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Original Article

ORIGINAL ARTICLE

Nutritional n-3 PUFA Deficiency Abolishes Endocannabinoid Gating of Hippocampal Long-Term Potentiation

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Abstract

Maternal n-3 polyunsaturated fatty acids (PUFAs), especially docosahexaenoic acid, is critical during perinatal brain development. How early postnatal n-3 PUFA deficiency impacts on hippocampal synaptic plasticity is mostly unknown. Here we compared activity-dependent plasticity at excitatory and inhibitory synapses in the CA1 region of the hippocampus in weaned pups whose mothers were fed with an n-3 PUFA-balanced or n-3 PUFA-deficient diet. Normally, endogenous cannabinoids (eCB) produced by the post-synapse dually control network activity by mediating the long-term depression of inhibitory inputs (iLTD) and positively gating NMDAR-dependent long-term potentiation (LTP) of excitatory inputs. We found that both iLTD and LTP were impaired in n-3 PUFA-deficient mice. Pharmacological dissection of the underlying mechanism revealed that impairment of NMDAR-dependent LTP was causally linked to and attributable to the ablation of eCB-mediated iLTD and associated to disinhibitory gating of excitatory synapses. The data shed new light on how n-3 PUFAs shape synaptic activity in the hippocampus and provide a new synaptic substrate to the cognitive impairments associated with perinatal n-3 deficiency.

Key words: endocannabinoid, hippocampus, iLTD, n-3 PUFA, synaptic plasticity

Introduction

Linoleic acid (LA) and α-linolenic acid (ALA) are essential n-6 and n-3 polyunsaturated fatty acids (PUFAs) that are exclusively provided by food (Innis 2008; Gibson et al. 2011). Their metabolites, the long chain PUFAs (LC-PUFAs), arachidonic acid (AA, 20:4n-6) and docosahexaenoic acid (DHA, 22:6n-3) are then esterified in phospholipids of cell membranes. AA and DHA are essential to the normal development and function of the brain (Bourre 2005; Bazinet and Layé 2014). As a consequence, imbalanced maternal dietary intake in PUFAs is a risk factor for neurodevelopmental disorders including autism and schizophrenia (Lauritzen et al. 2001; Bazinet and Layé 2014; Joffre et al. 2014). DHA is particularly important for development of synaptic circuits in the hippocampus (Calderon and Kim 2004; Cao et al. 2009), and perinatal n-3 PUFA deficiency leads to strong cognitive impairments (Moriguchi et al. 2000; Catalan et al. 2002; Moranis et al. 2012). Conversely, n-3 PUFAs dietary supplementation potently rescues synaptic plasticity in the hippocampus following perinatal intoxication (Patten et al. 2013) or aging (Lynch et al. 2007; Kelly et al. 2011). The effects

of developmental n-3 PUFAs deficiency on hippocampal synaptic plasticity, a mechanism that is considered to be the physical substrate of learning and memory in this structure (Morris et al. 1986; Bliss and Collingridge 1993; Lynch 2004; Mayford et al. 2012), have never been explored.

Activity-dependent modifications in synaptic efficiency, namely synaptic plasticity, are essential for neuronal development, learning and memory formation (Lynch 2004; Neves et al. 2008). In the hippocampus, a crucial site for learning and memory (Scoville and Milner 1957; Jarrard 1993; Burgess et al. 2002; Squire et al. 2004), long-term potentiation (LTP) is induced by stimulation of glutamatergic Schaffer Collateral (SC) afferents in the CA1 region (Lynch 2004; Mayford et al. 2012). This form of synaptic plasticity depends on N-methyl-D-aspartate (NMDA) glutamatergic receptors and is gated by endocannabinoids (eCBs) acting at inhibitory synapses. eCBs are signaling lipids that are produced "on demand" from LC-PUFAs present in phospholipids in the neuronal cell membranes in response to neuronal activity. Activity-dependent synthesis of eCBs underlies various forms of synaptic plasticity (Heifets and Castillo 2009; Castillo et al. 2012) and is necessary for optimal brain functions (Leweke and Koethe 2008; Hill and Gorzalka 2009; Lutz 2009). In the hippocampus, this unique mechanism is called heterosynaptic inhibitory long-term depression (iLTD) (Carlson et al. 2002; Chevaleyre and Castillo 2003; Castillo et al. 2012). Heterosynaptic iLTD relies on activation of G protein-coupled receptors CB_1 receptors, which in turn reduce gamma-aminobutyric acid (GABA) release from inhibitory synapses.

Here, we tested the hypothesis that a developmental n-3 PUFA deficiency alters hippocampal synaptic plasticity. To this aim, mice were fed from the first gestational day with a diet deficient in n-3 PUFA and synaptic plasticity was analyzed at the SC-CA1 synapses of 21 days-old male pups. We found that n-3 PUFAs dietary deficiency completely abolishes synaptic plasticity in the hippocampus by impairing eCB gating of LTP. These results highlight the importance of maternal dietary intake for optimal synaptic functions.

Materials and Methods

Animals

All experiments were performed according to the criteria of the European Communities Council Directive (Local Animal Care and Use Committee agreement #5012094-A). Parents of tested mice (CD1) were obtained from Janvier Labs (Le Genest-Saint-Isle, France) and tested mice stayed with the mother until electrophysiological recordings. Experiments were performed on P18–P24 CD1 male mice. Mice were maintained under standard housing conditions on corn cob litter at a controlled temperature (23 ± 1°C) and humidity (40%) in an animal room with a 12-h light/dark cycle (0700–1900 h), with ad libitum access to food and water.

Diets

Mothers were fed with a diet containing 6% of rapeseed oil (rich in ALA, 18:3n-3; "n-3 bal.") or 6% fat in the form of sunflower oil (rich in LA, 18:2n-6; "n-3 def.") from the first gestational day (Lafourcade et al. 2011; Madore et al. 2014). Diets were isocaloric and differed only in the oil content. Pellets were prepared by UPAE-INRA (Jouy-en-Josas, France) and stored at 4°C. Fatty acid composition was regularly controlled via gas chromatography analyses of organic extracts from manufactured food pellets as previously described (Lafourcade et al. 2011; Labrousse et al. 2012; Madore et al. 2014).

Hippocampus Slice Preparation

Coronal hippocampal slices (350 μm) were prepared from P18–P24 mice fed with n-3 PUFA-balanced diet or n-3 PUFA-deficient diet. Slices were prepared as previously described (Delpech et al. 2015) with a vibrating blade microtome (VT1000S, Leica Microsystems) in an oxygenated artificial cerebrospinal fluid (ACSF) containing in (mM): 23 $NaHCO_3$, 87 NaCl, 75 sucrose, 25 glucose, 4 $MgCl_2$, 2.5 KCl, 0.5 $CaCl_2$, 1.25 NaH_2PO_4. Slices were stored at room temperature and recordings were started after at least 1 h of rest. ACSF solution for storage contained (in mM): 23 $NaHCO_3$, 130 NaCl, 11 Glucose, 2.4 $MgCl_2$, 2.5 KCl, 1.2 $CaCl_2$, 1.2 NaH_2PO_4. ACSF solution for recordings contained (in nM): 23 $NaHCO_3$, 130 NaCl, 11 glucose, 1.2 $MgCl_2$, 2.5 KCl, 2.4 $CaCl_2$, 1.2 NaH_2PO_4.

Electrophysiological Recordings

Recordings were performed at 28°C using a temperature control system (TC-344B, Warner Instrument Corporation) and slices were continuously superfused at 3–4 mL/min with recording ACSF. Synaptic events were evoked by stimulation of the SC pathway afferents (150 μs, 0.1 Hz, bipolar concentric electrode from Phymep, and stimulator A365 from WPI). Extracellular field excitatory postsynaptic potentials (fEPSPs) were recorded in the CA1 stratum radiatum as previously described (Delpech et al. 2015). Recordings were performed with borosilicate glass pipettes of 5–10 MΩ resistance filled with recording ACSF. For whole-cell patch-clamp recordings, CA1 pyramidal neurons were visualized using an infrared microscope (BX-51WI, Olympus). EPSCs were recorded at −70 mV with pipettes filled with balanced intracellular solutions (in mM): 128 cesium methane-sulfonate (CH_3O_3SCs, for AMPA/NMDA recordings) or 128 potassium gluconate (KGlu, for current clamp recordings), 20 NaCl, 1 $MgCl_2$, 1 EGTA, 0.3 $CaCl_2$, 2 Na^{2+}-ATP, 0.3 Na^+-GTP, 10 glucose buffered with 10 HEPES, pH 7.3, osmolarity 290 mOsm. IPSCs were recorded at −45 mV in the continuous presence of NMDA and AMPA/KA receptor antagonists (APV and DNQX) with the following intracellular solution (in mM): 150 cesium chloride, 2 $MgCl_2$, 1 EGTA, 3 Na_2-ATP, 0.3 Na-GTP, 0.2 cAMP, buffered with 10 HEPES, pH 7.3, osmolarity 290 mOsm. If access resistance (no compensation, <25 MΩ) changed by >20%, the experiment was rejected.

Data Acquisition and Analysis

Signals were amplified using Multiclamp 700B amplifier (Molecular Devices) controlled with pClamp 10.3 software via a Digidata 1440A interface (Molecular Devices). Data were digitized at 20 kHz for current clamp recordings and 200 kHz for voltage-clamp recordings and filtered at 5 kHz.

High frequency stimulation (HFS) consists in 2 stimulation trains at 100 Hz for 1 s with 20 -s interval between the two stimulation trains. Baseline responses were recorded for 10 min at 0.1 Hz with a stable response. After plasticity protocol, synaptic events were recorded for up to 45 min at 0.1 Hz. For each experiment, fEPSP slopes or IPSC amplitudes were measured 10 min before plasticity protocol (HFS) and up to 45 min after the protocol using Clampfit 10.3 (Molecular Devices).

For AMPA/NMDA ratio, AMPA component was measured from EPSCs at −70 mV and NMDA component was measured 40 ms after the start of dual component EPSCs at +40 mV (Kasanetz and Manzoni 2009). For plasticity protocols, stable synaptic responses (IPSC or fEPSP) were recorded for 10 min at 0.1 Hz, with a stimulus intensity of 40%–50% of the maximum fEPSP for field recordings. To perform current – voltage ($I-V$) curves and to test neuronal pyramidal neuron excitability, a series of hyperpolarizing and depolarizing current steps starting at −400 pA with 50 pA

increment were applied in current clamp configuration immediately after breaking in the cell in whole cell. For inhibitory spontaneous activities, spontaneous IPSCs (sIPSCs) were measured for 5 min at −45 mV. Amplitude and interevent interval time were analyzed with Axograph X using a double exponential template: $f(t) = \exp(-t/\text{rise}) + \exp(-t/\text{decay})$, rise = 0.5 ms and decay = 3 ms. The threshold of amplitude detection was set at 5 pA (Kasanetz and Manzoni 2009).

Drugs

All products for solutions and picrotoxin (PTX, 100 µM) were from Sigma Aldrich. TTX (0.5 µM), AM251 (4 µM), APV (50 µM), DNQX (20 µM), JZL184 (1 µM), CP 55 940 (10 µM) were provided by Tocris Bioscience. Drugs were dissolved in DMSO and added in ACSF recording solution at a final DMSO concentration of 0.001%.

Statistical Analysis

All values are given mean ± SEM. Statistical analysis was performed with Prism 5.0 (GraphPad software). Multiple comparisons were made with 1- or 2-factor parametric or nonparametric ANOVA, as appropriate, with post hoc Bonferroni's test. Single comparisons were made with Student's t, Mann–Whitney or Wilcoxon-signed-rank tests. Kolmogorov–Smirnov tests were used for comparing cumulative distributions.

Results

Long-Term Synaptic Plasticity is Altered in the Hippocampus of n-3 PUFA-Deficient Mice

The relative contribution of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) and NMDAR to synaptic currents is an indicator of synaptic plasticity in particular in those depending on the incorporation or removal of AMPAR in the post-synaptic density (e.g., LTP or LTD, respectively) (Kasanetz and Manzoni 2009). AMPAR- and NMDAR-evoked EPSCs ratio was first evaluated. This index was significantly higher in the n-3 PUFA-deficient mice compared with the n-3 PUFA-balanced mice, suggesting that the n-3 PUFA-deficient diet altered the overall gain of SC-CA1 synapses (Fig. 1A). Input/output relationships in n-3 PUFA-balanced and n-3 PUFA-deficient mice were then compared to probe the functional connectivity of CA1 pyramidal neurons. The excitability of SC-CA1 synapses was unaltered in n-3 PUFA-deficient mice (Fig. 1B). Activity NMDAR-dependent LTP is probably the most widely expressed and extensively studied form of synaptic plasticity (Lynch 2004). LTP was measured at the SC-CA1 synapse in n-3 PUFA-balanced and deficient mice using a HFS protocol (2 trains at 100 Hz for 1 s with 20-s interval). In the hippocampus of n-3 PUFA-balanced mice, HFS induced a significant LTP (Fig. 1C,D) that was completely prevented by the NMDAR antagonist APV (50 µM) (Fig. 1E), confirming its dependency on NMDAR (Harris et al. 1984; Bashir et al. 1991; Dudek and

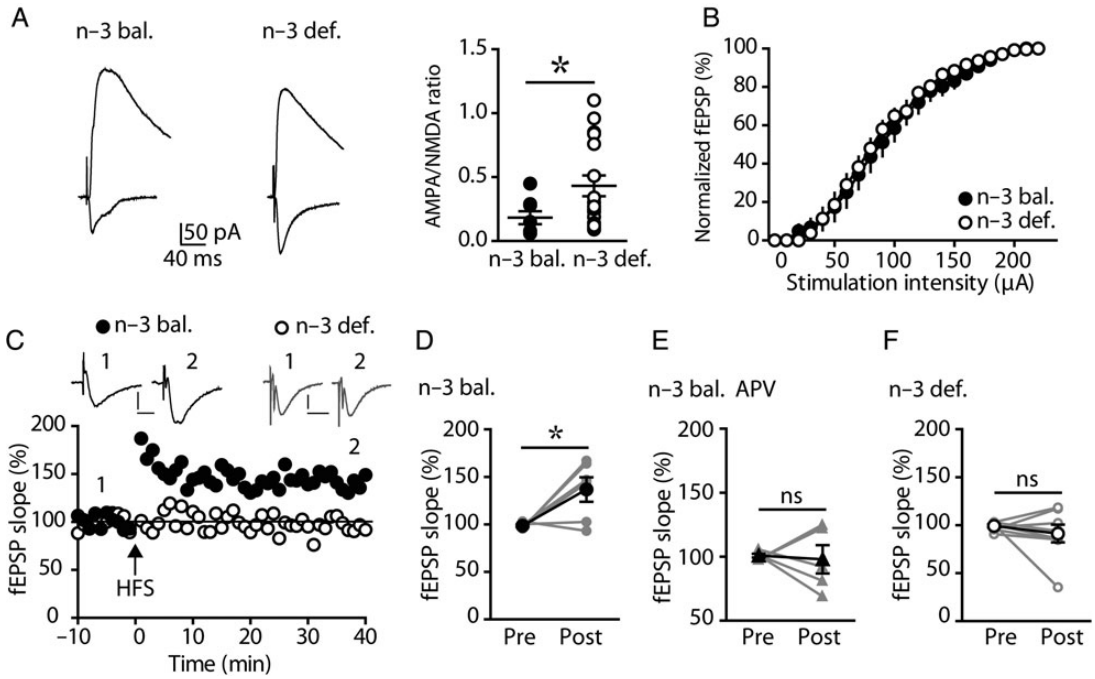

Figure 1. NMDAR-dependent functional LTP is impaired at SC-CA1 synapses of n-3 PUFA-deficient mice. (*A*) AMPA/NMDA ratio is increased in n-3 PUFA-deficient mice. Left: representative recordings of AMPA and NMDA EPSCs taken from both groups. Right: Dot plot of AMPAR-mediated EPSC/NMDAR-mediated EPSC ratio in CA1 pyramidal neurons for n-3 PUFA-balanced (n-3 bal., black circles: 0.18 ± 0.05, $n = 8$ neurons) and deficient (n-3 def., white circles: 0.43 ± 0.08, $n = 17$ neurons) mice ($P = 0.0334$, Mann–Whitney test). Mean ± SEM, each dot represents one neuron. (*B*) Input/output curves (mean ± SEM) of evoked fEPSPs in CA1 stratum radiatum of n-3 PUFA-balanced mice ($n = 9$, black circles) and deficient mice ($n = 10$, white circles) for increasing stimulation intensities. fEPSPs were normalized with the minimum value for each recording being "0" and the maximal value being "100" (min-to-max normalization). 2-way ANOVA with "stimulation intensity" ($F_{22,391} = 164.7$, $P < 0.0001$) and "diet" ($F_{1,391} = 2.835$, $P = 0.0930$) as factors, interaction: $F_{22,391} = 0.2705$, $P = 0.9997$. (*C–F*) NMDAR-dependent LTP is abolished in n-3 PUFA-deficient mice. (*C*) Representative traces from averaged fEPSP response before (1) and 40 min after plasticity induction (2) in mice from both groups. Scale bar: 0.2 mV, 5 ms (left), 0.2 mV, 10 ms (right). Representative time courses of mean fEPSPs were normalized to baseline. HFS of SC induced a LTP of evoked fEPSPs recorded in stratum radiatum in CA1 of n-3 PUFA-balanced (black circles) but not deficient mice (white circles). (*D–F*) Normalized fEPSP slope (as a percentage of the baseline) before (Pre) and 40 min after HFS (Post); ns, $P > 0.05$, *$P < 0.05$, paired *t*-test before and 40 min after HFS for each recording. (*D*) n-3 PUFA-balanced mice, black circles: before: 98% ± 1% of baseline fEPSP versus after HFS: 136% ± 13%, $n = 6$, $P = 0.0415$. (*E*) n-3 PUFA-balanced mice + APV: before: 101% ± 1% of basal fEPSP versus after HFS: 98% ± 11%, $n = 5$, $P = 1.0000$. (*F*) n-3 PUFA-deficient mice, white circles: 98% ± 2% of baseline fEPSP versus after HFS: 90% ± 9%, $n = 8$, $P = 0.4281$.

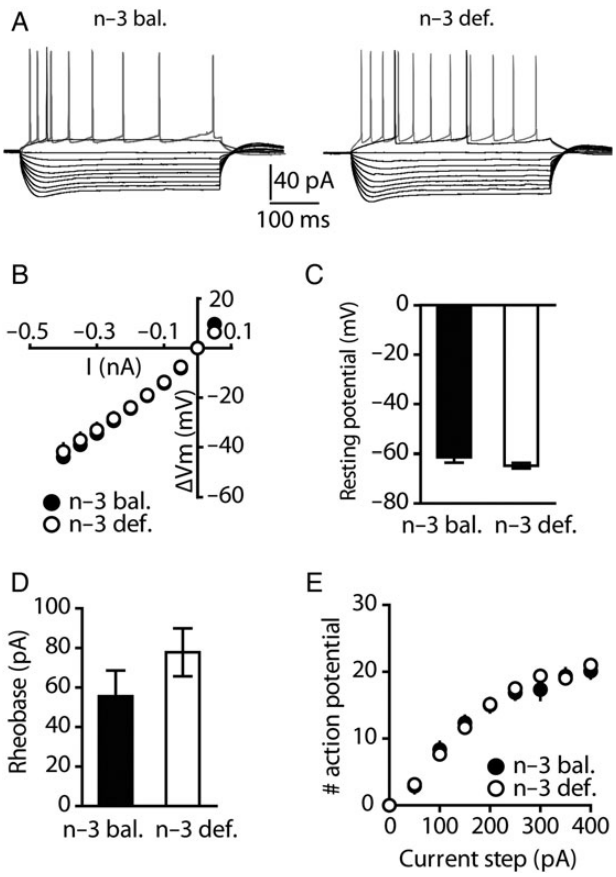

Figure 2. Intrinsic properties of CA1 pyramidal neurons in n-3 PUFA-balanced and deficient mice. (*A*) Typical membrane responses to somatic current steps of n-3 PUFA-balanced (left) and deficient (right) CA1 pyramidal neurons. Stimulation protocol consists in 500 ms-long steps of current with increment of 50 pA, starting at −400 pA. (*B*) Summary of all the current–voltage (*I–V*) curves recorded in the 2 groups showing no difference between n-3 PUFA-balanced ($n = 9$ cells, black circles) and deficient ($n = 10$ cells, white circles) mice. 2-way ANOVA with "current" ($F_{11,\ 204} = 115.5$, $P < 0.0001$) and "diet"($F_{1,204} = 0.1740$ $P = 0.6770$) as factors, interaction: $F_{11,204} = 0.2373$, $P = 0.9946$. (*C*) The resting membrane potential (n-3 PUFA-balanced: −61.3 ± 2.2 mV, $n = 9$ vs. n-3 PUFA deficient: −64.7 ± 1.1 mV, $n = 10$) and (*D*) the rheobase (n-3 PUFA-balanced: 56 ± 13 pA, $n = 9$ vs. n-3 PUFA deficient: 78 ± 12 pA, $n = 9$) were similar in both groups. $P > 0.05$, Mann–Whitney test. (*E*) Summary of current-firing curves indicating that the number of evoked action potentials in response to somatic current steps was similar in pyramidal neurons from n-3 PUFA deficient ($n = 8$) and balanced mice ($n = 8$). 2-way ANOVA with "current" ($F_{8,120} = 202.4$, $P < 0.0001$) and "diet" ($F_{1,120} = 0.03538$, $P = 0.8533$) as factors, interaction: $F_{8,120} = 0.6916$, $P = 0.6983$.

Bear 1992; Mulkey and Malenka 1992; Malenka 1994). In contrast, in n-3 PUFA-deficient mice, a HFS protocol did not trigger any significant LTP (Fig. 1*C–F*). Overall, the AMPA/NMDA ratio and LTP, but not the over excitability of SC-CA1 synapses were altered in the hippocampus of n-3 PUFA-deficient mice.

Membrane Properties of CA1 Pyramidal Neurons are not Altered in the Hippocampus of n-3 PUFA-Deficient Mice

Basic intrinsic properties of CA1 pyramidal neurons were then studied to decipher the lack of synaptic plasticity in the hippocampus of n-3 PUFA-deficient mice. Independently of the diet, all recorded pyramidal neurons showed similar membrane profiles in response to a series of somatic current steps (Fig. 2*A*) with an inward rectification, shown as a shift from linearity in the $I - V$ plots (Fig. 2*B*). The resting membrane potentials (Fig. 2*C*), the minimal current necessary to evoke action potential firing (rheobase) (Fig. 2*D*), and the number of action potentials in response to somatic current steps (Fig. 2*E*) were similar in n-3 PUFA-balanced and deficient mice. Thus, basic intrinsic properties of CA1 neurons were not altered by n-3 PUFA deficiency.

Inhibition of GABA Transmission Rescues Synaptic Plasticity in the Hippocampus of n-3 PUFA-Deficient Mice

GABAergic transmission is known to control the level of synaptic excitability in pyramidal neurons and to adjust the overall plasticity threshold. First, the effect of $GABA_A$ receptors blockade by bath application of PTX (100 µM) was evaluated on basic properties of SC-CA1 synapses. Input/output relationships were similar in n-3 PUFA-balanced mice with or without bath application of PTX (Fig. 3*A*). However, SC-CA1 synaptic excitability in the hippocampus of n-3 PUFA-deficient mice was strongly enhanced after bath application of PTX (Fig. 3*B*), indicating that GABA transmission participated in the LTP impairment. HFS LTP was compared in the presence or absence of PTX to test this hypothesis. Remarkably, LTP at the SC-CA1 synapses of n-3 PUFA-deficient mice was completely restored by bath application of PTX (Fig. 3*C*,*D*). Co-application of APV and PTX totally prevented LTP induction (Fig. 3*C*,*E*), demonstrating the NMDAR-dependency of the restored synaptic plasticity in n-3 PUFA-deficient animals. Thus, LTP in the hippocampus of n-3 PUFA-deficient mice is disrupted in a GABA-dependent manner and is rescued by blockade of GABAergic transmission.

Heterosynaptic Plasticity in CA1 Region is Altered in the Hippocampus of n-3 PUFA-Deficient Mice

Restoration of LTP with PTX in the hippocampus of n-3 PUFA-deficient mice suggests that the altered NMDAR-dependent LTP of SC-CA1 synapse is due to an increase of basal inhibitory synaptic transmission, which prevents NMDAR from inducing LTP. Fast $GABA_A$R-mediated spontaneous IPSCs (sIPSCs) were then compared in the hippocampus of the 2 groups. No significant difference in the cumulative distribution plots of either amplitude and frequency of sIPSCs was found (Fig. 4*A*) suggesting that n-3 PUFA deficiency had no effect on the basal inhibitory activity.

In the hippocampus, heterosynaptic LTD of inhibitory transmission (iLTD) is mediated by eCBs produced at excitatory synapses and acting on CB_1R on GABAergic interneurons (Chevaleyre and Castillo 2003). To determine whether heterosynaptic inhibitory plasticity was altered in the hippocampus of n-3 PUFA-deficient mice, IPSCs were isolated in CA1 pyramidal neurons with APV (50 µM) and DNQX (20 µM) and we measured the effects of the HFS protocol. Heterosynaptic iLTD was readily induced in the hippocampus of n-3 PUFA-balanced mice (Fig. 4*B*,*C*) and, as expected of a eCB-mediated phenomenon, inhibited by AM251 (4 µM), a specific CB_1R antagonist. In marked contrast, the HFS protocol did not induce a significant iLTD in n-3 PUFA-deficient mice (Fig. 4*B*,*C*).

This lack of iLTD in n-3 PUFA-deficient mice could be due to an alteration of CB_1R functionality or to an alteration in eCB production. To assess CB_1R functionality, we recorded stable IPSCs and applied the CB1R agonist CP 55 940 (10 µM) in the bath. CP 55 940 significantly reduced IPSCs amplitude 30 min after bath application independently of the diet (Fig. 4*D*,*E*). To test whether the lack of iLTD was due to an alteration of eCB production, we applied JZL184 (1 µM), an inhibitor of eCBs degradation that enhances synaptic levels of 2-AG, the main hippocampal eCB (Long et al. 2009).

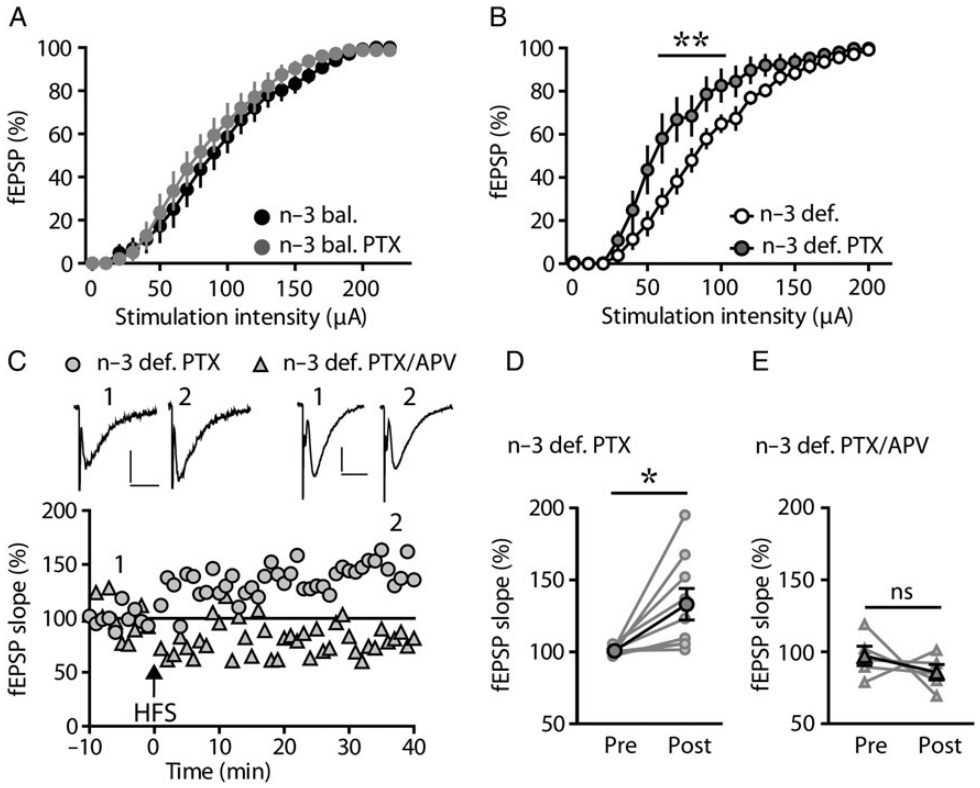

Figure 3. The lack of NMDAR-dependent LTP in n-3 PUFA-deficient mice is due to an increase in GABA inhibition. (*A* and *B*) fEPSPs were normalized with the minimum value for each recording being "0" and the maximal value being "100" (min-to-max normalization). (*A*) PTX does not change synaptic excitability in CA1 of n-3 PUFA-balanced mice. Input/output curves of evoked fEPSPs in CA1 stratum radiatum of n-3 PUFA-balanced mice without ($n = 9$, black circles) and with PTX ($n = 5$, gray circles) for increasing stimulation intensities. Two-way ANOVA with "stimulation intensity" ($F_{20,240} = 178.2$, $P < 0.0001$) and "PTX" ($F_{1,240} = 0.6338$, $P = 0.4414$) as factors, interaction: $F_{20,240} = 0.6227$, $P = 0.8942$. (*B*) PTX increased synaptic excitability in CA1 of n-3 PUFA-deficient mice. Input/output curves of evoked fEPSPs in CA1 stratum radiatum of n-3 PUFA-deficient mice with ($n = 10$, gray circles) and without PTX ($n = 6$, white circles) for increasing stimulation intensities. **$P < 0.01$, a 2-way ANOVA with "stimulation intensity" ($F_{20,294} = 132.4$, $P < 0.0001$) and "PTX" ($F_{1,294} = 61.57$, $P < 0.0001$) as factors, interaction: $F_{20,294} = 2.346$, $P = 0.0012$. Bonferroni post-test revealed a significant difference between groups for stimulation intensities of 50 pA ($t = 3.927$, $P < 0.01$), 60 pA ($t = 4.58$, $P < 0.001$), 70 pA ($t = 4.510$, $P < 0.001$), 80 pA ($t = 3.239$, $P < 0.05$), and 90 pA ($t = 3.250$, $P < 0.05$). (*C*), PTX restored NMDAR-dependent LTP in n-3 PUFA-deficient mice. Representative traces from normalized fEPSP response before (1) and 40 min after plasticity induction (2) in either treatment. Scale bar: 0.1 mV, 10 ms (left), 0.2 mV, 10 ms (right). Representative time courses of mean fEPSPs were normalized to baseline. (*D* and *E*) Normalized fEPSP slope (as a percentage of the baseline) before (Pre) and 40 min after HFS (Post); ns, $P > 0.05$, *$P < 0.05$, paired *t*-test before and 40 min after HFS for each recording. (*D*) HFS of SC induced a LTP of evoked fEPSPs recorded in CA1 stratum radiatum of n-3 PUFA-deficient mice in the presence of PTX (gray circles: before: 100 ± 1% of baseline fEPSP vs. after HFS: 133 ± 11% $n = 9$, $P = 0.0166$). (*E*) LTP is completely prevented by APV application (gray triangles: before: 97 ± 8% of baseline fEPSP vs. after HFS: 85 ± 5%, $n = 5$, $P = 0.4375$).

Bath application of JZL184 totally restored a robust iLTD in the hippocampus of n-3 PUFA-deficient mice (Fig. 4F,G). Control experiments showed that the iLTD induced in the presence of JZL184 was blocked by AM251 (Fig. 4F,G).

Enhancement of 2-AG Signaling Normalizes LTP in the Hippocampus of n-3 PUFA-Deficient Mice

Could iLTD participate in the LTP impairment observed in n-3 PUFA-deficient mice? We found that bath application of JZL184 was sufficient to fully prevent LTP impairment in the hippocampus of n-3 PUFA-deficient mice (Fig. 5A,B) and verified that this effect was abolished by AM251 (Fig. 5C,D). JZL184-induced LTP in n-3 PUFA-deficient mice was also blocked by APV, in agreement with our working hypothesis (Fig. 5C,D).

Discussion

In this study, we have shown that NMDAR-dependent LTP is altered in the hippocampus of n-3 PUFA-deficient mice and that the alteration of LTP is due to the ablation of heterosynaptic CB_1R-dependent iLTD (Fig. 6). Our results further reinforce the idea that perinatal exposure to n-3 PUFA deficiency is deleterious to CB1R-dependent plasticity and behavior (Mingam et al. 2008; Lafourcade et al. 2011; Moranis et al. 2012; Delpech et al. 2015; Lepinay et al. 2015).

In our study, loss of NMDAR-dependent LTP was not explained by changes in excitatory synaptic transmission or intrinsic membrane properties of pyramidal neurons. Previous studies suggested that the lack of LTP in the hippocampus of n-3 PUFA-deficient mice was due to decreased NMDAR subunits expression (Cao et al. 2009). In the present work, we demonstrated that the AMPA/NMDA ratio is increased in the hippocampus of n-3 PUFA-deficient mice. However, blockade of GABAergic transmission completely restored NMDAR-dependent LTP, suggesting that the increase in AMPA/NMDA ratio cannot by itself explain the lack of LTP in n-3 PUFA-deficient mice.

Changes in GABA level have been shown to affect LTP induction in the hippocampus (Vaillend and Billard 2002; Kleschevnikov et al. 2004; Fernandez et al. 2007). Moreover, DHA modulates GABAR-mediated responses in the hippocampus (Nabekura et al. 1998; Cao et al. 2009), while AA directly facilitates GABA release in

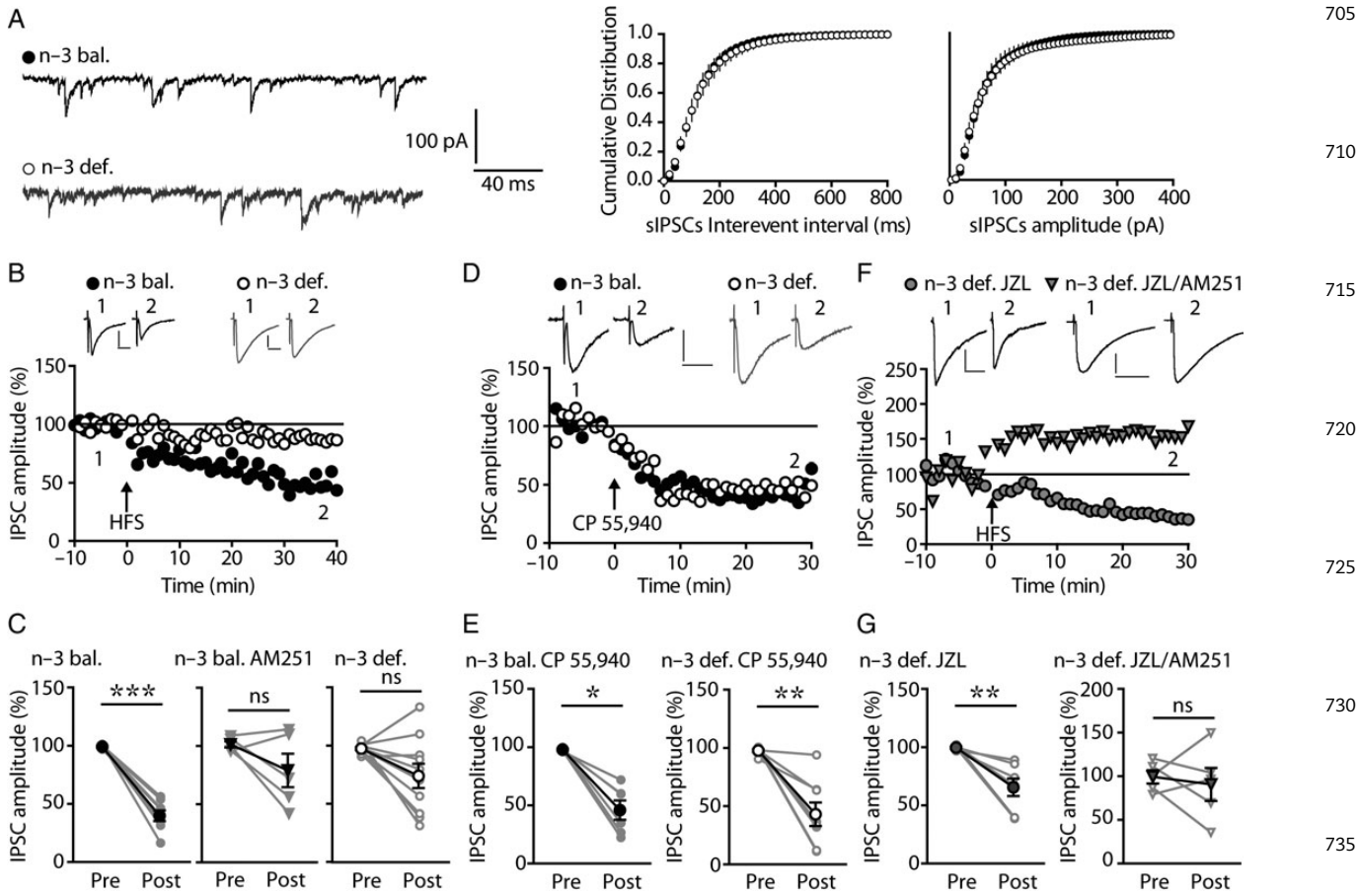

Figure 4. eCB-mediated GABA inhibition in the CA1 is altered in n-3 PUFA-deficient mice. (*A*) Left, representative trace of sIPSCs recordings in n-3 PUFA-balanced (top) and deficient (bottom) mice. Right, cumulative sIPSCs interevent interval (left) and amplitude (right) distributions showed no difference in frequency and amplitude (n-3 bal., $n = 8$, white circles; n-3 def., $n = 8$, black circles). $P > 0.05$, Kolmogorov–Smirnov tests. (*B*) iLTD was abolished in n-3 PUFA-deficient mice. Representative traces averaged from IPSC response before (1) and 40 min after plasticity induction (2) in mice from either diet. Scale bar: 100 pA, 10 ms. Representative time courses of mean IPSCs were normalized to baseline. (*C*) Summary of individual experiments showing percent change in IPSC pre and post iLTD in n-3 balanced mice (black circles: before: 99 ± 1% of baseline fEPSP vs. after HFS: 40 ± 5%, $n = 8$, $P < 0.0001$), n-3 balanced mice with AM251 (black triangle: before: 101 ± 3% of baseline fEPSP vs. after HFS: 79 ± 14%, $n = 5$, $P = 0.1827$) and n-3 deficient mice (white circles: before: 98 ± 1% of baseline fEPSP vs. after HFS: 74 ± 10%, $n = 10$, $P = 0.0504$). (*D* and *E*) Application of the cannabimimetic CP55,940 (10 µM) induced depression of evoked IPSCs recorded in CA1 pyramidal neurons of n-3 PUFA-balanced (black circles: before 98 ± 1% of baseline fEPSP vs. after CP55,940: 46 ± 8%, $n = 6$, $P = 0.0313$) and deficient mice (white circles: before: 98 ± 1% of baseline fEPSP vs. after CP55,940: 43 ± 10%, $n = 8$, $P = 0.0078$). Representative traces averaged from IPSC response before (1) and 30 min after CP55,940 application (2) in mice from either diet. Scale bar: 100 pA, 40 ms. Representative time courses of mean IPSCs were normalized to baseline. Representative time courses of mean IPSCs was normalized to baseline. (*F*) JZL184 restored iLTD in the hippocampus of n-3 PUFA-deficient mice. Representative traces averaged from IPSC response before (1) and 30 min after plasticity induction (2) in either treatment. Scale bar: 200 pA, 100 ms (left), 100 pA, 40 ms (right). Representative time courses of mean IPSCs were normalized to baseline. (*G*) HFS of SC induced a iLTD of evoked IPSCs recorded CA1 pyramidal neurons of n-3 PUFA-deficient mice after JZL184 treatment (gray circles; before: 100 ± 1% of baseline fEPSP vs. after HFS: 66 ± 9%, $n = 7$ $P = 0.0054$), which is prevented by CB_1R antagonist AM251 application (gray triangles: before: 100 ± 8% vs. after HFS: 91 ± 19%, $n = 5$, $P = 0.8125$).

hippocampal synaptosome (Cunha and Ribeiro 1999). A recent study also demonstrated that acute application of DHA was able to reduce hippocampal excitability through a GABA-dependent mechanism (Taha et al. 2013). Here, we postulated that restoration of LTP by blockade of GABAergic transmission in the hippocampus of n-3 PUFA-deficient mice occurs through the enhancement of neuronal excitability. Interestingly, basal GABAergic synaptic transmission was similar in the hippocampus of n-3 PUFA-deficient and balanced mice but pharmacological disinhibition increases synaptic excitability in CA1 of n-3 PUFA-deficient mice only. We conclude from this result that impairment of LTP may involve activity-dependent GABAergic transmission and not just basal GABAergic activity.

In the hippocampus, it is noteworthy that LTP at SC-CA1 synapses is gated by heterosynaptic iLTD in GABAergic interneurons, which is dependent on eCB production and CB_1R activation (Carlson et al. 2002; Chevaleyre and Castillo 2003, 2004; Castillo et al. 2012). Our work revealed that n-3 PUFA-deficient mice lack this form of eCB-dependent iLTD. This important result reveals that the eCB system is in a unique position to link dietary fatty acids and synaptic activity in the hippocampus.

The link between dietary LC-PUFAs and brain eCBs has already been shown since modulation of dietary LC-PUFAs during adulthood efficiently modifies levels of 2-AG and anandamide (Watanabe et al. 2003; Artmann et al. 2008). In addition, our group recently demonstrated that developmental n-3 PUFA deficiency abolishes eCB-dependent synaptic plasticity in the prefrontal cortex and in the nucleus accumbens (Lafourcade et al. 2011; Larrieu et al. 2012). Here, we demonstrated that n-3 PUFA deficiency affects the eCB system in the hippocampus, which consequently alters the gating of LTP at SC-CA1 synapses. As suggested by previous studies (Lafourcade et al. 2011; Larrieu et al.

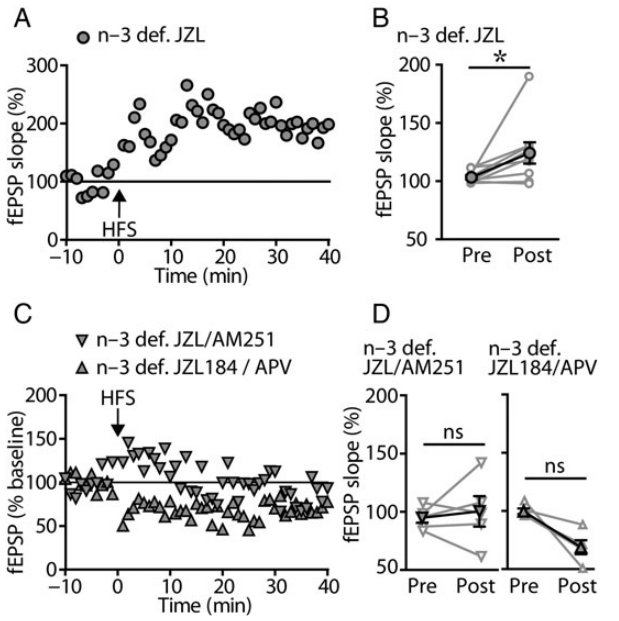

Figure 5. Enhancement of eCB signaling gates NMDAR-LTP in n-3 PUFA-deficient mice. (*A*) JZL184 restored normal LTP of fEPSPs depended on CB_1R in n-3 PUFA-deficient mice. Representative time course of mean fEPSP were normalized to baseline. (*B*) HFS of SC induced LTP of evoked fEPSPs recorded in CA1 stratum radiatum of n-3 PUFA-deficient mice in the presence of JZL184 (gray circles: before: 103 ± 2% of baseline fEPSP vs. after HFS: 124 ± 9%, $n = 9$, $P = 0.0117$). (*C*) This LTP was completely prevented by AM251, a CB_1R antagonist and by co-application of APV. Representative time courses of mean fEPSP were normalized to baseline. (*D*) HFS of SC does not induce LTP of evoked fEPSPs recorded in CA1 stratum radiatum of n-3 PUFA-deficient mice in the presence of JZL184 and AM251 (gray triangles: before: 94 ± 4% of baseline fEPSP vs. after HFS: 100 ± 13% $n = 5$, $P = 0.8125$) or by co-application of APV (gray triangles: before: 100 ± 2% of baseline fEPSP vs. after HFS: 69 ± 6%, $n = 5$, $P = 0.0625$). Error bars represent S.E.M.

2012; Moranis et al. 2012; Madore et al. 2014), the developmental n-3 PUFA dietary deficiency leads to increased AA and n-6 docosapentaenoic acid (DPA n-6) levels, and decreased DHA levels in the brain (Fig. 6). Interestingly, the present work reveals that developmental n-3 PUFA deficiency primarily affects eCB production in the hippocampus, and not CB_1R functionality, in contrast to what has been shown previously in other brain structures (Lafourcade et al. 2011; Larrieu et al. 2012). We hypothesize that developmental n-3 PUFA deficiency induces homeostatic changes that can be either CB_1R desensitization (Lafourcade et al. 2011; Larrieu et al. 2012) or decreased eCB production, as we demonstrated in the present study. It remains to determine why adaptation of the eCB system to n-3 PUFA deficiency differs depending on the brain structure.

To definitively prove that LTP in n-3 PUFA-deficient mice was altered by a deficit of eCB on-demand production, we enhanced 2-AG signaling with JZL184, an inhibitor of monoacylglycerol lipase. Several studies revealed that inhibition of this enzyme facilitates eCB-LTD (Marrs et al. 2010) and rescues eCB-LTD in mouse models of Fragile X Syndrome (Jung et al. 2012) and Down syndrome (Thomazeau et al. 2014). Here, we observed that iLTD was restored by JZL184 and remarkably, LTP was also completely restored by JZL184. Thus, enhancement of eCB signaling was able to rescue the lack of LTP linked to diet through a normalization of heterosynaptic plasticity (Fig. 6).

In conclusion, we have demonstrated that the loss of LTP in the hippocampus of n-3 PUFA-deficient mice is due to an alteration of eCB-mediated heterosynaptic iLTD. This result reveals that disinhibition mediated by eCB is thwarted by n-3 PUFA deficiency leading to impairment of synaptic plasticity. The mechanism we deciphered could explain cognitive and memory impairment that are observed in n-3 PUFA-deficient animals (Catalan et al. 2002; Moranis et al. 2012). It remains to determine whether these developmental alterations can be restored by n-3 PUFA supplementation during adulthood. Our results highlight the importance of

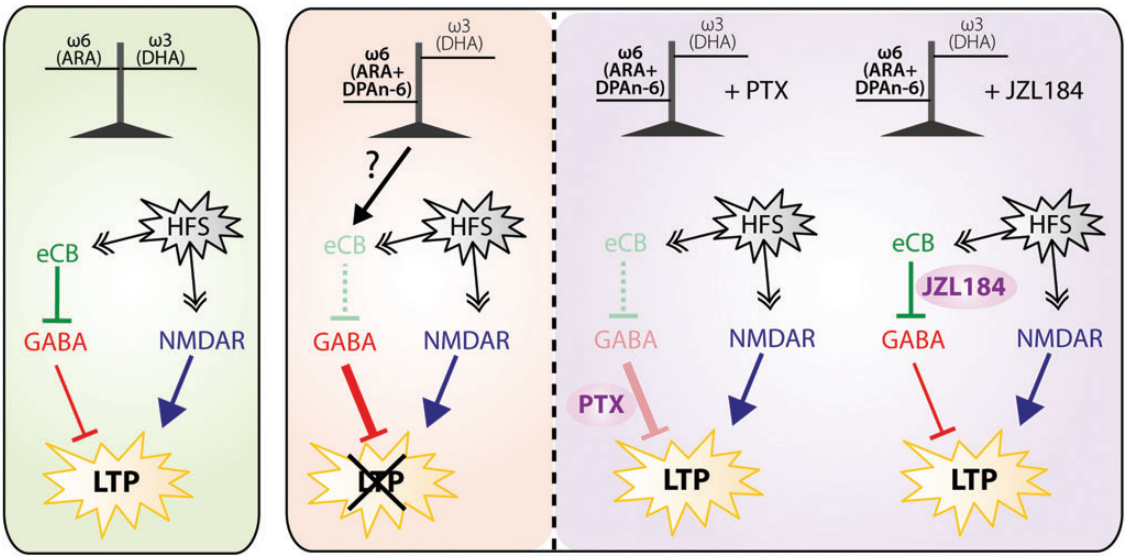

Figure 6. Scheme summarizing the putative mechanism involved in LTP alteration in the hippocampus of n-3 PUFA-deficient mice. Left: In n-3 PUFA-balanced mice, glutamate released during HFS activates metabotropic receptor and the release of 2-AG, which in turn stimulates presynaptic inhibitory CB_1R located on GABAergic interneurons (Chevaleyre and Castillo 2003). Subsequent inhibition of GABA release disinhibits the excitatory network and facilitates LTP. Right: Developmental n-3 PUFA dietary deficiency leads to increased AA and n-6 DPA n-6, and decreased DHA levels in the brain (Lafourcade et al. 2011; Larrieu et al. 2012; Moranis et al. 2012; Madore et al. 2014). In n-3 PUFA-deficient mice, the disinhibitory eCB gating is absent and LTP strongly impaired. PTX, an inhibitor of $GABA_A$ receptors or JZL184, an inhibitor of MAGL restore LTP in n-3 PUFA-deficient mice.

balanced diet during perinatal periods of brain development. Moreover, this work reveals that eCB could serve as a novel therapeutic target for treatment of cognitive disorders linked to dietary n-3 PUFA imbalance.

Author Contributions

A.T., C.B.B., O.J.M., and S.L. designed research; A.T. and C.B.B. performed research; A.T., C.B.B. analyzed the data; A.T., C.B.B., O.J.M., and S.L. wrote the manuscript; O.J.M. and S.L. supervised the entire project.

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Notes

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References

Artmann A, Petersen G, Hellgren LI, Boberg J, Skonberg C, Nellemann C, Hansen SH, Hansen HS. 2008. Influence of dietary fatty acids on endocannabinoid and N-acylethanolamine levels in rat brain, liver and small intestine. Biochim Biophys Acta. 1781:200–212.

Bashir ZI, Alford S, Davies SN, Randall AD, Collingridge GL. 1991. Long-term potentiation of NMDA receptor-mediated synaptic transmission in the hippocampus. Nature. 349:156–158.

Bazinet RP, Layé S. 2014. Polyunsaturated fatty acids and their metabolites in brain function and disease. Nat Rev Neurosci. 15:771–785.

Bliss TV, Collingridge GL. 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature. 361:31–39.

Bourre JM. 2005. Where to find omega-3 fatty acids and how feeding animals with diet enriched in omega-3 fatty acids to increase nutritional value of derived products for human: what is actually useful? J Nutr Health Aging. 9:232–242.

Burgess N, Maguire EA, O'Keefe J. 2002. The human hippocampus and spatial and episodic memory. Neuron. 35:625–641.

Calderon F, Kim HY. 2004. Docosahexaenoic acid promotes neurite growth in hippocampal neurons. J Neurochem. 90:979–988.

Cao D, Kevala K, Kim J, Moon HS, Jun SB, Lovinger D, Kim HY. 2009. Docosahexaenoic acid promotes hippocampal neuronal development and synaptic function. J Neurochem. 111:510–521.

Carlson G, Wang Y, Alger BE. 2002. Endocannabinoids facilitate the induction of LTP in the hippocampus. Nat Neurosci. 5:723–724.

Castillo PE, Younts TJ, Chavez AE, Hashimotodani Y. 2012. Endocannabinoid signaling and synaptic function. Neuron. 76:70–81.

Catalan J, Moriguchi T, Slotnick B, Murthy M, Greiner RS, Salem N Jr. 2002. Cognitive deficits in docosahexaenoic acid-deficient rats. Behav Neurosci. 116:1022–1031.

Chevaleyre V, Castillo PE. 2003. Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. Neuron. 38:461–472.

Chevaleyre V, Castillo PE. 2004. Endocannabinoid-mediated metaplasticity in the hippocampus. Neuron. 43:871–881.

Cunha RA, Ribeiro JA. 1999. Facilitation of GABA release by arachidonic acid in rat hippocampal synaptosomes. Eur J Neurosci. 11:2171–2174.

Delpech JC, Thomazeau A, Madore C, Bosch-Bouju C, Larrieu T, Lacabanne C, Remus-Borel J, Aubert A, Joffre C, Nadjar A, et al. 2015. Dietary n-3 PUFAs Deficiency Increases Vulnerability to Inflammation-Induced Spatial Memory Impairment. Neuropsychopharmacology. 40:2774–2787.

Dudek SM, Bear MF. 1992. Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. Proc Natl Acad Sci U S A. 89:4363–4367.

Fernandez F, Morishita W, Zuniga E, Nguyen J, Blank M, Malenka RC, Garner CC. 2007. Pharmacotherapy for cognitive impairment in a mouse model of Down syndrome. Nat Neurosci. 10:411–413.

Gibson RA, Muhlhausler B, Makrides M. 2011. Conversion of linoleic acid and alpha-linolenic acid to long-chain polyunsaturated fatty acids (LCPUFAs), with a focus on pregnancy, lactation and the first 2 years of life. Matern Child Nutr. 7(Suppl 2):17–26.

Harris EW, Ganong AH, Cotman CW. 1984. Long-term potentiation in the hippocampus involves activation of N-methyl-D-aspartate receptors. Brain Res. 323:132–137.

Heifets BD, Castillo PE. 2009. Endocannabinoid signaling and long-term synaptic plasticity. Annu Rev Physiol. 71:283–306.

Hill MN, Gorzalka BB. 2009. The endocannabinoid system and the treatment of mood and anxiety disorders. CNS Neurol Disord Drug Targets. 8:451–458.

Innis SM. 2008. Dietary omega 3 fatty acids and the developing brain. Brain Res. 1237:35–43.

Jarrard LE. 1993. On the role of the hippocampus in learning and memory in the rat. Behav Neural Biol. 60:9–26.

Joffre C, Nadjar A, Lebbadi M, Calon F, Laye S. 2014. n-3 LCPUFA improves cognition: the young, the old and the sick. Prostaglandins Leukot Essent Fatty Acids. 91:1–20.

Jung KM, Sepers M, Henstridge CM, Lassalle O, Neuhofer D, Martin H, Ginger M, Frick A, DiPatrizio NV, Mackie K, et al. 2012. Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. Nat Commun. 3:1080.

Kasanetz F, Manzoni OJ. 2009. Maturation of excitatory synaptic transmission of the rat nucleus accumbens from juvenile to adult. J Neurophysiol. 101:2516–2527.

Kelly L, Grehan B, Chiesa AD, O'Mara SM, Downer E, Sahyoun G, Massey KA, Nicolaou A, Lynch MA. 2011. The polyunsaturated fatty acids, EPA and DPA exert a protective effect in the hippocampus of the aged rat. Neurobiol Aging. 32:2318 e2311–2315.

Kleschevnikov AM, Belichenko PV, Villar AJ, Epstein CJ, Malenka RC, Mobley WC. 2004. Hippocampal long-term potentiation suppressed by increased inhibition in the Ts65Dn mouse, a genetic model of Down syndrome. J Neurosci. 4:8153–8160.

Labrousse VF, Nadjar A, Joffre C, Costes L, Aubert A, Gregoire S, Bretillon L, Laye S. 2012. Short-term long chain omega3 diet protects from neuroinflammatory processes and memory impairment in aged mice. PLoS One. 7:e36861.

Lafourcade M, Larrieu T, Mato S, Duffaud A, Sepers M, Matias I, De Smedt-Peyrusse V, Labrousse VF, Bretillon L, Matute C, et al. 2011. Nutritional omega-3 deficiency abolishes endocannabinoid-mediated neuronal functions. Nat Neurosci. 14:345–350.

Larrieu T, Madore C, Joffre C, Laye S. 2012. Nutritional n-3 polyunsaturated fatty acids deficiency alters cannabinoid receptor signaling pathway in the brain and associated anxiety-like behavior in mice. J Physiol Biochem. 68:671–681.

Lauritzen L, Hansen HS, Jorgensen MH, Michaelsen KF. 2001. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res. 40:1–94.

Lepinay AL, Larrieu T, Joffre C, Acar N, Garate I, Castanon N, Ferreira G, Langelier B, Guesnet P, Bretillon L, et al. 2015. Perinatal high-fat diet increases hippocampal vulnerability to the adverse effects of subsequent high-fat feeding. Psychoneuroendocrinology. 53:82–93.

Leweke FM, Koethe D. 2008. Cannabis and psychiatric disorders: it is not only addiction. Addict Biol. 13:264–275.

Long JZ, Nomura DK, Vann RE, Walentiny DM, Booker L, Jin X, Burston JJ, Sim-Selley LJ, Lichtman AH, Wiley JL, et al. 2009. Dual blockade of FAAH and MAGL identifies behavioral processes regulated by endocannabinoid crosstalk in vivo. Proc Natl Acad Sci U S A. 106:20270–20275.

Lutz B. 2009. From molecular neurodevelopment to psychiatry: new insights in mechanisms underlying Cannabis-induced psychosis and schizophrenia. Eur Arch Psychiatry Clin Neurosci. 259:369–370.

Lynch MA. 2004. Long-term potentiation and memory. Physiol Rev. 84:87–136.

Lynch AM, Loane DJ, Minogue AM, Clarke RM, Kilroy D, Nally RE, Roche OJ, O'Connell F, Lynch MA. 2007. Eicosapentaenoic acid confers neuroprotection in the amyloid-beta challenged aged hippocampus. Neurobiol Aging. 28:845–855.

Madore C, Nadjar A, Delpech JC, Sere A, Aubert A, Portal C, Joffre C, Laye S. 2014. Nutritional n-3 PUFAs deficiency during perinatal periods alters brain innate immune system and neuronal plasticity-associated genes. Brain Behav Immun. 41:22–31.

Malenka RC. 1994. Synaptic plasticity in the hippocampus: LTP and LTD. Cell. 78:535–538.

Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, Bodor AL, Muccioli GG, Hu SS, Woodruff G, et al. 2010. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. Nat Neurosci. 13:951–957.

Mayford M, Siegelbaum SA, Kandel ER. 2012. Synapses and memory storage. Cold Spring Harbor Perspectives in Biology. 4.

Mingam R, Moranis A, Bluthe RM, De Smedt-Peyrusse V, Kelley KW, Guesnet P, Lavialle M, Dantzer R, Laye S. 2008. Uncoupling of interleukin-6 from its signalling pathway by dietary n-3-polyunsaturated fatty acid deprivation alters sickness behaviour in mice. Eur J Neurosci. 28:1877–1886.

Moranis A, Delpech JC, De Smedt-Peyrusse V, Aubert A, Guesnet P, Lavialle M, Joffre C, Laye S. 2012. Long term adequate n-3 polyunsaturated fatty acid diet protects from depressive-like behavior but not from working memory disruption and brain cytokine expression in aged mice. Brain Behav Immun. 26:721–731.

Moriguchi T, Greiner RS, Salem N Jr. 2000. Behavioral deficits associated with dietary induction of decreased brain docosahexaenoic acid concentration. J Neurochem. 75:2563–2573.

Morris RG, Anderson E, Lynch GS, Baudry M. 1986. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature. 319:774–776.

Mulkey RM, Malenka RC. 1992. Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron. 9:967–975.

Nabekura J, Noguchi K, Witt MR, Nielsen M, Akaike N. 1998. Functional modulation of human recombinant gamma-aminobutyric acid type A receptor by docosahexaenoic acid. J Biol Chem. 273:11056–11061.

Neves G, Cooke SF, Bliss TV. 2008. Synaptic plasticity, memory and the hippocampus: a neural network approach to causality. Nat Rev Neurosci. 9:65–75.

Patten AR, Brocardo PS, Christie BR. 2013. Omega-3 supplementation can restore glutathione levels and prevent oxidative damage caused by prenatal ethanol exposure. J Nutr Biochem. 24:760–769.

Scoville WB, Milner B. 1957. Loss of recent memory after bilateral hippocampal lesions. J Neurol Neurosurg Psychiatry. 20:11–21.

Squire LR, Stark CE, Clark RE. 2004. The medial temporal lobe. Annu Rev Neurosci. 27:279–306.

Taha AY, Zahid T, Epps T, Trepanier MO, Burnham WM, Bazinet RP, Zhang L. 2013. Selective reduction of excitatory hippocampal sharp waves by docosahexaenoic acid and its methyl ester analog ex-vivo. Brain Res. 1537:9–17.

Thomazeau A, Lassalle O, Iafrati J, Souchet B, Guedj F, Janel N, Chavis P, Delabar J, Manzoni OJ. 2014. Prefrontal deficits in a murine model overexpressing the Down syndrome candidate gene dyrk1a. J Neurosci. 34:1138–1147.

Vaillend C, Billard JM. 2002. Facilitated CA1 hippocampal synaptic plasticity in dystrophin-deficient mice: role for GABAA receptors? Hippocampus. 12:713–717.

Watanabe S, Doshi M, Hamazaki T. 2003. n-3 Polyunsaturated fatty acid (PUFA) deficiency elevates and n-3 PUFA enrichment reduces brain 2-arachidonoylglycerol level in mice. Prostaglandins Leukot Essent Fatty Acids. 69:51–59.