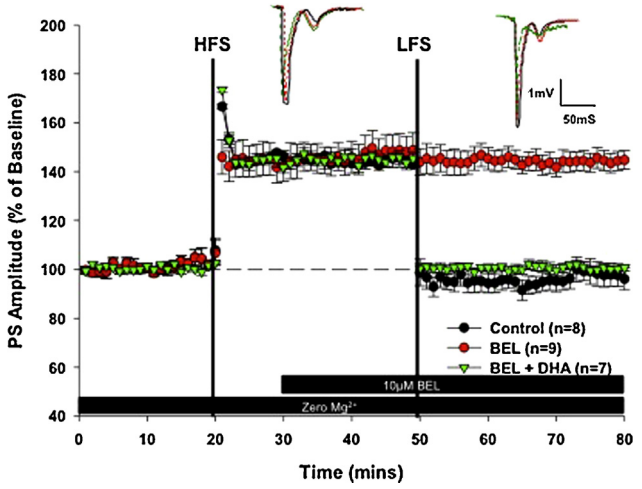

Fig. 7. The effect of DHA following selective iPLA2 inhibition on corticostriatal depotentiation. The effect of 30 μm DHA following selective iPLA2 inhibition via 10 μm BEL on corticostriatal depotentiation. PS = population spike, HFS = high frequency stimulation, LFS = low-frequency stimulation, BEL = bromoenol lactone, DHA = docosahexaenoic acid.

3.5. Discussion

Since initial reports, over three decades ago, linking increased physiological level of DHA with reduced incidence of ischaemic heart disease (Bang et al., 1976), interest in the physiological mechanisms of DHA has increased. Studies have demonstrated a positive correlation between DHA consumption, physiological levels and cognitive performance (Bauer et al., 2014a,b; de Groot et al., 2007; Titova et al., 2013). It has been well established that synaptic plasticity represents the most appropriate cellular model of learning and memory in the nervous system (Bliss and Collingridge, 1993). Critically, a number of studies have demonstrated that experimental manipulations that block LTP similarly produce impairment in cognitive function (Davis et al., 1992; Silva et al., 1992a,b).

In this study we demonstrate for the first time that corticostriatal LTP is dependant on iPLA2 signalling, which can be reversed with application of DHA. Our findings build on previous evidence from Fujita et al., who demonstrate within the CA1 region of the hippocampus, that iPLA2 signalling is required for the expression of corticostriatal LTP, where LTD and PPF are unaffected (Fujita et al., 2001). We show that acute application of 30 μM DHA results in a significant facilitation of croticostriatal LTP, with no significant effect on LTD. The mechanistic actions of DHA on synaptic physiology are a matter for debate. Traditionally, it was considered that DHA acted directly on the neuronal membrane, through interaction with phosphatidyl and plasmenyl components of the membrane increase membrane fluidity, enabling rapid translocation of lipid rafts (Farooqui and Horrocks, 2004). Within neuronal membranes this would play a significant role in enabling rapid translocation of membrane receptors and channel protiens. Significantly, it has been shown that dietary DHA treatment results in an increased expression of a range of glutametergic excitatory receptors (Dyall et al., 2007). However, it must be noted that such studies have occurred in conditions where animals have been fed high DHA diets over a period of time, and thus may not be applicable in conditions such as this study, where DHA is applied acutely to the tissue.

Over the last decade a significant argument has grown that DHA plays a critical signalling function in synaptic plasticity. Our findings show that inhibition of iPLA2 activity inhibits the expression of corticostriatal LTP, whilst inhibition of cPLA2 has no significant effect. Given that iPLA2 selectively cleaves DHA from the membrane, whilst cPLA2 selectively cleaves AA, our findings suggest that DHA is a critical component in corticostriatal LTP, though not a critical component of corticostriatal LTD. Further, we demonstrate that ablation of corticostriatal LTP following iPLA2 inhibition is reversed following application of DHA, but not AA. Therefore suggesting that selective release of DHA from the neuronal membrane by iPLA2 is critical for corticostriatal LTP, through subsequent DHA signalling. Yet, it still remains unclear how cytosolic DHA signals during corticostriatal LTP? Though previous studies have demonstrated that DHA and BEL have an effect on LTD (Young et al., 2000, 1998), we confirm previous findings and demonstrate that acute application of DHA has no effect on corticostriatal LTD (Fujita et al., 2001). Therefore, given that corticostriatal LTP is NMDA receptor dependent, and that corticostriatal LTD is NMDA receptor independent (Calabresi et al., 2000), the NMDA receptor provides a plausible potential signalling target for DHA facilitated LTP. Interestingly, previous studies have argued that DHA either modifies NMDA receptor dependent activity, or has no effect (Fujita et al., 2001; Young et al., 2000, 1998). Where Fujita et al. show an increase in NMDA mediated EPSC's, and Young et al. show no effect of DHA on NMDA mediated EPSC's. However, this discrepancy can be accountable to differing modality of DHA application, where the Young et al. study used animals undergoing chronic DHA dietary supplementation, and Fujita et al. used acute application of DHA to normal tissue slices. Therefore suggesting that DHA can have differing effects on neuronal signalling during LTP, dependent on dietary or acute application. Suggesting that DHA can have differing effects on cellular receptors based on short-term, or long-term application.

To further investigate the role of corticostriatal synaptic plasticity we investigated the role of iPLA2 during depotentiation. We demonstrate that inhibition of iPLA2 inhibits depotentiation, which is restored following application of DHA, suggesting that DHA plays a critical role in depotentiation. Previous studies have demonstrated that depotentiation is a critical facet of synaptic plasticity (Picconi et al., 2003). Initially considered reliant on appropriate PKA and PP1 phosphorylation of AMPA and NMDA receptors (Huang et al., 2001), recent evidence has suggested the role of other transmitter systems (Picconi et al., 2003). The mechanisms of depotentiation of corticostriatal synaptic plasticity have become a topic of significant interest over recent years. Within 6-OHDA lesion models of Parkinsons disease (PD) chronic L-DOPA treatment results in the sporadic expression of dyskinesia within a sub-group of the lesion treated animals (Picconi et al., 2003). Interestingly, it has been shown that animals lacking expression of dyskinesia display normal depotentiation, whilst dyskinetic animals fail to display depotentiation (Picconi et al., 2003). Furthermore, in non-dyskinetic animals depotentiation is blocked by either D1 receptor activation or PP1 inhibition (Picconi et al., 2003). In this present study we show that iPLA2 inhibition results in the inhibition of corticostriatal depotentiation, which is restored following application of DHA. Therefore suggesting that DHA plays a key role in corticostriatal depotentiation. Interestingly, application of DHA has been linked with increased PP1 activity in the Jurkat cell line (Siddiqui et al., 2003). Therefore, though it is clear that the mechanisms of DHA signalling during corticostriatal depotentiation requires further investigation, on plausible mechanism is via regulation of PP1 activity.

4. Conclusion

In conclusion, our findings show that acute application of DHA facilitates corticostriatal LTP, with no effect on LTD, basal transmission, or PPF. Selective inhibition of iPLA2 via BEL, impairs the expression of LTP, with no effect on LTD. Further, LTP is restored

following application of DHA, but not AA. Whilst selective inhibition of cPLA2 via AACOCF3 has no effect on corticostriatal LTP or LTD, suggesting regulation of corticostriatal LTP is dependent on iPLA2 mediated release of DHA. Finally, depotentiation of corticostriatal LTP is impaired following iPLA2 inhibition, by BEL, is restored following DHA treatment.

References

Aires, V., Hichami, A., Filomenko, R., Plé, A., Rébé, C., Bettaieb, A., Khan, N.A., 2007. Docosahexaenoic acid induces increases in [Ca2+]i via inositol 1,4,5-triphosphate production and activates protein kinase C gamma and -delta via phosphatidylserine binding site: implication in apoptosis in U937 cells. Mol. Pharmacol. 72, 1545–1556, http://dx.doi.org/10.1124/mol.107.039792.

Anderson, W.W., Collingridge, G.L., 2007. Capabilities of the WinLTP data acquisition program extending beyond basic LTP experimental functions. J. Neurosci. Methods 162, 346–356, http://dx.doi.org/10.1016/j.jneumeth.2006.12.018.

Bang, H.O., Dyerberg, J., Hjøorne, N., 1976. The composition of food consumed by Greenland Eskimos. Acta Med. Scand. 200, 69–73.

Bauer, I., Crewther, S., Pipingas, A., Sellick, L., Crewther, D., 2014a. Does omega-3 fatty acid supplementation enhance neural efficiency? A review of the literature. Hum. Psychopharmacol. 29, 8–18, http://dx.doi.org/10.1002/hup.2370.

Bauer, I., Hughes, M., Rowsell, R., Cockerell, R., Pipingas, A., Crewther, S., Crewther, D., 2014b. Omega-3 supplementation improves cognition and modifies brain activation in young adults. Hum. Psychopharmacol. 29, 133–144, http://dx.doi.org/10.1002/hup.2379.

Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39, http://dx.doi.org/10.1038/361031a0.

Brown, W.J., Chambers, K., Doody, A., 2003. Phospholipase A2 (PLA2) enzymes in membrane trafficking: mediators of membrane shape and function. Traffic 4, 214–221.

Calabresi, P., Centonze, D., Gubellini, P., Marfia, G.A., Pisani, A., Sancesario, G., Bernardi, G., 2000. Synaptic transmission in the striatum: from plasticity to neurodegeneration. Prog. Neurobiol. 61, 231–265.

Calabresi, P., Pisani, A., Mercuri, N.B., Bernardi, G., 1992. Long-term potentiation in the striatum is unmasked by removing the voltage-dependent magnesium block of NMDA receptor channels. Eur. J. Neurosci. 4, 929–935.

Chabot, C., Gagné, J., Giguère, C., Bernard, J., Baudry, M., Massicotte, G., 1998. Bidirectional modulation of AMPA receptor properties by exogenous phospholipase A2 in the hippocampus. Hippocampus 8, 299–309, doi:10.1002/(SICI)1098-1063(1998)8:3<299::AID-HIPO11>3.0.CO;2-W.

Davis, S., Butcher, S.P., Morris, R.G., 1992. The NMDA receptor antagonist D-2-amino-5-phosphonopentanoate (D-AP5) impairs spatial learning and LTP in vivo at intracerebral concentrations comparable to those that block LTP in vitro. J. Neurosci. 12, 21–34.

de Groot, R.H.M., Hornstra, G., Jolles, J., 2007. Exploratory study into the relation between plasma phospholipid fatty acid status and cognitive performance. Prostaglandins Leukot. Essent. Fatty Acids 76, 165–172, http://dx.doi.org/10.1016/j.plefa.2007.01.001.

de Urquiza, A.M., Liu, S., Sjöberg, M., Zetterström, R.H., Griffiths, W., Sjövall, J., Perlmann, T., 2000. Docosahexaenoic acid, a ligand for the retinoid X receptor in mouse brain. Science 290, 2140–2144.

Denys, A., Aires, V., Hichami, A., Khan, N.A., 2004. Thapsigargin-stimulated MAP kinase phosphorylation via CRAC channels and PLD activation: inhibitory action of docosahexaenoic acid. FEBS Lett. 564, 177–182, http://dx.doi.org/10.1016/S0014-5793(04)00361-8.

Diamond, J.S., 2001. Neuronal glutamate transporters limit activation of NMDA receptors by neurotransmitter spillover on CA1 pyramidal cells. J. Neurosci. 21, 8328–8338.

Dyall, S.C., Michael, G.J., Whelpton, R., Scott, A.G., Michael-Titus, A.T., 2007. Dietary enrichment with omega-3 polyunsaturated fatty acids reverses age-related decreases in the GluR2 and NR2B glutamate receptor subunits in rat forebrain. Neurobiol. Aging 28, 424–439, http://dx.doi.org/10.1016/j.neurobiolaging.2006.01.002.

Exton, J.H., 1994. Phosphatidylcholine breakdown and signal transduction. Biochim. Biophys. Acta 1212, 26–42.

Farooqui, A.A., Horrocks, L.A., 2004. Brain phospholipases A2: a perspective on the history. Prostaglandins Leukot. Essent. Fatty Acids 71, 161–169, http://dx.doi.org/10.1016/j.plefa.2004.03.004.

Farooqui, A.A., Horrocks, L.A., Farooqui, T., 2000. Deacylation and reacylation of neural membrane glycerophospholipids. J. Mol. Neurosci. 14, 123–135.

Fujita, S., Ikegaya, Y., Nishikawa, M., Nishiyama, N., Matsuki, N., 2001. Docosahexaenoic acid improves long-term potentiation attenuated by phospholipase A(2) inhibitor in rat hippocampal slices. Br. J. Pharmacol. 132, 1417–1422, http://dx.doi.org/10.1038/sj.bjp.0703970.

Hölscher, C., Canevari, L., Richter-Levin, G., 1995. Inhibitors of PLA2 and NO synthase cooperate in producing amnesia of a spatial task. Neuroreport 6, 730–732.

Hölscher, C., Rose, S.P., 1994. Inhibitors of phospholipase A2 produce amnesia for a passive avoidance task in the chick. Behav. Neural Biol. 61, 225–232.

Huang, C.C., Liang, Y.C., Hsu, K.S., 2001. Characterization of the mechanism underlying the reversal of long term potentiation by low frequency stimulation at hippocampal CA1 synapses. J. Biol. Chem. 276, 48108–48117, http://dx.doi.org/10.1074/jbc.M106388200.

Kudo, I., Murakami, M., 2002. Phospholipase A2 enzymes. Prostaglandins Other Lipid Mediat. 68–69, 3–58.

Mashimo, M., Hirabayashi, T., Murayama, T., Shimizu, T., 2008. Cytosolic PLA2(alpha) activation in Purkinje neurons and its role in AMPA-receptor trafficking. J. Cell. Sci. 121, 3015–3024, http://dx.doi.org/10.1242/jcs.032987.

Massicotte, G., Oliver, M.W., Lynch, G., Baudry, M., 1990. Effect of bromophenacyl bromide, a phospholipase A2 inhibitor, on the induction and maintenance of LTP in hippocampal slices. Brain Res. 537, 49–53.

Mazzocchi-Jones, D., Doebroessy, M., Dunnett, S.B., 2009. Embryonic striatal grafts restore bi-directional synaptic plasticity in a rodent model of Huntington's disease. Eur. J. Neurosci. 30, 2134–2142, http://dx.doi.org/10.1111/j.1460-9568.2009.07006.x.

Mazzocchi-Jones, D., Doebroessy, M., Dunnett, S.B., 2011. Environmental enrichment facilitates long-term potentiation in embryonic striatal grafts. Neurorehabil. Neural Repair 25, 548–557, http://dx.doi.org/10.1177/1545968311402090.

Murakami, M., Shimbara, S., Kambe, T., Kuwata, H., Winstead, M.V., Tischfield, J.A., Kudo, I., 1998. The functions of five distinct mammalian phospholipase A2S in regulating arachidonic acid release. Type IIa and type V secretory phospholipase A2S are functionally redundant and act in concert with cytosolic phospholipase A2. J. Biol. Chem. 273, 14411–14423.

Oregan, M.H., Perkins, L.M., Phillis, J.W., 1995. Arachidonic acid and lysophosphatidylcholine modulate excitatory transmitter amino acid release from the rat cerebral cortex. Neurosci. Lett. 193, 85–88.

Picconi, B., Centonze, D., Håkansson, K., Bernardi, G., Greengard, P., Fisone, G., Cenci, M.A., Calabresi, P., 2003. Loss of bidirectional striatal synaptic plasticity in L-DOPA-induced dyskinesia. Nat. Neurosci. 6, 501–506, http://dx.doi.org/10.1038/nn1040.

Rapoport, S.I., Chang, M.C., Spector, A.A., 2001. Delivery and turnover of plasma-derived essential PUFAs in mammalian brain. J. Lipid Res. 42, 678–685.

Scott, B.L., Bazan, N.G., 1989. Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. Proc. Natl. Acad. Sci. U. S. A. 86, 2903–2907.

Scott, D.L., White, S.P., Otwinowski, Z., Yuan, W., Gelb, M.H., Sigler, P.B., 1990. Interfacial catalysis: the mechanism of phospholipase A2. Science 250, 1541–1546.

Siddiqui, R.A., Jenski, L.J., Harvey, K.A., Wiesehan, J.D., Stillwell, W., Zaloga, G.P., 2003. Cell-cycle arrest in Jurkat leukaemic cells: a possible role for docosahexaenoic acid. Biochem. J. 371, 621–629, http://dx.doi.org/10.1042/BJ20021667.

Silva, A.J., Paylor, R., Wehner, J.M., Tonegawa, S., 1992a. Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. Science 257, 206–211.

Silva, A.J., Stevens, C.F., Tonegawa, S., Wang, Y., 1992b. Deficient hippocampal long-term potentiation in alpha-calcium-calmodulin kinase II mutant mice. Science 257, 201–206.

Strokin, M., Sergeeva, M., Reiser, G., 2003. Docosahexaenoic acid and arachidonic acid release in rat brain astrocytes is mediated by two separate isoforms of phospholipase A2 and is differently regulated by cyclic AMP and Ca2+. Br. J. Pharmacol. 139, 1014–1022, http://dx.doi.org/10.1038/sj.bjp.0705326.

Titova, O.E., Sjögren, P., Brooks, S.J., Kullberg, J., Ax, E., Kilander, L., Riserus, U., Cederholm, T., Larsson, E.-M., Johansson, L., Ahlström, H., Lind, L., Schiöth, H.B., Benedict, C., 2013. Dietary intake of eicosapentaenoic and docosahexaenoic acids is linked to gray matter volume and cognitive function in elderly. Age (Dordr) 35, 1495–1505, http://dx.doi.org/10.1007/s11357-012-9453-3.

Young, C., Gean, P.W., Chiou, L.C., Shen, Y.Z., 2000. Docosahexaenoic acid inhibits synaptic transmission and epileptiform activity in the rat hippocampus. Synapse 37, 90–94, doi:10.1002/1098-2396(200008)37:2<90::AID-SYN2>3.0.CO;2-Z.

Young, C., Gean, P.W., Wu, S.P., Lin, C.H., Shen, Y.Z., 1998. Cancellation of low-frequency stimulation-induced long-term depression by docosahexaenoic acid in the rat hippocampus. Neurosci. Lett. 247, 198–200.