

Amplification of mGlu₅-Endocannabinoid Signaling Rescues Behavioral and Synaptic Deficits in a Mouse Model of Adolescent and Adult Dietary Polyunsaturated Fatty Acid Imbalance

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Energy-dense, yet nutritionally poor food is a high-risk factor for mental health disorders. This is of particular concern during adolescence, a period often associated with increased consumption of low nutritional content food and higher prevalence of mental health disorders. Indeed, there is an urgent need to understand the mechanisms linking unhealthy diet and mental disorders. Deficiency in n-3 polyunsaturated fatty acid (PUFAs) is a hallmark of poor nutrition and mood disorders. Here, we developed a mouse model of n-3 PUFA deficiency lasting from adolescence into adulthood. Starting nutritional deficits in dietary n-3 PUFAs during adolescence decreased n-3 PUFAs in both mPFC and nucleus accumbens, increased anxiety-like behavior, and decreased cognitive function in adulthood. Importantly, we discovered that endocannabinoid/mGlu₅-mediated LTD in the mPFC and accumbens was abolished in adult n-3-deficient mice. Additionally, mPFC NMDAR-dependent LTP was also lacking in the n-3-deficient group. Pharmacological enhancement of the mGlu₅/eCB signaling complex, by positive allosteric modulation of mGlu₅ or inhibition of endocannabinoid 2-arachidonylethanolamide degradation, fully restored synaptic plasticity and normalized emotional and cognitive behaviors in malnourished adult mice. Our data support a model where nutrition is a key environmental factor influencing the working synaptic range into adulthood, long after the end of the perinatal period. These findings have important implications for the identification of nutritional risk factors for disease and design of new treatments for the behavioral deficits associated with nutritional n-3 PUFA deficiency.

Key words: accumbens; endocannabinoid; LTD; LTP; mGluR5; prefrontal cortex

Significance Statement

In a mouse model mimicking n-3 PUFA dietary deficiency during adolescence and adulthood, we found strong increases in anxiety and anhedonia which lead to decreases in specific cognitive functions in adulthood. We found that endocannabinoid/mGlu₅-mediated LTD and NMDAR-dependent LTP were lacking in adult n-3-deficient mice. Acute positive allosteric modulation of mGlu₅ or inhibition of endocannabinoid degradation normalized behaviors and synaptic functions in n-3 PUFA-deficient adult mice. These findings have important implications for the identification of nutritional risk for disease and the design of new treatments for the behavioral deficits associated with nutritional n-3 PUFAs' imbalance.

Introduction

Poverty and food insecurity are associated with lower food expenditures, low fruit and vegetable consumption, and overall

lower-quality diets. Such diets are more affordable than are prudent diets based on lean meats, fish, fresh vegetables, and fruit. The dietary status of adults in Western countries is a serious rising

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public health concern. This is particularly preoccupying in the low-income segment of the population that is widely exposed to low-nutritional content food containing insufficient quantities of long-chain ω -3 polyunsaturated fatty acids (n-3 PUFAs).

Perinatal and adolescent nutritional n-3 PUFA deficiency is a major environmental risk factor associated with neuropsychiatric disorders, including major depression, bipolar disorder, and schizophrenia (Simopoulos, 2009; Bazinet and Layé, 2014; Bosch-Bouju et al., 2016). ω -3 deficiencies during adolescence may interfere with normal late development and increase the vulnerability to behavioral deficits during adulthood (Drewnowski, 2004). For example, in adolescents, multigenerational dietary n-3 PUFA deficiency causes neurobehavioral deficits that are distinct from those developed in adulthood (Bondi et al., 2014). Thus, n-3 PUFA deficiency during early adolescence may modify the normal developmental trajectory and cause alterations emerging in adulthood. However, the behavioral and *a fortiori* synaptic consequences of dietary n-3 PUFA deficiency in adults resulting from a PUFA-imbalanced diet during adolescence have seldom been studied (Lalancette-Hébert et al., 2011; Joffe et al., 2014; Delpech et al., 2015).

Developing brains rely solely on, and thus are exquisitely sensitive to, maternal PUFA levels (Bazinet and Layé, 2014). Following lifelong dietary imbalance, our previous work revealed alterations of the endocannabinoid (eCB) system that were paralleled by heightened expression of anxiety and depression-like behaviors in mice (Lafourcade et al., 2011; Larrieu et al., 2012, 2014; Thomazeau et al., 2017) and highlighted the involvement of the nucleus accumbens and the mPFC. However, no data are available as to whether the observed emotional deficits are principally related to the effects of n-3 PUFA deficiency during perinatal and/or postnatal periods. This is an important question because nutritional independence from parental supervision often starts at adolescence, a period of ongoing structural and functional refinements of the frontostriatal circuitry implicated in behavioral control (Galvan et al., 2006) and higher cognitive functions (Caballero and Tseng, 2016; Caballero et al., 2016). As a consequence, the full understanding of the pathophysiological underpinnings of dietary n-3 deficiency-induced behavioral and synaptic alterations requires more specific distinction between these two temporal stages.

To clarify the role of nutritional PUFAs in the refinement of adult synaptic circuitry and behavior, we developed a model of

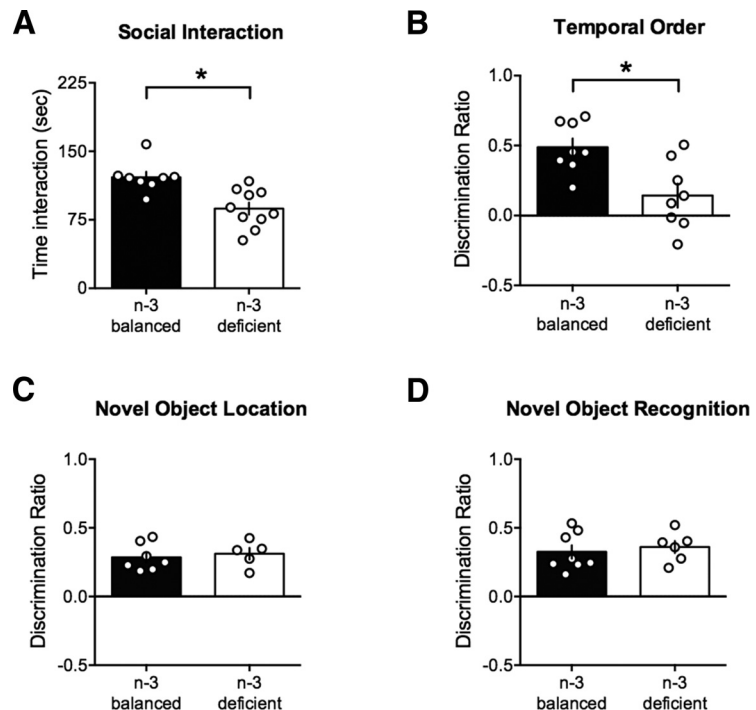


Figure 1. Nutritional n-3 deficiency starting in adolescence reduces sociability and temporal order memory at adulthood. **A**, Social interaction test: n-3 PUFA-deficient group mice spent less time to interact with a congener (n-3-balanced: 121.60 ± 5.94 , $n = 8$; n-3-deficient: 87.34 ± 6.59 , $n = 10$; $t_{(16)} = 3.77$, $p < 0.01$, t test) and had less contact with their congeners than mice fed with an n-3 diet (51.88 ± 2.40 , $n = 8$; and 29.50 ± 1.68 , $n = 10$; $t_{(16)} = 7.85$, $p < 0.01$, t test; n-3-balanced and n-3-deficient mice, respectively; data not shown). **B**, Temporal order: there was a marked reduction of discrimination in the temporal order memory task in the n-3-deficient group (n-3-balanced: 0.49 ± 0.06 , $n = 8$; n-3-deficient: 0.14 ± 0.08 , $n = 8$; $t_{(14)} = 3.25$; $p < 0.05$, t test). **C**, Novel object location test: Discrimination between the object that had changed position, and the object that had remained in a constant position was similar in n-3-balanced and n-3 PUFA-deficient groups (n-3-balanced: 0.29 ± 0.04 , $n = 7$; n-3-deficient: 0.31 ± 0.04 , $n = 5$; $t_{(10)} = 0.47$, $p > 0.05$, t test). **D**, Novel object recognition test: n-3 PUFAs deficient adult mice discriminated similarly between the novel and familiar objects after 5 min delay (n-3-balanced: 0.33 ± 0.05 , $n = 8$; n-3-deficient: 0.36 ± 0.04 , $n = 6$; $t_{(12)} = 0.52$, $p > 0.05$, t test). Furthermore, n-3-deficient adult mice were also able to discriminate between novel and familiar objects after 2 h delay (0.253 ± 0.167 , $n = 10$; and 0.273 ± 0.196 , $n = 9$; $t_{(17)} = 0.82$, $p > 0.05$, t test; n-3-balanced and n-3-deficient mice, respectively, data not shown). Scatter dot plot represents each animal. Error bars indicate SEM. $*p < 0.05$.

n-3 PUFA deficiency starting at adolescence. To this end, mice that had received balanced food until this point in life were exposed to an n-3 PUFA-deficient diet from early adolescence through adulthood.

We discovered severe alterations of the emotional and cognitive repertoire in adult mice exposed to n-3 PUFA deficiency from early adolescence that were paralleled by specific deficits in mGlu₅/eCB synaptic plasticity of the mPFC and the accumbens. We found that pharmacological enhancement of the mGlu₅/eCB-signaling macromolecular complex similarly normalized behavioral and synaptic deficits.

Materials and Methods

Animals

Animals were treated in compliance with the European Communities Council Directive (86/609/EEC) and the United States National Institutes of Health *Guide for the care and use of laboratory animals*. All mice arrived at P21 and were group-housed and acclimated to laboratory conditions before experiments with 12 h light/dark cycles and *ad libitum* access to food and water. All behavioral and synaptic plasticity experiments were performed on male C57BL/6J mice between P90 and P120.

Diets. Male C57BL/6J mice (Janvier) were fed standard laboratory chow through 1 week after weaning; at P28, male C57BL/6J mice were divided into two groups that received a diet containing 6% of mixed oils containing rapeseed oil (rich in α -linolenic acid, 18:3n-3; “n-3”) or 6%

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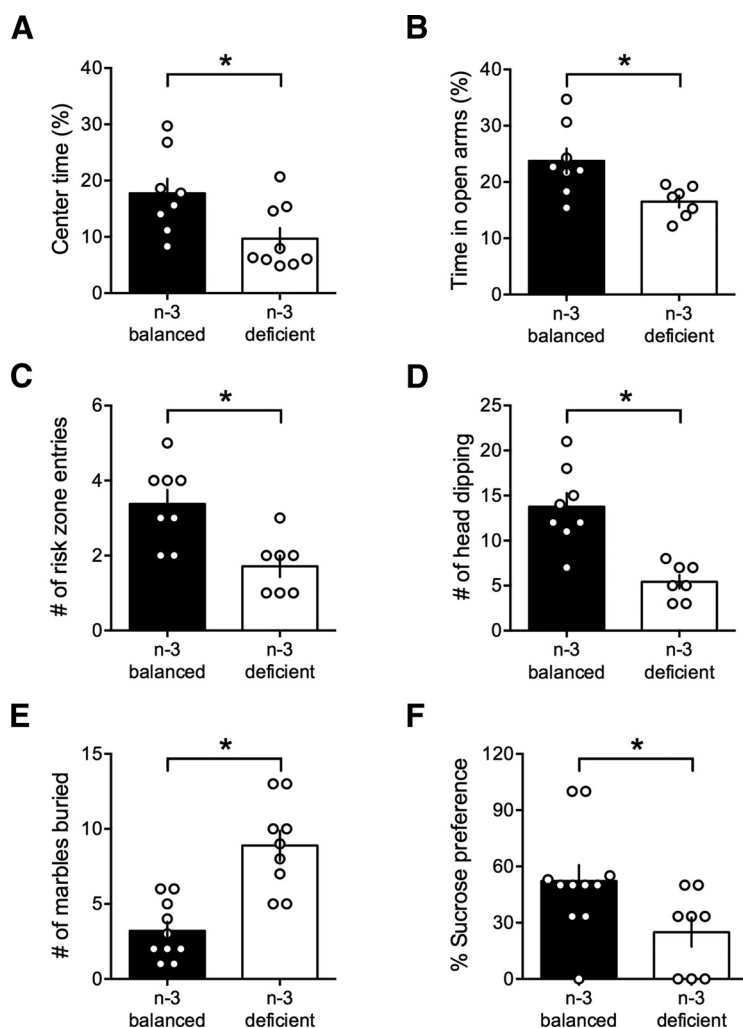


Figure 2. Nutritional n-3 deficiency starting at adolescence blunts emotional behaviors. **A**, Open field test: there was a marked reduction of the time spent in the center of the open field arena in the n-3 PUFA-deficient group that indicated increased anxiety (n-3-balanced: 17.76 ± 2.59 , $n = 8$; n-3-deficient: 9.67 ± 1.91 , $n = 9$; $t_{(15)} = 2.51$, $p < 0.05$, t test). We controlled that the increased anxiety-like behavior was not linked to impaired total motor locomotion, and found no significant change in the distance traveled between n-3-balanced and n-3-deficient mice (2780 ± 117.8 cm, $n = 8$; and 2779 ± 228.8 cm, $n = 9$; $t_{(15)} = 0.02$, $p > 0.05$, t test; n-3-balanced and n-3-deficient mice, respectively). **B–D**, n-3 deficiency augmented all traits of anxiety in the elevated plus maze test. There was a marked reduction of the percentage of time spent in the open arm (**B**; n-3-balanced: 23.74 ± 2.32 , $n = 8$; n-3-deficient: 16.53 ± 1.04 , $n = 7$; $t_{(13)} = 2.80$, $p < 0.05$, t test), the frequency of entries in the risk zone in the open arms (**C**; n-3-balanced: 3.38 ± 0.38 , $n = 8$; n-3-deficient: 1.71 ± 0.29 , $n = 7$; $t_{(13)} = 3.44$, $p < 0.01$, t test) and the frequency of head dipping (**D**; n-3-balanced: 13.75 ± 1.53 , $n = 8$; n-3-deficient: 5.43 ± 0.75 , $n = 7$; $t_{(13)} = 4.65$, $p < 0.001$, t test). **E**, Dietary n-3 PUFA deprivation in adulthood increased anxiety-like behavior: in the marble burying test, the n-3-deficient group buried twice as many marbles in bedding than the n-3-balanced mice (n-3-balanced: 3.20 ± 0.61 , $n = 10$; n-3-deficient: 8.89 ± 0.99 , $n = 9$; $t_{(17)} = 4.99$, $p < 0.001$, t test). **F**, The marked reduction in sucrose preference of the n-3-deficient group is compatible with increased anhedonia in these mice (n-3-balanced: 52.24 ± 8.55 , $n = 11$; n-3-deficient: 25.00 ± 7.72 , $n = 8$; $t_{(17)} = 2.27$, $p < 0.05$, t test). Basal fluid intake was similar in both groups (during the 2 h test period: 5.55 ± 0.72 ml, $n = 11$; and 5.63 ± 0.53 ml, $n = 8$; $t_{(17)} = 0.08$, $p > 0.05$, t test; n-3-balanced and n-3-deficient mice, respectively; data not shown). Scatter dot plot represents each animal. Error bars indicate SEM. * $p < 0.05$.

fat in the form of sunflower oil (rich in linolenic acid, 18:2n-6; “n-3-deficient”) (Lafourcade et al., 2011; Madore et al., 2014; Mingam et al., 2008). Diets were isocaloric and differed only in the oil composition. Pellets were prepared by UPAE-Institut national de la recherche agronomique 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; Moranis et al., 2012; Madore et al., 2014).

Body weight and food intake. To evaluate the possible effects of the different diets on body weight, mice fed with n-3 and n-3 PUFA-deficient diets were weighed weekly from P28 until commencement of the exper-

iments at P90. Daily food intake was also calculated as average daily food consumption in grams per day for each week.

Fatty acid analysis. Fatty acid compositions were analyzed in PFC and nucleus accumbens as previously described and expressed as the percentage of total fatty acids (Labrousse et al., 2012; Madore et al., 2014; Delpech et al., 2015; Joffe et al., 2016). Briefly, total lipids were extracted according to the method developed by Folch et al. (1957) and were submitted to fatty acid methylation using 7% boron trifluoride in methanol according to Morrison and Smith (1964). Fatty acid methyl esters were analyzed using gas chromatography as described by Joffe et al. (2016). They were identified by comparison with commercial standards. The proportion of each fatty acid was expressed as a percentage of total fatty acids.

Behavior

All mice were male, experimentally naïve, and used only once.

Social interaction. The test was performed as previously published (Lafourcade et al., 2011; Larrieu et al., 2012). Mice were transferred to the experimental arena (40 × 40 cm). A social exploration session comprised 5 min without target where the arena contained an empty wire mesh cage in the corner followed by 5 min exposure of an unfamiliar adult CD-1 male enclosed in the wire mesh cage placed in the corner of the arena. The frequency of active investigatory behavior (mainly sniffing the anogenital region, mouth, ears, trunk, and tail) was manually counted by an experimenter blind to the conditions. A video tracking system (Ethovision XT, Noldus Information Technology) was used to measure the time spent in the interaction zone surrounding the target box (Lafourcade et al., 2011; Larrieu et al., 2012).

Temporal order. Mice were habituated to the experimental arena (40 × 40 cm) without objects for 10 min daily for 2 d before testing. This task consisted of two sample phases and one test trial (Barker et al., 2007). In each sample phase, mice were allowed to explore two copies of an identical object for a total of 4 min. Different objects were used for sample Phases 1 and 2, with a delay between the sample phases of 1 h. After 3 h from sample Phase 2, mice performed the test trial (4 min duration) where a third copy of the objects from sample Phase 1 and a third copy of the objects from sample Phase 2 were used. The positions of the objects in the test and the objects used in sample Phase 1 and sample Phase 2 were counterbalanced between the mice. An intact temporal order memory requested the subjects to spend more

time exploring the object from Sample 1 (i.e., the object presented less recently) compared with the object from Sample 2 (i.e., the “new” object). The discrimination ratio was calculated as the difference in time spent by each animal exploring the object from sample Phase 1 compared with the object from sample Phase 2 divided by the total time spent exploring both objects in the test phase.

Novel object location. In this test, mice ability to recognize that a previously known object had changed location was assessed (Barker et al., 2007). After 2 consecutive days of 10 min habituation/d to the experimental arena (40 × 40 cm) without objects, mice were exposed to objects

Table 1. Prefrontal cortex fatty acid composition in adult C57Bl6/J mice fed n-3-balanced (n-3) or deficient (n-3-deficient) diet expressed as % of total fatty acid (n = 4 per group)

| Diet fatty acids | n-3 | n-3-deficient | Statistical effect |
|------------------|--------------|---------------|--------------------|
| 14:0 | 0.2 ± 0.01 | 0.2 ± 0.01 | NS |
| 15:0 | 0.05 ± 0.002 | 0.06 ± 0.005 | NS |
| 16:0 | 22.5 ± 0.37 | 22.6 ± 0.61 | NS |
| 17:0 | 0.1 ± 0.01 | 0.1 ± 0.01 | NS |
| 18:0 | 22.1 ± 0.33 | 22.1 ± 0.42 | NS |
| 20:0 | 0.2 ± 0.01 | 0.2 ± 0.02 | NS |
| 22:0 | 0.2 ± 0.02 | 0.2 ± 0.04 | NS |
| 24:0 | 0.2 ± 0.03 | 0.3 ± 0.06 | NS |
| Saturated | 45.6 ± 0.10 | 45.7 ± 0.49 | NS |
| 16:1n-9 | 0.2 ± 0.01 | 0.2 ± 0.01 | NS |
| 16:1n-7 | 0.6 ± 0.02 | 0.6 ± 0.03 | NS |
| 17:1n-9 | 0.04 ± 0.016 | 0.03 ± 0.005 | NS |
| 18:1trans | 0.06 ± 0.009 | 0.06 ± 0.006 | NS |
| 18:1n-9 | 11.9 ± 0.20 | 11.8 ± 0.33 | NS |
| 18:1n-7 | 3.1 ± 0.04 | 3.2 ± 0.02 | NS |
| 20:1n-9 | 0.6 ± 0.03 | 0.6 ± 0.09 | NS |
| 20:1n-7 | 0.14 ± 0.009 | 0.18 ± 0.019 | NS |
| 22:1n-9 | 0.06 ± 0.005 | 0.06 ± 0.012 | NS |
| 24:1n-9 | 0.4 ± 0.07 | 0.6 ± 0.01 | NS |
| Monounsaturated | 17.1 ± 0.28 | 17.4 ± 0.57 | NS |
| 18:2n-6 | 0.3 ± 0.01 | 0.5 ± 0.02 | <0.0001 |
| 20:2n-6 | 0.1 ± 0.01 | 0.1 ± 0.01 | NS |
| 20:3n-6 | 0.3 ± 0.01 | 0.3 ± 0.01 | <0.0001 |
| 20:4n-6 | 9.8 ± 0.15 | 10.1 ± 0.19 | NS |
| 22:4n-6 | 2.1 ± 0.08 | 2.4 ± 0.05 | <0.01 |
| 22:5n-6 | 0.6 ± 0.02 | 2.6 ± 0.21 | <0.0001 |
| n-6 | 13.2 ± 0.17 | 16.0 ± 0.29 | <0.0001 |
| 22:5n-3 | 0.2 ± 0.01 | 0.1 ± 0.01 | <0.01 |
| 22:6n-3 | 17.4 ± 0.09 | 14.0 ± 0.42 | <0.0001 |
| n-3 | 17.6 ± 0.09 | 14.1 ± 0.42 | <0.0001 |
| 20:3n-9 | 0.1 ± 0.00 | 0.1 ± 0.01 | <0.001 |
| Polysaturated | 30.9 ± 0.21 | 30.2 ± 0.60 | NS |
| DMA16:0 | 1.7 ± 0.05 | 1.7 ± 0.10 | NS |
| DMA18:0 | 3.3 ± 0.01 | 3.2 ± 0.08 | NS |
| DMA18:1n-9 | 0.7 ± 0.03 | 0.8 ± 0.10 | NS |
| DMA18:1n-7 | 0.7 ± 0.03 | 0.9 ± 0.10 | NS |
| DMA | 6.4 ± 0.07 | 6.6 ± 0.36 | NS |
| n-6/n-3 | 0.7 ± 0.01 | 1.1 ± 0.03 | <0.0001 |
| AA/DHA | 0.6 ± 0.01 | 0.7 ± 0.01 | <0.0001 |
| u.i. | 174.8 ± 0.77 | 167.7 ± 2.71 | <0.05 |

DMA, Dimethylacetal; AA, arachidonic acid, 20:4 n-6; DHA, docosahexaenoic acid, 22:6; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; LA, linoleic acid, 18:2 n-6; n-6 DPA, docosapentaenoic acid, 22:5 n-6; n-3 DPA, docosapentaenoic acid, 22:5 n-3. Adolescence to adulthood n-3 deprivation modifies mPFC fatty acid composition. Fatty acid composition in mice fed with n-3-balanced or n-3-deficient diet from PND 28 to PND 90. Results are expressed as percentage of total fatty acids (n = 4).

Table 2. Nucleus accumbens fatty acid composition in adult C57Bl6/J mice fed n-3-balanced or n-3-deficient diet expressed as percentage of total fatty acid (n = 4 per group)

| Diet fatty acids | n-3 | n-3-deficient | Statistical effect |
|------------------|--------------|---------------|--------------------|
| 14:0 | 0.2 ± 0.02 | 0.2 ± 0.02 | NS |
| 15:0 | 0.03 ± 0.012 | 0.06 ± 0.07 | NS |
| 16:0 | 19.2 ± 0.61 | 21.0 ± 0.79 | NS |
| 17:0 | 0.12 ± 0.005 | 0.13 ± 0.000 | NS |
| 18:0 | 23.1 ± 0.55 | 22.2 ± 0.39 | NS |
| 20:0 | 0.3 ± 0.03 | 0.3 ± 0.01 | NS |
| 22:0 | 0.3 ± 0.04 | 0.3 ± 0.02 | NS |
| 24:0 | 0.6 ± 0.13 | 0.3 ± 0.02 | NS |
| Saturated | 43.8 ± 0.44 | 44.5 ± 0.44 | NS |
| 16:1n-9 | 0.15 ± 0.002 | 0.15 ± 0.005 | NS |
| 16:1n-7 | 0.6 ± 0.03 | 0.6 ± 0.01 | NS |
| 17:1n-9 | 0.11 ± 0.008 | 0.07 ± 0.026 | NS |
| 18:1trans | 0.09 ± 0.013 | 0.07 ± 0.007 | NS |
| 18:1n-9 | 13.2 ± 0.03 | 13.1 ± 0.10 | NS |
| 18:1n-7 | 3.3 ± 0.04 | 3.4 ± 0.03 | 0.05 |
| 20:1n-9 | 1.1 ± 0.06 | 1.0 ± 0.07 | NS |
| 20:1n-7 | 0.2 ± 0.018 | 0.2 ± 0.010 | NS |
| 22:1n-9 | 0.18 ± 0.048 | 0.09 ± 0.012 | NS |
| 24:1n-9 | 1.0 ± 0.11 | 0.8 ± 0.05 | NS |
| Monounsaturated | 20.0 ± 0.18 | 19.5 ± 0.23 | NS |
| 18:2n-6 | 0.3 ± 0.02 | 0.5 ± 0.02 | <0.01 |
| 20:2n-6 | 0.13 ± 0.023 | 0.14 ± 0.018 | NS |
| 20:3n-6 | 0.4 ± 0.03 | 0.3 ± 0.01 | <0.05 |
| 20:4n-6 | 10.4 ± 0.17 | 10.6 ± 0.23 | NS |
| 22:4n-6 | 2.7 ± 0.06 | 2.7 ± 0.04 | NS |
| 22:5n-6 | 0.5 ± 0.09 | 1.8 ± 0.05 | <0.0001 |
| n-6 | 14.4 ± 0.23 | 16.0 ± 0.30 | <0.01 |
| 22:5n-3 | 0.4 ± 0.06 | 0.1 ± 0.04 | <0.05 |
| 22:6n-3 | 14.3 ± 0.31 | 12.1 ± 0.22 | <0.01 |
| n-3 | 14.7 ± 0.32 | 12.2 ± 0.19 | <0.001 |
| 20:3n-9 | 0.16 ± 0.032 | 0.07 ± 0.015 | <0.05 |
| Polysaturated | 29.2 ± 1.15 | 28.3 ± 0.45 | NS |
| DMA16:0 | 1.9 ± 0.22 | 2.3 ± 0.17 | NS |
| DMA18:0 | 3.3 ± 0.11 | 3.4 ± 0.06 | NS |
| DMA18:1n-9 | 0.8 ± 0.05 | 0.9 ± 0.03 | NS |
| DMA18:1n-7 | 1.0 ± 0.06 | 1.1 ± 0.03 | NS |
| DMA | 7.0 ± 0.41 | 7.7 ± 0.23 | NS |
| n-6/n-3 | 1.0 ± 0.01 | 1.3 ± 0.02 | <0.0001 |
| AA/DHA | 0.72 ± 0.01 | 0.88 ± 0.01 | <0.0001 |
| u.i. | 174.8 ± 0.77 | 167.7 ± 2.71 | <0.05 |

DMA, Dimethylacetal; AA, arachidonic acid, 20:4 n-6; DHA, docosahexaenoic acid, 22:6; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; LA, linoleic acid, 18:2 n-6; n-6 DPA, docosapentaenoic acid, 22:5 n-6; n-3 DPA, docosapentaenoic acid, 22:5 n-3. Adolescence to adulthood n-3 deprivation modifies mPFC fatty acid composition. Fatty acid composition in mice fed with n-3-balanced or n-3-deficient diet from PND 28 to PND 90. Results are expressed as percentage of total fatty acids (n = 4).

A1 and A2, which were placed in the far corners of the arena (i.e., sample phase 4 min duration). After a delay of 5 min (test phase), a copy of object A1 was placed in the same position as the original object A1, whereas a copy of object A2 was placed in a different corner than the original A2, diagonally across from A1. Thus, both objects in the test phase were equally familiar, but one was in a new location. The position of the moved object was counterbalanced between mice. The discrimination ratio was calculated as the difference in time spent by each animal exploring the object that changed position compared with the object that remained in the same position divided by the total time spent exploring all objects.

Novel object recognition. After 2 d of 10 min habituation to the experimental arena, mice were exposed to the procedure of an acquisition or sample phase, followed by a preference test after a delay of either 5 min or 2 h (Barker et al., 2007). In the sample phase, duplicate copies (A1 and A2) of an object were placed near the two corners at either end of one side of the arena (8 cm from each adjacent wall). Mice were placed into the arena facing the center of the opposite wall and allowed a total of 4 min in

the arena. At test (4 min duration), mice were placed in the arena, presented with two objects in the same positions: one object (A3) was a third copy of the set of the objects used in the sample phase, and the other object was a novel object (B). The positions of the objects in the test and the objects used as novel or familiar were counterbalanced between the mice. The discrimination ratio was calculated as the difference in time spent by each animal exploring the novel compared with the familiar object divided by the total time spent exploring both objects.

Open-field. The test was performed as we previously published (Lafourcade et al., 2011; Jung et al., 2012). The apparatus consisted of a Plexiglas arena (40 × 40 cm) illuminated by fluorescent bulbs at a height of 2 m above the floor of the open field apparatus (light intensity of 30 Lux). The floor was cleaned between each trial to avoid olfactory clues. Each mouse was transferred to the open-field facing a corner and was allowed to freely explore the experimental area for 10 min. A video tracking system (Ethovision XT, Noldus Information Technology) recorded the exact track of each mouse as well as total distance traveled (Lafourcade et al., 2011; Larrieu et al., 2012).

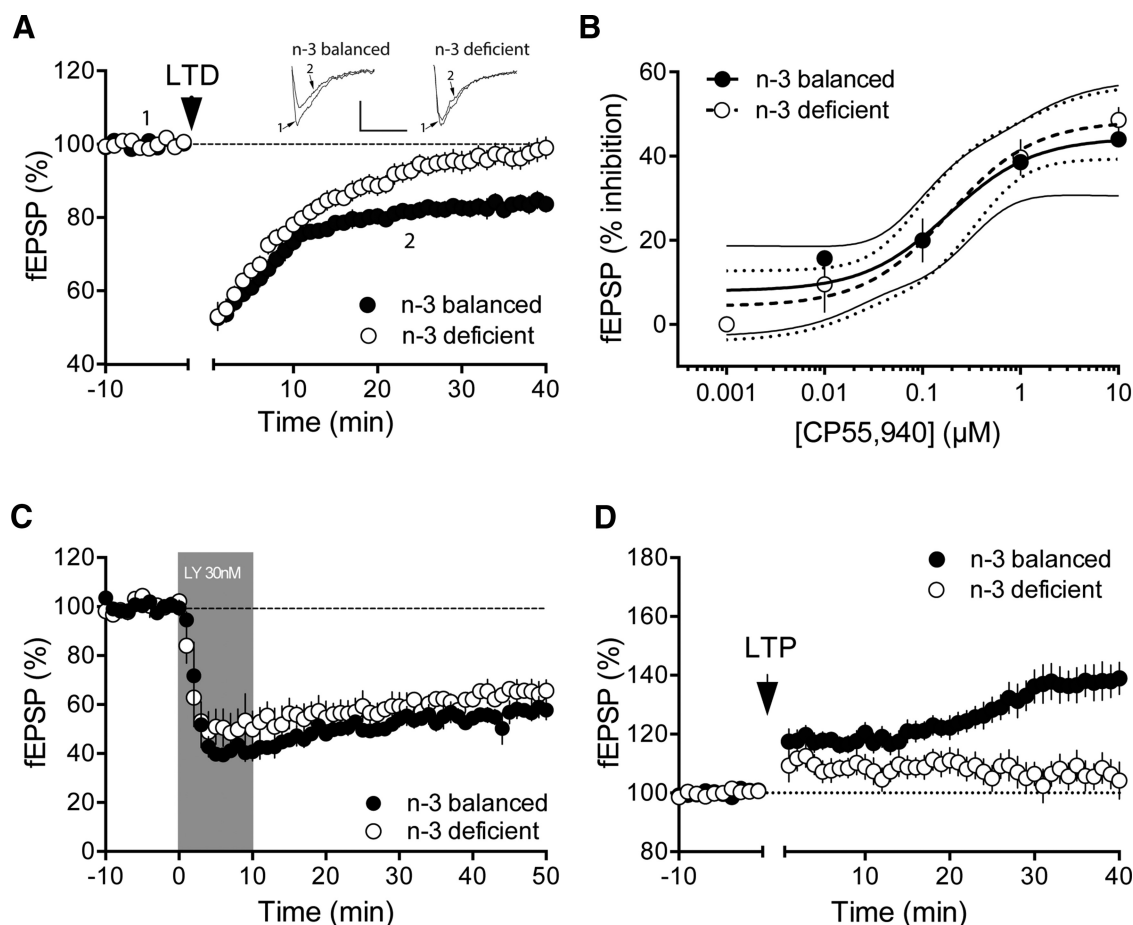


Figure 3. n-3 PUFA deficiency starting at adolescence reduces the working synaptic range in adult mPFC. **A**, Average time courses of mean EPSPs showing that low-frequency stimulation (10 min at 10 Hertz, arrow) of excitatory inputs induced an LTD of evoked EPSPs recorded in the mPFC in n-3-balanced ($n = 15$; black circles), but not in n-3-deficient ($n = 21$; white circles) mice. Top, Example traces from baseline and 40 min after LTD induction (n-3-balanced; 10 ms, 0.2 mV n-3-deficient). Calibration: 10 ms, 0.1 mV. **B**, Nutritional n-3 deficiency did not modify synaptic CB1R potency or efficiency in the mPFC. Dose–response curve for the cannabinimetic CP55,940 in n-3-balanced (black symbols, $n = 3–7$ animals, $EC_{50} = 0.18$, top value 44.33%, 95% CI for $EC_{50} = 0.03167–1.068$) and n-3-deficient mice (white symbols, $n = 3–5$ animals, $EC_{50} = 0.19$, top value 48.35%, 95% CI for $EC_{50} = 0.05193–0.6623$). fEPSP amplitudes were measured 30 min after application of CP55,940. Each point is expressed as the percentage of inhibition of its basal value. Error bars indicate SEM. **C**, Average time courses of mean EPSPs showing that, after bath perfusion with the mGluR2/3-specific agonist LY379268 (30 nM), n-3 PUFA deficiency did not alter mGluR_{2–3}-dependent LTD in the mPFC of n-3-deficient diet mice ($n = 3$; black and white circles). **D**, Average time courses of mean EPSPs showing that TBS induces LTP at layer V/VI mPFC synapses in n-3-balanced diet mice (black circles), but not n-3 PUFA-deficient mice (white circles) ($n = 16$ animals in n-3-balanced group; $n = 11$ animals in n-3-deficient mice).

Elevated plus maze. The elevated plus maze apparatus comprised two open arms ($30 \times 8 \text{ cm}^2$) and two closed arms ($30 \times 8 \times 15 \text{ cm}^3$) that extended from a common central platform ($8 \times 8 \text{ cm}^2$). The test was performed as previously described (Jung et al., 2012; Manduca et al., 2015). Mice were individually placed on the central platform of the maze facing an open arm and allowed to freely explore the apparatus for 5 min. The 5 min test period was recorded by the video tracking system Ethovision XT (Noldus Information Technology). The following parameters were analyzed: percentage of time spent in the open arms expressed as seconds spent on the open arms of the maze/seconds spent on the open + closed arms $\times 100$; number of entries into the risk-zone reflected by open arm extremities; number of head dipping events (i.e., the animal protruded the head over the ledge of an open arm and down toward the floor while its body is in the closed arms, central platform or in the open arms).

Marble burying. To quantify behavioral traits of obsessive-like behavior (Woods-Kettelberger et al., 1997) and anxiety (Njung'e and Handley, 1991), 20 clean blue glass marbles were spaced in five rows of four, on 5 cm layer of sawdust bedding, which was lightly pressed down to make a flat even surface, in a plastic cage ($30 \times 20 \text{ cm}$) (light intensity of 30 Lux). Mice were individually placed facing a corner for a 20 min session. The number of marbles buried was counted at the end of the session. The

number of marbles buried was plotted. Buried was defined by having over two-thirds of the total top surface of the marble covered by bedding.

Sucrose preference. Anhedonia was assessed in the sucrose consumption test (Monleon et al., 1995; Bessa et al., 2009). Mice were individually housed in a laboratory cage and exposed for 72 h to a bottle containing a sucrose solution (1% in tap water, Sigma), placed into the middle of the cage wire top, followed by 18 h of water deprivation and a 2 h exposure to two identical bottles (one filled with 1% sucrose solution and the other with water). Bottles were placed at opposite ends of the cage and counterbalanced across groups to avoid side bias. Sucrose preference was calculated as the ratio of the volume of sucrose versus total volume (sucrose + water) $\times 100$ consumed during the 2 h test.

Drug treatments. Six hours before behavioral testing, mice were given intraperitoneal injections of vehicle (22.5:77.5 2-hydroxypropyl- β -cyclodextrin [HBC] or Tween 80: $H_2O = 1:9$), the monoacylglycerol lipase inhibitor 4-nitrophenyl-4-[bis(1,3-benzodioxol-5-yl) (hydroxy) methyl]piperidine-1-carboxylate (JZL184) (16 mg per kg in HBC) (Jung et al., 2012) or the positive allosteric modulator of mGlu₅ receptors, 3-cyano-*N*-(1,3-diphenyl-1H-pyrazol-5-yl) benzamide (CDPPB) (10 mg per kg in Tween 80: H_2O) (Won et al., 2012). JZL184 was freshly dissolved in HBC vehicle and mixed thoroughly during 30 min. CDPPB was dissolved in Tween 80: H_2O to a final concentration of 1 mg/ml.

Statistical analysis. All the behavioral parameters were expressed as mean \pm SEM. Group comparisons used *t* test and two-way ANOVA (for different diets and pharmacological treatments), followed by Student-Newman-Keuls *post hoc* tests where appropriate.

Physiology

Slice preparation. Adult male mice (P90–P120) were anesthetized with isoflurane and killed as previously described (Robbe et al., 2002; Lafourcade et al., 2011; Jung et al., 2012; Martin et al., 2016a). The brain was sliced (300 μ m) in the coronal plane with a vibratome (Integraslice, Campden Instruments) in a sucrose-based solution at 4°C (in mM as follows: 87 NaCl, 75 sucrose, 25 glucose, 2.5 KCl, 4 MgCl₂, 0.5 CaCl₂, 23 NaHCO₃ and 1.25 NaH₂PO₄). Immediately after cutting, slices containing the mPFC or accumbens were stored for 1 h at 32°C in a low calcium ACSF that contained (in mM) as follows: 130 NaCl, 11 glucose, 2.5 KCl, 2.4 MgCl₂, 1.2 CaCl₂, 23 NaHCO₃, 1.2 NaH₂PO₄, and were equilibrated with 95% O₂/5% CO₂ and then at room temperature until the time of recording.

Electrophysiology. Whole-cell patch-clamp of visualized layer 5 pyramidal neurons mPFC and accumbens medium spiny neurons (MSNs) and field potential recordings were made in coronal slices containing the mPFC or the accumbens as previously described (Robbe et al., 2002; Lafourcade et al., 2011; Jung et al., 2012; Martin et al., 2016a). During the recording, slices were placed in the recording chamber and superfused at 2 ml/min with either low Ca²⁺ ACSF for mPFC or normal ACSF for the accumbens. All experiments were done at 32°C or room temperature for mPFC and accumbens, respectively. The superfusion medium contained picrotoxin (100 μ M) to block GABA Type A (GABA-A) receptors. All drugs were added at the final concentration to the superfusion medium.

For whole-cell patch-clamp experiments, neurons were visualized using an upright microscope with infrared illumination. The intracellular solution was based on K⁺ gluconate (in mM as follows: 145 K⁺ gluconate, 3 NaCl, 1 MgCl₂, 1 EGTA, 0.3 CaCl₂, 2 Na₂⁺ATP, and 0.3 Na⁺ GTP, 0.2 cAMP, buffered with 10 HEPES). To quantify the AMPA/NMDA ratio, we used a CH₃O₃SCs-based solution (in mM as follows: 128 CH₃O₃SCs, 20 NaCl, 1 MgCl₂, 1 EGTA, 0.3 CaCl₂, 2 Na₂⁺ATP, and 0.3 Na⁺ GTP, 0.2 cAMP, buffered with 10 HEPES, pH 7.2, osmolarity 290–300 mOsm). The pH was adjusted to 7.2 and osmolarity to 290–300 mOsm. Electrode resistance was 4–6 MOhm.

Whole-cell patch-clamp recordings were performed with an Axopatch-200B amplifier as previously described (Robbe et al., 2002; Kasantz and Manzoni, 2009; Lafourcade et al., 2011; Jung et al., 2012; Thomazeau et al., 2014, 2017; Martin et al., 2016b). Data were low pass filtered at 2 kHz, digitized (10 kHz, DigiData 1440A, Axon Instruments), collected using Clampex 10.2 and analyzed using Clampfit 10.2 (all from Molecular Device).

A –2 mV hyperpolarizing pulse was applied before each evoked EPSC to evaluate the access resistance, and those experiments in which this parameter changed >25% were rejected. Access resistance compensation was not used, and acceptable access resistance was <30 MOhm. The potential reference of the amplifier was adjusted to zero before breaking into the cell. Cells were held at –76 mV.

Current-voltage (*I*–*V*) curves were made by a series of hyperpolarizing to depolarizing current steps immediately after breaking into the cell. Membrane resistance was estimated from the *I*–*V* curve around resting membrane potential (Kasantz et al., 2010; Thomazeau et al., 2014; Martin et al., 2016a).

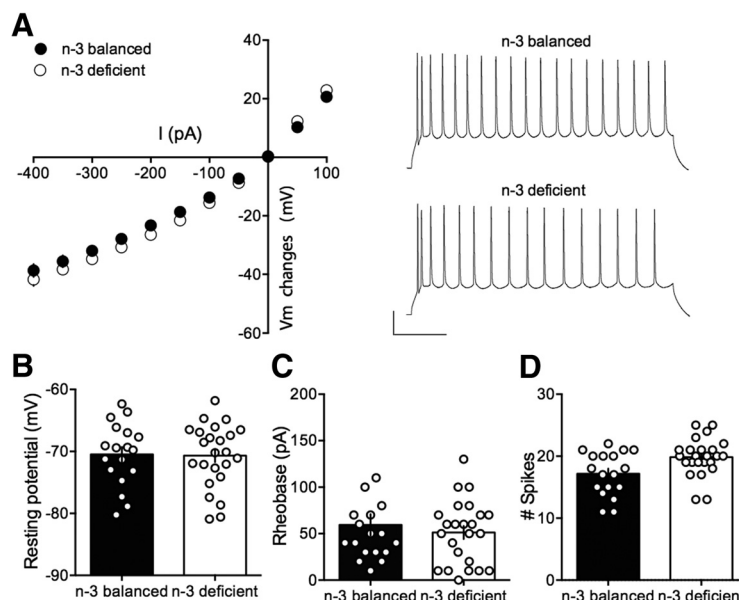


Figure 4. Intrinsic properties of layer 5 mPFC pyramidal neurons in n-3 and n-3 PUFA-deficient mice. **A**, Left, Summary of all the *I*–*V* curves recorded in the two dietary groups showing no difference between n-3-balanced group ($n = 18$ cells/10 mice, black symbols) and n-3-deficient mice ($n = 24$ cells/13 mice, white symbols; $F_{(26,1072)} = 0.438$, $p > 0.05$, two-way ANOVA). Right, Typical membrane responses to somatic current steps of mPFC neurons from n-3-balanced or n-3-deficient mice. Sample spike trains in response to 300 pA depolarizing current step from n-3-balanced and n-3-deficient mice. **B**, The resting membrane potential from n-3-balanced mice (black bar; -70.48 ± 1.19 , $n = 18$ cells/10 mice) and n-3-deficient mice (white bar; -70.68 ± 1.04 , $n = 24$ cells/13 mice) and **C** the rheobase (59.44 ± 11.45 , $n = 18$ cells/10 mice from n-3-balanced mice, black bar; 69 ± 96.95 , $n = 24$ cells/13 mice from n-3-deficient mice, white bar) was similar in both groups ($p > 0.05$, Mann–Whitney *U* test). **D**, The number of evoked action potentials (number of spikes) in response to 300 pA depolarizing current step (n-3-balanced: 117.17 ± 0.83 , $n = 18$ cells/10 mice, black bar symbols; n-3-deficient: 19.83 ± 0.62 , $n = 24$ cells/13 mice black bar symbols) was similar in both groups ($p > 0.05$, Mann–Whitney *U* test). Error bars indicate SEM.

For extracellular field experiments, the recording pipette was filled with ACSF. The glutamatergic nature of the field EPSP (fEPSP) was systematically confirmed at the end of the experiments using the ionotropic glutamate receptor antagonist CNQX (20 μ M), which specifically blocked the synaptic component without altering the nonsynaptic component (data not shown).

Both EPSC/fEPSP area and amplitude were analyzed. Stimulation was performed with a glass electrode filled with ACSF and placed ~ 200 μ m in the dorsal-medial direction of the recorded cell. The stimulus intensity was adjusted $\sim 60\%$ of maximal intensity after performing an input–output curve (baseline EPSC amplitudes ranged between 50 and 150 pA). Stimulation frequency was set at 0.1 Hz.

To determine the AMPA/NMDA ratio, the cells were voltage-clamped to 40 mV, and a stable dual response (AMPA + NMDA current) to afferent stimulation was recorded. The EPSC was isolated after bath application of the AMPAR antagonist NBQX (10 μ M). The AMPAR EPSC was obtained by digital subtraction of the NMDAR EPSC from the dual response.

Spontaneous AMPAR-mediated EPSC (sEPSCs) were recorded at –76 mV in whole-cell voltage-clamp configuration using Axoscope 10 (Molecular Devices). sEPSCs were filtered at 2 kHz and digitized at 20 kHz. sEPSC amplitude and interinterval 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. The threshold of amplitude detection was set at 5 pA.

Data acquisition and analysis. The magnitude of plasticity was calculated 38–40 min after induction as percentage of baseline responses. sEPSCs were analyzed with Axograph X (Axograph). Statistical analysis of data was performed with Prism (GraphPad Software) using tests indicated in the main text after outlier subtraction. All values are given as mean \pm SEM, and statistical significance was set at $p < 0.05$.

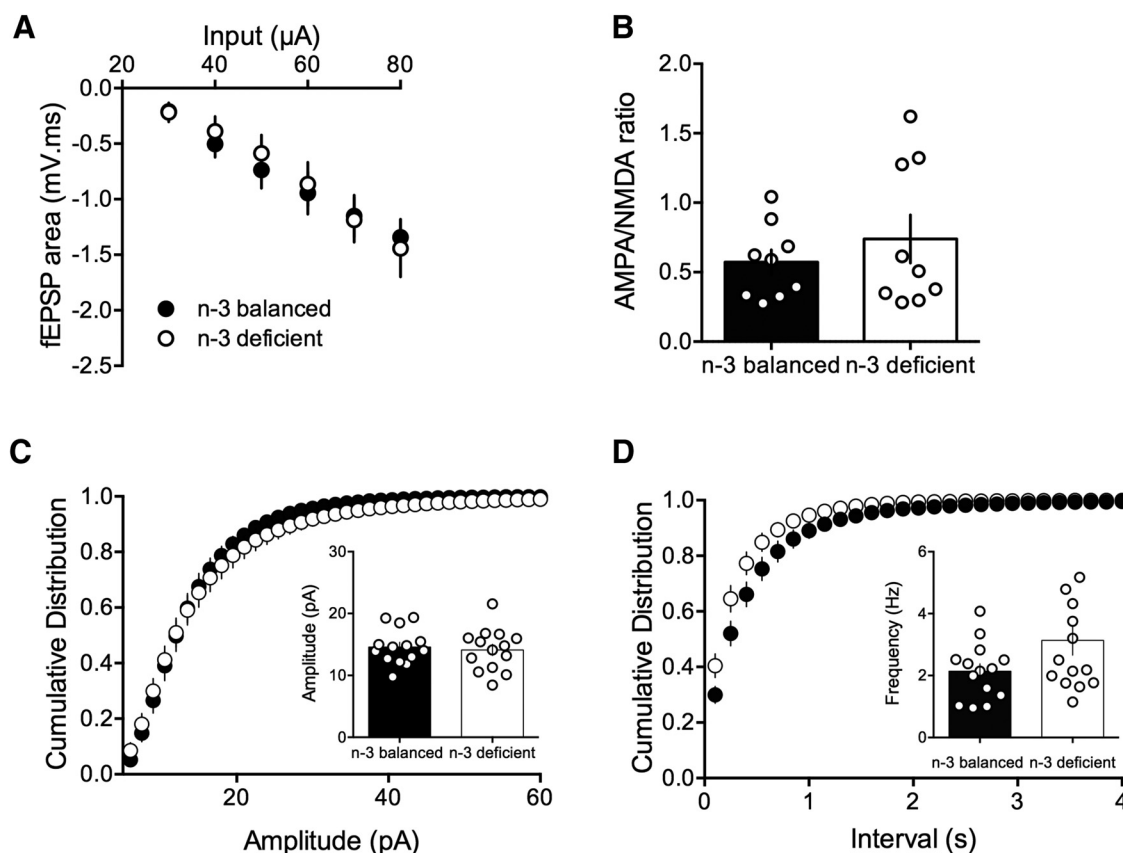


Figure 5. Nutritional n-3 deficiency does not change the basic synaptic properties in mPFC pyramidal neurons. **A**, Input–output profile from n-3-balanced and n-3 PUFA-deficient mice from mPFC layer 5 fEPSPs. Averaged fEPSP area measured as a factor of stimulus intensity ($F_{(6,63)} = 0.154$, $p > 0.05$, two-way ANOVA; n-3-balanced group $n = 5$ mice, n-3-deficient group $n = 6$ mice). **B**, AMPAR/NMDAR ratios measured in the n-3-balanced were similar to that measured in n-3 PUFA-deficient mice (n-3-balanced: 0.5725 ± 0.09 , $n = 9$ cells from 9 mice; n-3-deficient: 0.739 ± 0.17 , $n = 9$ cells from 9 mice; $p > 0.05$, Mann–Whitney U test). **C**, Sample traces from layer 5 mPFC pyramidal neurons clamped at -70 mV from n-3-balanced and n-3-deficient mice. Calibration: 0.25 s, 20 pA. sEPSCs were recorded in the whole-cell configuration in accumbens MSNs clamped at -70 mV. AMPAR-mediated mEPSC amplitudes were not changed in n-3-deficient mice (black symbols, $n = 14$ cells/12 mice; white symbols, $n = 14$ cells/9 mice). The cumulative distribution (Kolmogorov–Smirnov test; not significant) and mean amplitude (inset) of the sEPSC were similar in the two dietary conditions ($p > 0.05$; paired t test). **D**, The frequency of spontaneous AMPAR-mediated events was indistinguishable in the two dietary conditions. Cumulative probability distributions of intervals between events were similar in the two dietary conditions (Kolmogorov–Smirnov test, not significant); mean frequency (inset) of the mEPSC was similar in both dietary conditions ($p > 0.05$; paired t test). Error bars indicate SEM.

Results

Body weight and food intake

Body weight was measured weekly in n-3 and n-3 PUFA-deficient mice from P28 until commencement of the experiments at P90. Average body weight of n-3 PUFA-deficient mice did not differ from diet-matched n-3 controls at any age ($F_{(\text{weeks} \times \text{diet})8,72} = 1.168$, $p = 0.330$, two-way ANOVA). Daily food intake was calculated as average daily food consumption in grams per day for each week. In n-3 PUFA-deficient mice, daily food consumption did not differ from mice fed with an n-3 diet ($F_{(\text{weeks} \times \text{diet})8,72} = 1.677$, $p = 0.119$, two-way ANOVA). Collectively, these data indicate that the n-3 PUFA-deficient diet per se did not affect body weight or food consumption in mice.

Starting n-3 PUFA deficiency at adolescence selectively impairs cognition at adulthood

We found that reducing dietary n-3 PUFAs starting at adolescence modified selected behaviors at adulthood. We previously showed the detrimental consequences of perinatal malnutrition on sociability (Lafourcade et al., 2011). Accordingly, we first evaluated social interactions in the two dietary conditions. We observed that n-3-deficient adult mice spent less time in social interactions (Fig. 1A) and had less contact with their congeners than mice fed an n-3 diet.

To explore whether the social deficits extended to the cognitive domain, we measured temporal order memory, a task that implicates both PFC and hippocampal circuits (Barker et al., 2007). Mice from the n-3 PUFA-deficient group presented a marked reduction in temporal order memory illustrated by a lack of discrimination in favor of the old object (normally perceived as new) over the most recent object (Fig. 1B). To further indicate impairment in hippocampal function, we next measured the consequences of n-3 deficiency on two tasks strongly associated with hippocampal integrity: location of novel object and novel object recognition (Barker et al., 2007). In marked contrast to temporal order memory and social interaction results, the hippocampal-dependent tasks were conserved in n-3 PUFA-deficient adults. Thus, in the novel object location test, n-3 and n-3 PUFA-deficient groups were indistinguishable in their ability to discriminate the object which had changed position from the object which had not (Fig. 1C). In the novel object recognition test, n-3-deficient adult mice were able to discriminate between novel and familiar objects either after 5 min or 2 h delay (Fig. 1D).

n-3 PUFA deficiency is anxiogenic in adulthood

Based on previous work from our group showing that lifelong dietary n-3 PUFA deficiency augmented anxiety and depression-

related behaviors (Lafourcade et al., 2011; Larrieu et al., 2012, 2014), we next compared the n-3 and n-3 PUFA-deficient mice for anxiety-like behaviors.

We found that dietary n-3 PUFA deficiency decreased the percentage of time spent exploring the center of an open-field compared with mice fed with the n-3 PUFA diet (Fig. 2A). The increased anxiety-like behavior was not linked to impaired total motor locomotion, and there was no significant change in the distance traveled between n-3 and n-3-deficient mice (data not shown).

Reminiscent of our previous report with the lifelong n-3 PUFA deficiency model (Lafourcade et al., 2011), this finding suggests that nutritional n-3 PUFA status is closely linked to an anxious state in mice. To confirm this idea, mice groups were compared in an elevated plus maze (Fig. 2B–D). The n-3-deficient mice displayed a clear aversion to the open arms of the elevated plus maze compared with n-3-balanced diet mice (Fig. 2B). Furthermore, both the number of entries in the risk zone (i.e., edge of the open arms; Fig. 2C) and head dippings were strongly reduced (Fig. 2D), suggesting increased risk-aversion in n-3-deficient mice.

Another behavioral marker of anxiety sometime used to identify behavioral traits of obsessive-like behavior (Woods-Kettelberger et al., 1997) is the marble-burying task (Njung'e and Handley, 1991). Mice from the n-3-deficient group buried substantially more marbles than n-3-balanced mice (Fig. 2E). Next, we investigated whether heightened anxiety was accompanied by decreased preference for sucrose, a hallmark of depression-like behavior (Monleon et al., 1995). As previously noted, motor locomotion was unchanged in n-3-deficient mice, and we verified that basal fluid intake was similar in both groups (data not shown). However, there was a massive reduction in the preference for sweet solution, a putative sign of anhedonia, in the n-3-deficient mice compared with mice fed with the n-3-balanced diet (Fig. 2F).

n-3 PUFA deficiency alters fatty acid levels in the mPFC and accumbens

Dietary intake of PUFAs is necessary for maintaining a healthy n-3 PUFAs status in the form of docosahexaenoic acid (DHA) in the brain (Bazin et al., 2014). We and others have reported that perinatal n-3 PUFA deficiency strongly affects brain DHA content (Lafourcade et al., 2011; Joffe et al., 2014); however, the effect of a n-3 PUFA-deficient diet starting at adolescence on brain PUFA content at adulthood has been poorly evaluated (Delpech et al., 2015). Thus, we measured the impact of early life n-3 PUFA dietary manipulation on PUFA content in PFC and accumbens in adulthood. Exposure to the n-3 PUFA-deficient diet from adolescence to adulthood is sufficient to increase n-6 and decrease n-3 PUFA content in the PFC (Table 1) and accumbens (Table 2). In particular, DHA (22:6 n-3) and docosapentaenoic acid (n-3 DPA, 22:5 n-3) levels were significantly decreased in the accumbens and PFC of n-3-deficient mice (Table 2). In addition,

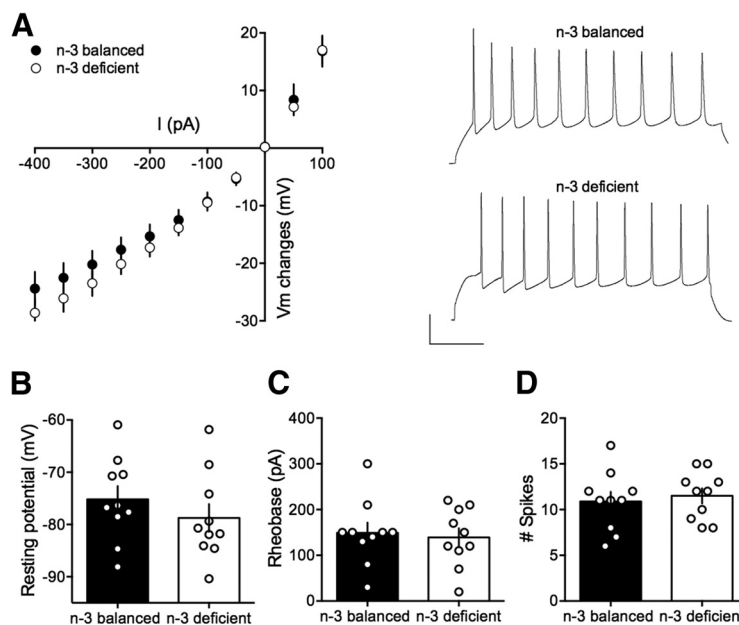


Figure 6. Intrinsic properties of accumbens MSNs in n-3 and n-3-deficient mice. **A**, Right, Summary of all the I – V curves recorded in the two dietary groups showing no difference between n-3-balanced ($n = 10$ cells/8 mice, black symbols) and n-3-deficient mice ($n = 10$ cells/6 mice, white symbols; $F_{(17,306)} = 0.697$, $p > 0.05$, two-way ANOVA). Left, Typical membrane responses to somatic current steps of accumbens MSNs from n-3-balanced or n-3-deficient mice. Sample spike trains in response to 300 pA depolarizing current step from n-3-balanced and n-3-deficient mice. **B**, The resting membrane potential from n-3-balanced mice (black bar; -75.18 ± 2.54 , $n = 10$ cells/8 mice), n-3-deficient mice (white bar; -78.73 ± 2.65 , $n = 10$ cells/6 mice), and **C** the rheobase (149.0 ± 22.58 , $n = 10$ cells from n-3-balanced mice, black bar; 139.0 ± 20.25 , $n = 10$ cells from n-3-deficient mice, white bar) were similar in both groups ($p > 0.05$, Mann–Whitney U test). **D**, The number of evoked action potentials in response to somatic current steps was similar in both dietary conditions (n-3-balanced: $n = 10$ cells/8 mice, black bar symbols; n-3-deficient: $n = 10$ cells/6 mice white bar symbols; $p > 0.05$, Mann–Whitney U test).

n-6 DPA (22:5 n-6) and LA (18:2 n-6) levels were significantly increased, whereas arachidonic acid (AA, 20:4 n-6) levels were similar (Table 2) as previously described in the hippocampus (Delpech et al., 2015). Thus, adolescent malnutrition modified lipid composition in our structures of interest. Correct perinatal nutrition does not protect from dietary deficits later in life, reinforcing the idea that it is necessary to maintain appropriate n-3 PUFA intake after weaning.

n-3 PUFA deficiency from adolescence to adulthood reduces the working synaptic range in adult mPFC

A maximum working synaptic range is indispensable to the expression of behavioral flexibility in response to an ever-changing world (Kasanez et al., 2013). Does nutrition affect the maximum synaptic working range at prefrontal excitatory synapses? In view of our results showing a reduction in prefrontal-dependent cognitive behaviors, we decided to interrogate two critical actors of bidirectional plasticity and reliable prefrontal endophenotypes of neuropsychiatric diseases: NMDAR-dependent LTP and endocannabinoid/mGlu₅-dependent LTD (Lafourcade et al., 2011; Kasanez et al., 2013; lafrati et al., 2014; Thomazeau et al., 2014; Martin et al., 2016a).

We found that, in mPFC slices prepared from n-3-balanced mice, tetanic stimulation induced a robust, eCB-mediated LTD (eCB-LTD) of excitatory synapses onto layer 5 mPFC pyramidal neurons that matched what we previously reported in mice fed a standard laboratory chow (Lafourcade et al., 2007; Thomazeau et al., 2014). This eCB-LTD was absent in n-3-deficient mice (Fig. 3A).

We next searched for functional alterations that could explain the lack of endocannabinoid/mGlu₅-LTD. Neither the potency

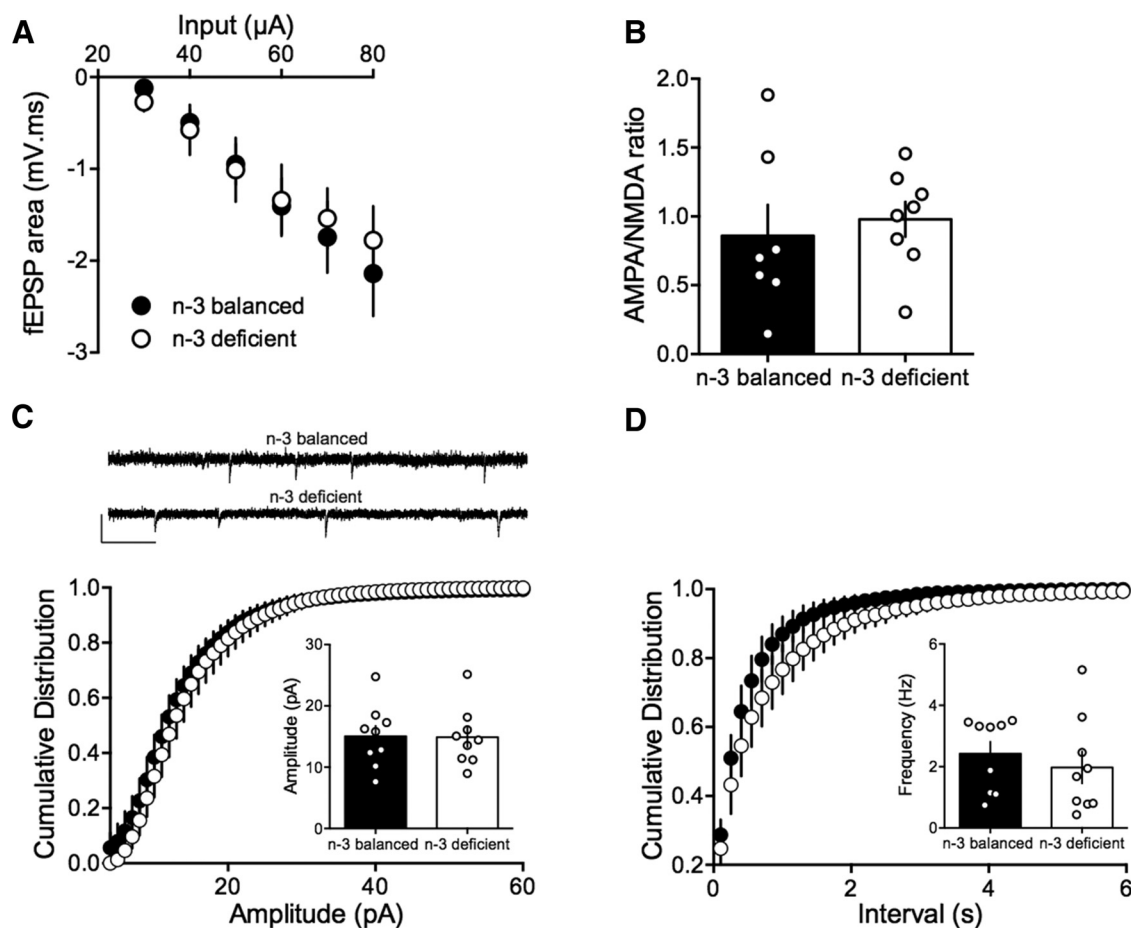


Figure 7. Nutritional n-3 deficiency does not change the basic synaptic properties in MSNs from the accumbens core. **A**, There was no change in synaptic excitability in the accumbens of n-3-deficient mice. Input–output curves of evoked fEPSP in accumbens neurons in n-3-balanced ($n = 9$ animals, black symbols) and in n-3-deficient mice ($n = 3$ animals, white symbols) for increasing stimulation intensities. Average mean fEPSPs are represented ($F_{(6,91)} = 0.182, p > 0.05$, two-way ANOVA). **B**, Summary histograms with superimposed scatterplots showing that the AMPAR-mediated EPSC/NMDAR-mediated EPSC ratio was similar in the two dietary groups ($0.86 \pm 0.22, n = 7$ cells from 7 n-3-balanced mice vs $0.98 \pm 0.13, n = 8$ cells from 8 n-3-deficient mice; $p > 0.05$, Mann–Whitney U test). **C, D**, Sample traces from MSN clamped at -70 mV from n-3-balanced and n-3-deficient mice. Calibration: 0.25 s, 20 pA. Quantal synaptic parameters were similar in both dietary conditions. Cumulative sEPSC amplitude (**C**) and interevent (**D**) distributions showed no difference between n-3-balanced (black symbols, $n = 9$ cells/4 mice) and n-3-deficient mice (white symbols, $n = 9$ cells/5 mice) ($p > 0.05$, Kolmogorov–Smirnov test). Insets, Summary histograms with superimposed scatterplots showing the average amplitudes and interevent values. Error bars indicate SEM.

nor the efficacy of presynaptic CB₁R was modified in the deficient group (Fig. 3B). The consequences of starting n-3 PUFA deficiency in adolescence and adulthood were different from those of gestational/perinatal deficiency where a marked reduction in CB₁R functions was reported (Lafourcade et al., 2011; Larrieu et al., 2012). Of note, the effects of n-3 PUFA depletion did not extend to all types of synaptic plasticity: mGlu_{2/3}-mediated LTD, a specific biomarker of cocaine addiction (Kasanez et al., 2013), was indistinguishable in n-3-balanced and n-3-deficient mice (Fig. 3C).

Theta-burst stimulation (TBS) induces an NMDAR-dependent LTP at mPFC pyramidal synapses. The absence of TBS-LTP in the mPFC is an endophenotype common to several models of neuropsychiatric diseases, including schizophrenia, autism, and mental retardation (Iafrati et al., 2014; Thomazeau et al., 2014; Martin et al., 2016a). TBS-LTP was readily induced in n-3-balanced mice similar to that observed in mice fed standard chow (Iafrati et al., 2014; Thomazeau et al., 2014; Martin et al., 2016a). In marked contrast, TBS-induced LTP was absent in n-3-deficient mice (Fig. 3D).

In search of mechanistic insights, we compared intrinsic firing properties of pyramidal neurons in the two dietary conditions. Independent of the dietary treatment, all recorded mPFC neu-

rons showed similar membrane reaction profiles in response to a series of somatic current steps, illustrated by superimposable I – V plots (Fig. 4A). The resting membrane potential (Fig. 4B) and the rheobase (Fig. 4C) were similar in all groups. The number of action potentials in response to somatic current steps was also similar in n-3-balanced and n-3-deficient mice (Fig. 4D). Thus, n-3 PUFA deficiency from adolescence did not impact the intrinsic properties of mPFC pyramidal cells in adulthood. This result is compatible with our observations following lifelong n-3 PUFA deficiency.

Circuit properties were next compared in the two dietary conditions. Input–output profiles were measured in both groups: fEPSPs evoked by electrical stimulation showed a consistent profile distribution in response to increasing stimulation intensity across different slices and mice: “input–output” curves from n-3-balanced and n-3-deficient mice were similar (Fig. 5A). The relative contribution of AMPAR and NMDAR to synaptic currents is an indicator of synaptic plasticity; in particular, those dependent upon the incorporation or removal of AMPAR in the postsynaptic density (Kasanez and Manzoni, 2009; Neuhofer et al., 2015). The ratio between AMPAR- and NMDAR-evoked EPSCs was similar in both dietary conditions, suggesting that n-3

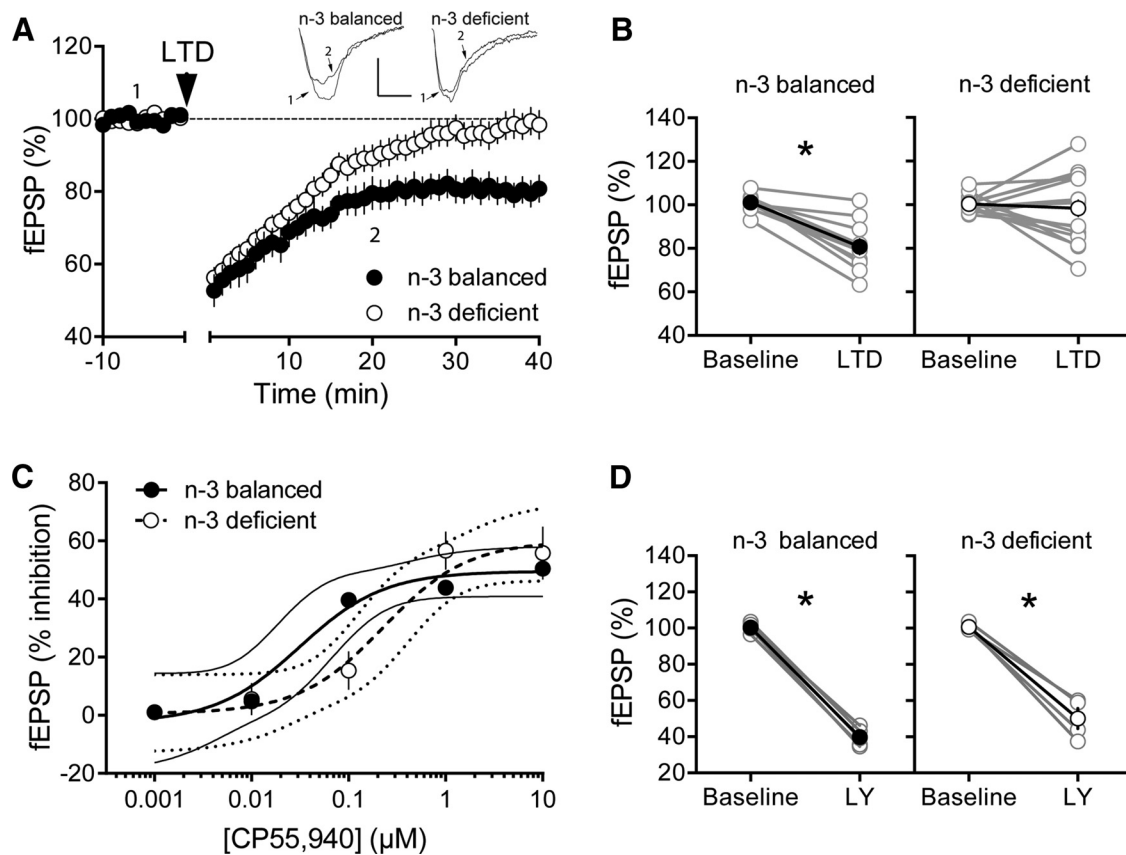


Figure 8. n-3 deficiency during adolescence ablates eCB-dependent plasticity. **A**, Example traces from baseline and 40 min after LTD induction. Calibration: 5 ms, 0.1 mV. Average time courses of mean EPSPs showing that low-frequency stimulation induced an LTD of evoked EPSPs recorded in accumbens MSNs in n-3-balanced (black circles), but not in n-3-deficient (white circles) mice. **B**, Individual experiments (gray) and group average (black represents n-3-balanced; white represents n-3-deficient), before (baseline) and after (40 min) LTD induction showing the lack of LTD in the n-3-deficient group. In n-3-balanced mice: 101.08 ± 1.22 , $n = 10$ before LTD, versus 80.81 ± 3.70 , $n = 10$, after LTD induction. In n-3-deficient mice: 100.31 ± 0.91 , $n = 16$ before LTD versus 98.38 ± 3.87 , $n = 16$, after LTD. **C**, Nutritional n-3 deficiency did not modify synaptic CB1R potency or efficiency in the accumbens. Dose-response curve for the cannabimimetic CP55,940 in n-3-balanced (black symbols, $n = 3$ –8 animals, $EC_{50} = 0.03 \mu M$, top value 49.54% , 95% CI for $EC_{50} = 0.006510$ – 0.1496) and n-3-deficient (white symbols, $n = 3$ –5 animals, $EC_{50} = 0.23 \mu M$, top value 59.99% , 95% CI for $EC_{50} = 0.05782$ – 0.9020) mice. fEPSP amplitudes were measured 30 min after application of CP55,940. Each point is expressed as the percentage of inhibition of its basal value. Error bars indicate SEM. **D**, n-3 PUFA deficiency does not alter mGlu $_{2/3}$ -dependent LTD in n-3-deficient mice. Individual experiments (gray) and group average (black: n-3-balanced; white: n-3-deficient), before (baseline) and after (40 min) bath perfusion with the mGlu $_{2/3}$ -specific agonist LY379268 (LY, 30 nM). In n-3-balanced mice: 100.40 ± 1.16 , $n = 5$ before LTD versus 39.79 ± 2.20 , $n = 5$, after LTD induction. In n-3-deficient mice: 100.70 ± 1.03 , $n = 4$ before LTD versus 50.07 ± 5.60 , $n = 4$, after LTD (n-3-balanced and n-3-deficient, $p < 0.05$; paired t test). Error bars indicate SEM. * $p < 0.05$.

PUFA deficiency did not modify the overall gain of prefrontal synapses (Fig. 5B). We next compared quantal events by recording spontaneous AMPAR-mediated EPSCs (sEPSCs) in mPFC neurons from both n-3-balanced and n-3-deficient mice. The mean amplitudes and distribution of sEPSC events (Fig. 5C) were similar in the two groups. We also compared the frequency of sEPSCs by comparing the interval between AMPAR events (Fig. 5D). No difference was found between the two dietary conditions. These results are consistent with what we observed in mice mPFC following lifelong n-3 PUFA deficiency (Lafourcade et al., 2011).

The data show that starting malnutrition at adolescence reduced the working synaptic range in adult mice from the n-3-deficient group. These synaptic aberrations in LTP or LTD were not caused by alterations of basal synaptic gain, neuronal excitability, postsynaptic AMPAR and NMDAR, or presynaptic CB $_1$ R coupling.

n-3 PUFA deficiency impairs eCB-mediated plasticity in the accumbens

We recently showed that eCB-mediated plasticity in the accumbens is instrumental in underlying depression-like behavior (Bosch-

Bouju et al., 2016). How n-3 PUFA deficiency impacts accumbens synapses and synaptic plasticity is currently not known.

The vast majority of accumbens neurons are so-called MSNs. Independent of the dietary treatment, all recorded MSNs showed similar membrane response profiles to a series of somatic current steps, illustrated by superimposable I – V plots (Fig. 6A). The resting membrane potential (Fig. 6B) and the rheobase (Fig. 6C) were similar in all groups. The number of action potentials in response to somatic current steps was also similar in n-3-balanced and n-3-deficient mice (Fig. 6D). Thus, n-3 PUFA deficiency from adolescence did not impact the intrinsic properties of MSNs in adulthood.

We next measured fEPSPs of accumbens MSNs to build input–output profiles in the two dietary conditions. fEPSPs evoked by electrical stimulation showed a consistent profile distribution in response to increasing stimulation intensity across different slices and mice; thus, “input–output” curves from n-3-balanced and n-3-deficient mice were similar (Fig. 7A). The ratio between AMPAR- and NMDAR-evoked EPSCs was similar in both groups, suggesting that n-3 PUFA deficiency did not modify the overall gain of accumbens excitatory synapses (Fig. 7B). We next

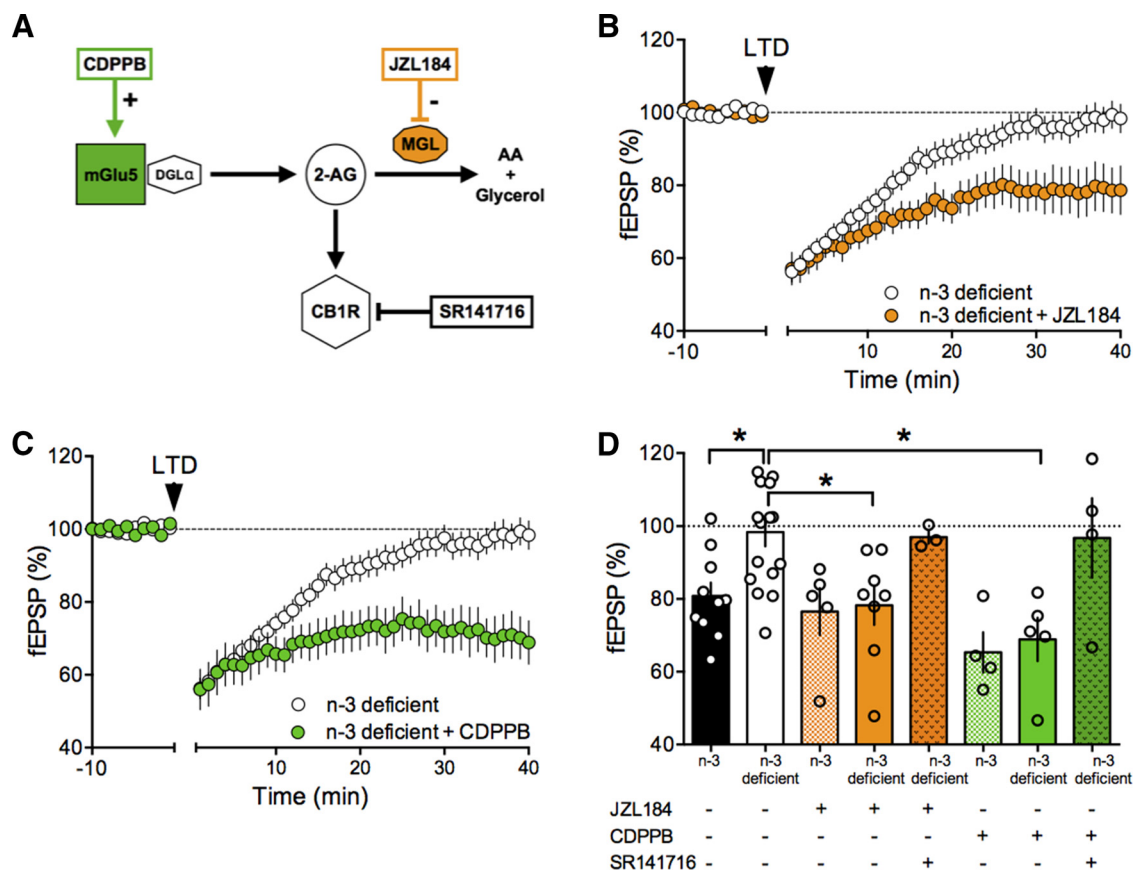


Figure 9. Acute pharmacological amplification of the mGlu₅-eCB signalosome restores eCB synaptic plasticity in the accumbens of n-3-deficient mice. **A**, Schematic representation of the mGlu₅-eCB signalosome and of the targets of the pharmacological agents used in the study. **B**, Time course of eCB-LTD in n-3-deficient accumbens slices preincubated for 45 min in 1 μ M JZL184 (orange circles). **C**, Time course of normalized fEPSP responses from n-3-deficient accumbens slices preincubated with 10 μ M CDPPB (green circles). **D**, Bar chart of fEPSP percentage change from baseline 38–40 min after 10 Hz stimulation in n-3 and n-3-deficient mice before and after treatment with JZL184 \pm SR141716A (5 μ M) (orange bars, $F_{(4,37)} = 4.960$, $p < 0.05$, one-way ANOVA) and CDPPB \pm SR141716A (green bars, $F_{(4,34)} = 7.357$, $p < 0.05$, one-way ANOVA). Error bars indicate SEM. * $p < 0.05$.

compared quantal events by recording sEPSCs in accumbens MSNs from both n-3-balanced and n-3-deficient mice. The mean amplitude of spontaneous events was similar in the two dietary conditions (Fig. 7C) at the resting membrane potential (-70 mV), where these events are principally mediated by AMPAR. Therefore, adult n-3 deficiency does not appear to affect AMPAR currents in accumbens MSNs. We also compared the frequency of sEPSCs by comparing the cumulative distribution of the interval between events (Fig. 7D). Both dietary conditions had a similar distribution and average interevent intervals.

In accumbens slices prepared from mice fed with a n-3-balanced diet, tetanic stimulation induced a robust eCB-LTD of excitatory synapses onto MSNs (Fig. 8A), which matched that observed in mice fed with normal chow (Robbe et al., 2002). In marked contrast, we found that this form of long-term synaptic depression was totally absent in n-3-deficient mice (Fig. 8A,B). Next, we estimated CB₁R presynaptic functions by building dose–response curves for the cannabinoid agonist CP55,940. In this model of n-3 deficiency from adolescence, maximal inhibitory effect of CP55,940 and its EC_{50} were comparable in both dietary conditions (Fig. 8C) and similar to what we previously reported in mice fed with standard laboratory chow (Mato et al., 2005). Of note, lifelong n-3 PUFA depletion strongly lowered the maximal efficacy of CB₁R in the mPFC (Puente et al., 2011). To determine whether n-3 deficiency impairments generalized to a second form of presynaptic LTD, we compared mGlu_{2/3}-triggered LTD

in the two dietary conditions (Mato et al., 2005; Lafourcade et al., 2011; Ksanetz et al., 2013). The mGlu_{2/3}-LTD was strongly expressed in n-3-balanced and n-3-deficient mice (Fig. 8D), leaving little doubt that n-3 deficiency had a minimal effect on the downstream presynaptic signaling cascades common to mGlu_{2/3} and CB₁R in the accumbens (Mato et al., 2005).

These data show that deprivation of dietary n-3 PUFAs from adolescence until adult life is sufficient to selectively abolish the development of mGlu₅-eCB-mediated synaptic plasticity in the mice accumbens.

Amplification of the mGlu₅-eCB signaling restores the working synaptic range in accumbens and mPFC of n-3-deficient mice

Pharmacological exploration of synaptic plasticity deficits in the brain slice preparation is a valuable approach to identify new therapeutic targets. Recent findings show that mGlu₅- and/or eCB-mediated signaling is a prime target for such intervention (Jung et al., 2012; Won et al., 2012; Martin et al., 2016a, b; Bosch-Bouju et al., 2016; Wei et al., 2016; Thomazeau et al., 2017).

The lack of eCB-mediated LTD in the mPFC and accumbens of mice deprived of dietary n-3 PUFAs prompted us to test the new hypothesis that amplification of some of its key molecular elements could restore synaptic plasticity (Fig. 9A, schematic).

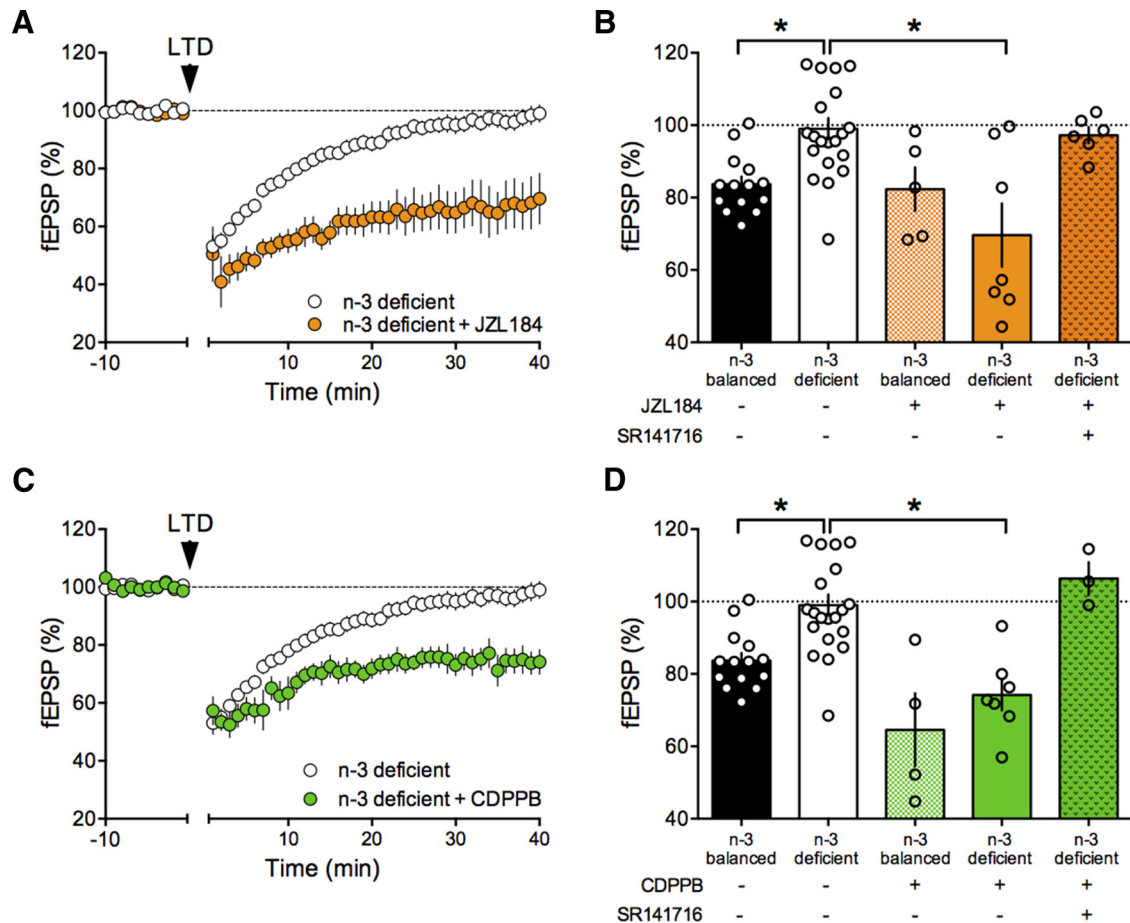


Figure 10. Pharmacological amplification mGlu₅-eCB signaling restores eCB synaptic plasticity in the mPFC of n-3-deficient mice. **A**, Time course of eCB-LTD in n-3-deficient mPFC slices in control conditions (white circles) or preincubated for 45 min in 1 μ M JZL184 (orange circles). **B**, Summary bar chart of fEPSP percentage change from baseline 38–40 min after 10 Hz stimulation in n-3-balanced and n-3-deficient mice before and after treatment with JZL184 (orange bars) \pm SR141716A (5 μ M) ($F_{(4,48)} = 8.062$, $p < 0.05$, one-way ANOVA). **C**, Time course of normalized fEPSP responses from mPFC slices in control conditions (white circles) or preincubated with CDPBB (green circles). **D**, Summary bar chart of fEPSP percentage change from baseline 38–40 min after 10 Hz stimulation in n-3-balanced and n-3-deficient mice with or without treatment with CDPBB \pm SR141716A ($F_{(4,44)} = 12.30$, $p < 0.05$, one-way ANOVA). Error bars indicate SEM. * $p < 0.05$.

We first tested whether inhibition of 2-arachidonylglycerol (2-AG)'s main degrading enzyme monoacylglycerol lipase (MGL) would allow 2-AG levels to reach the threshold for LTD induction in n-3-deficient mice (Fig. 9A). Accumbens slices were incubated with the MGL inhibitor JZL184 (1 μ M) for 45–90 min before LTD induction. This treatment was sufficient to restore eCB-LTD in the accumbens of n-3-deficient mice (Fig. 9B–D). JZL184 had no effect in mice fed with a n-3-balanced diet (Fig. 9D). The CB₁R antagonist SR141716A (5 μ M) blocked the JZL184 rescue, showing that CB₁R activation mediates the effects of the MGL inhibitor in the accumbens (Fig. 9D). Next, we reasoned that amplification of the most upstream element of the eCB signaling complex (i.e., mGlu₅) might also restore LTD. To this aim, we used CDPBB (10 μ M), a well-described positive allosteric modulator (PAM) of mGlu₅ (Nickols and Conn, 2014) (Fig. 9A). CDPBB fully restored the ability of excitatory accumbens synapses to express LTD in the n-3 PUFA-deficient group (Fig. 9C) while having no effect in the n-3 PUFA-balanced group (Fig. 9D). Importantly, we verified that CB₁R ultimately mediated the effects of CDPBB; preincubation with SR141716A prevented the CDPBB rescue (Fig. 9D).

We found that JZL184 allowed the expression of normal LTD in the mPFC of n-3-deficient mice (Fig. 10A) while having no effect in slices prepared from the n-3-balanced-group (Fig. 10B). The JZL184 rescue was fully prevented by the CB₁R antagonist

(Fig. 10B). mPFC LTD was also restored in slices acutely exposed to the mGlu₅ PAM and CDPBB (Fig. 10C). Antagonizing CB₁R similarly prevented this rescue of LTD (Fig. 10D), suggesting that, as in the accumbens, CDPBB acted on mGlu₅ to increase eCB production. Finally, we tested the response of LTP to the two LTD rescuing compounds, JZL184 and CDPBB. Enhancing 2-AG levels also restored LTP (Fig. 11A), an effect that was not blocked by the CB₁R antagonist (Fig. 11B). Postsynaptic mGlu₅ positively modulate NMDAR and mGlu₅ PAMs have been shown to rescue NMDAR-dependent synaptic plasticity and behavior in autism and Huntington models (Won et al., 2012; Doria et al., 2015). We found that CDPBB restored LTP in the n-3-deficient group (Fig. 11C,D). CB₁R antagonism did not prevent the recovery effect of CDPBB on LTP (Fig. 11D; see Discussion). An integrated interpretation of these results is that CDPBB potentiated mGlu₅, which in turn enhanced NMDAR responses, allowing the expression of full-blown LTP in n-3-deficient mice.

Amplification of the mGlu₅-eCB signaling complex rescues the behavioral deficits associated with n-3 PUFA deficiency

We next tested whether pharmacological enhancements of the mGlu₅-eCB signaling complex could normalize behavioral deficits.

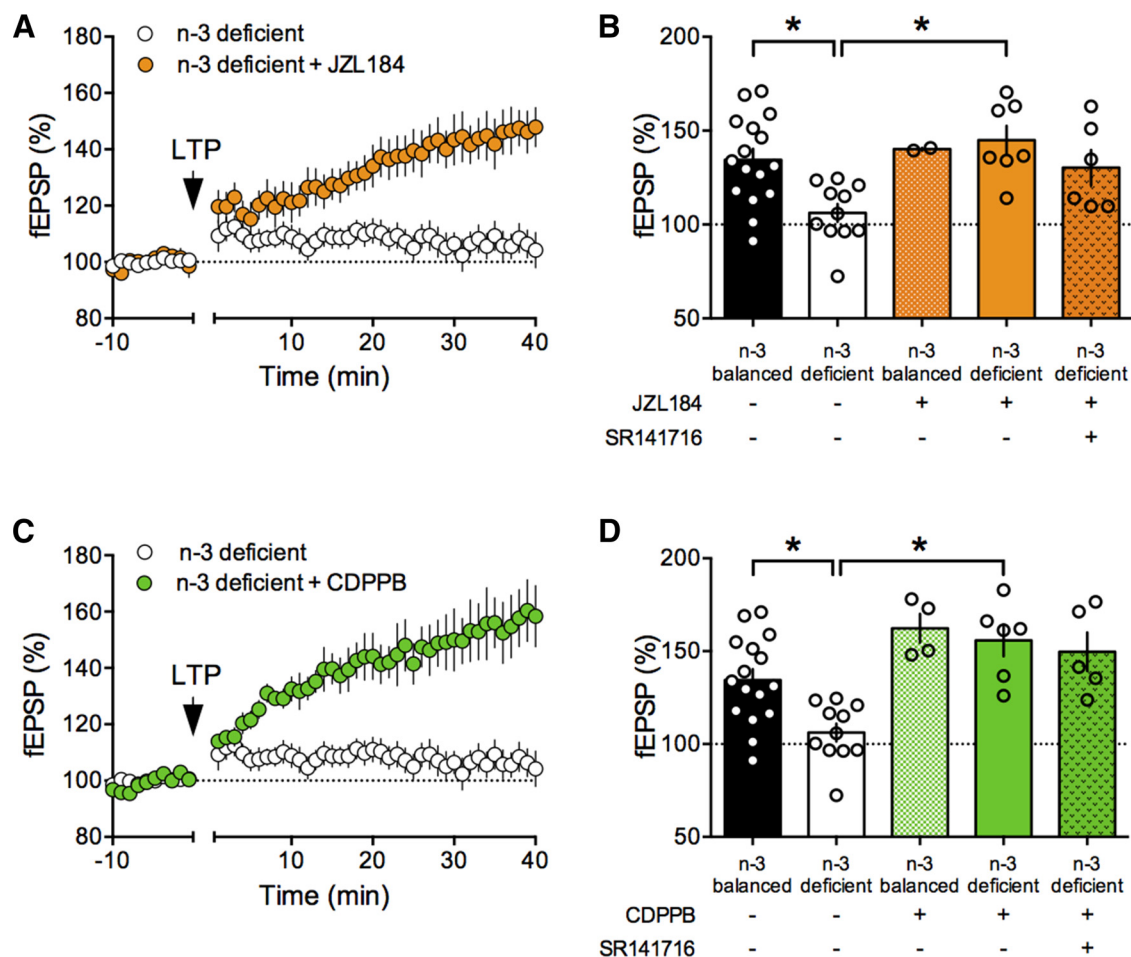


Figure 11. Pharmacological amplification mGlu₅-eCB signaling restores mPFC LTP in n-3-deficient mice. **A**, Time course of LTP in n-3-deficient mPFC slices in control conditions (white circles) or preincubated with JZL184 (45 min, 1 μ M, orange circles). **B**, Summary bar chart of fEPSP percentage change from baseline 38–40 min after LTP induction in n-3-balanced and n-3-deficient mice before and after treatment with JZL184 (orange bars) \pm SR141716A (5 μ M) ($F_{(4,37)} = 4.834$, $p < 0.05$, one-way ANOVA). **C**, Time course of LTP in mPFC slices in control conditions (white circles) or preincubated with CDPPB (green circles). **D**, Summary bar chart of fEPSP percentage change from baseline 38–40 min after LTP induction in n-3-balanced and n-3-deficient mice with or without treatment with CDPPB \pm SR141716A ($F_{(4,37)} = 9.346$, $p < 0.05$, one-way ANOVA). Error bars indicate SEM. * $p < 0.05$.

Inhibition of 2-AG degradation corrects cognitive and emotional deficits in n-3-deficient mice

Acute pharmacological enhancement of 2-AG with JZL184 (16 mg/kg, i.p.) ameliorated cognitive deficits in n-3-deficient mice. Social interaction (Fig. 12A) and temporal order memory deficits (Fig. 12B) were normalized, suggesting that the rescue effects of JZL184 extended to the cognitive domain. Furthermore, in the n-3-deficient group, time spent in the center of an open field (Fig. 12C), time spent in the open arm of an elevated plus maze (Fig. 12D), number of entries in the risk zone (Fig. 12E), head dipping in an elevated plus maze (Fig. 12F), number of marbles buried (Fig. 12G), and sucrose preference (Fig. 12H) were normalized. Of note, JZL184 had no effect on behaviors in the n-3-balanced group, indicating selectivity of the drug's effects to the disease state at the dose used. Thus, blocking 2-AG's degradation normalized emotional and cognitive deficits induced by the n-3 PUFAs deficient diet.

Potentiation of mGlu₅ rescues cognitive and emotional behaviors in n-3-deficient mice

Modulators of mGlu₅ receptors may be useful in the treatment of neuropsychiatric disease (Belzung, 2001; Won et al., 2012; Doria et al., 2015). In the n-3-deficient group, social cognition and

temporal order memory (Fig. 13A,B), time spent in the center of an open field (Fig. 13C), time spent in the open arm (Fig. 13D), number of entries in the risk zone and head dipping in an elevated plus maze (Fig. 13E,F), number of marbles buried (Fig. 13G), and sucrose preference (Fig. 13H) were normalized following injection of CDPPB (10 mg/kg, i.p.). We determined that, in the same conditions, CDPPB had no effect on these behaviors in the n-3-balanced group, indicating selectivity of the drug's effects to the disease-state at the dose used.

Discussion

Here, we developed a new mouse model of n-3 PUFA deficiency commencing in adolescence and continuing through adulthood to better understand the consequences of nutritional imbalance during a period of ongoing structural and functional refinements in frontostriatal circuitry. The results are threefold: (1) we found that starting n-3 PUFA-deficient diet at adolescence increases anxiety-related behavior and anhedonia in adults in agreement with the idea that malnutrition is accompanied by enhanced susceptibility to emotional distress. We also discovered that cognitive functions are impeded by nutritional deficits after the neurodevelopmental period. (2) The behavioral deficits were paralleled by a reduction of the working range at excitatory syn-

apses in the mPFC and accumbens where mGlu₅/eCB-LTD and NMDAR-dependent LTP were abolished. (3) Pharmacological enhancement of the mGlu₅/eCB signaling complex following positive allosteric modulation of mGlu₅ or inhibition of 2-AG degradation normalized the behavioral repertoire and synaptic plasticity in n-3-deficient mice. These findings strongly suggest that an n-3 PUFA-balanced diet is necessary throughout life to maintain optimal frontostriatal function, cognition and emotional behavior.

Nutrition influences nutrition. Adolescent exposure to dietary n-3 PUFA deficiency does not influence adult spatial memory, despite reducing DHA in the hippocampus (Delpech et al., 2015). In contrast, perinatal exposure to a deficient n-3 PUFA diet triggers adult memory deficits (Moranis et al., 2012; Lépinay et al., 2015). Nutritional n-3 PUFA deficits during adolescence impact mPFC development (Bondi et al., 2014). Here we show, for the first time, that nutritional n-3 deficiency impairs mPFC-dependent cognition, suggesting that adolescent nutrition is a determinant of adult behaviors and that balanced nutrition during the perinatal period does not protect from later dietary deficits. Furthermore, we also observed excessive anxiety and reduced sucrose preference in adult n-3-deficient mice, showing that, when dietary n-3 PUFA deficiency starts in adolescence, it impedes emotional behaviors later in life. These findings support the idea that malnutrition is accompanied by enhanced susceptibility to emotional distress and that adolescent nutritional n-3 PUFA deficiency is a major environmental risk factor associated with neuropsychiatric disorders (Simopoulos, 2009; Bazinet and Layé, 2014; Bosch-Bouju et al., 2016).

In mammals, the mPFC is one of the most evolved brain regions, and its malfunctions are a common denominator in several neuropsychiatric diseases, including (but not limited to) mental retardation, autism, schizophrenia, depression, and addiction (Goto et al., 2010). Previously, our laboratories showed that lifelong n-3 deficiency impairs eCB-mediated synaptic plasticity in the mPFC (Lafourcade et al., 2007; Larrieu et al., 2012). The idea that dietary PUFAs strongly control mPFC functions was reinforced by the results that n-3 deficiency starting at adolescence led to specific alterations of cognitive tasks classically associated with mPFC but not hippocampus. The lack of mPFC eCB-LTD cannot be readily explained by a massive reduction of CB₁R function as previously observed after an entire life of n-3 PUFA deficiency (Lafourcade et al., 2011). In marked contrast, presynaptic CB₁R potency was normal in the mPFC, suggesting that peri-

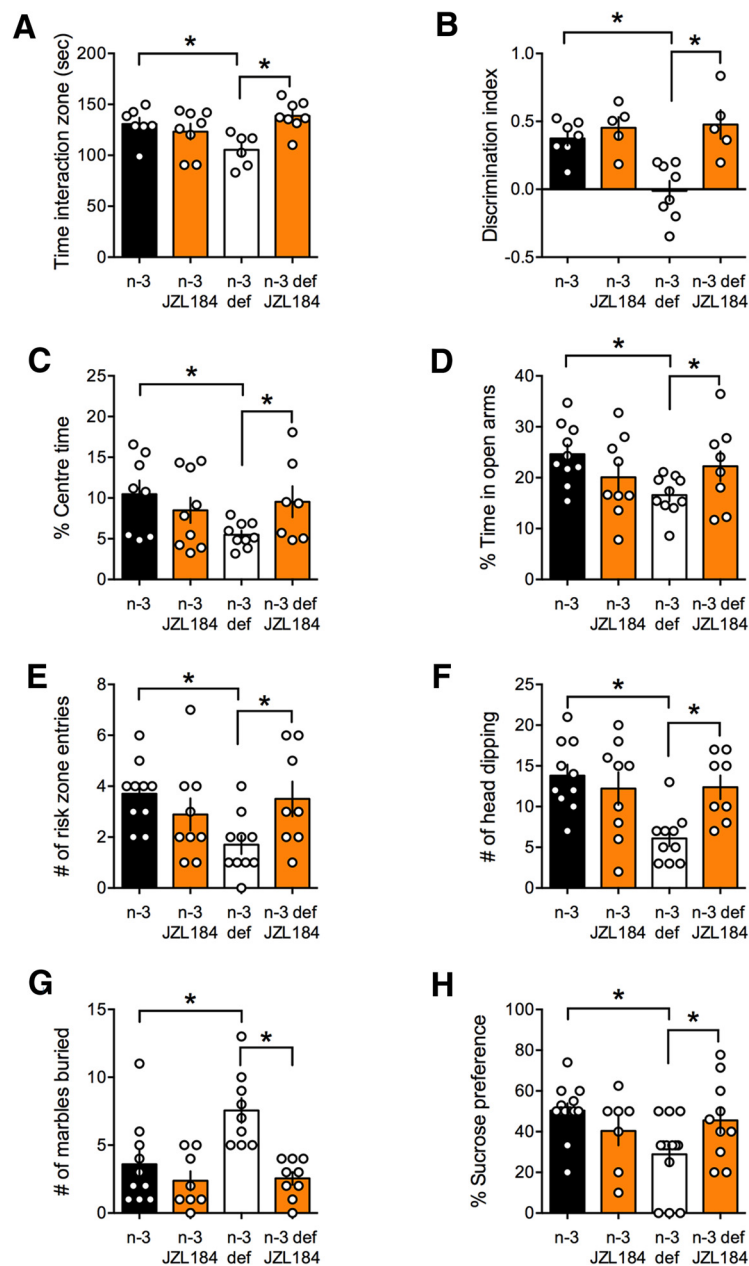


Figure 12. Inhibition of 2-AG degradation corrects cognitive and emotional deficits in n-3-deficient mice. JZL184 (16 mg/kg, i.p.) rescued cognitive deficits observed in the social interaction (**A**; $F_{(\text{dietxtreat})} = 9.596, p < 0.05$) and temporal order memory (**B**; $F_{(\text{dietxtreat})} = 6.948, p < 0.05$) tasks in n-3 PUFA-deficient mice. Enhancing 2-AG levels also normalized anxiety-related behaviors as measured by (**C**; $F_{(\text{dietxtreat})} = 4.201, p < 0.05$) the time spent in the center of the open field; (**D**; $F_{(\text{dietxtreat})} = 5.569, p < 0.05$) the percentage of time spent in the open arm; (**E**; $F_{(\text{dietxtreat})} = 6.362, p < 0.05$) the frequency of entries in the risk zone in the open arms of the elevated plus maze test; and (**F**; $F_{(\text{dietxtreat})} = 7.136, p < 0.05$) the frequency of head dipping. JZL184 corrected the deficits in the marble burying test (**G**; $F_{(\text{dietxtreat})} = 5.291, p < 0.05$) and reduced anhedonia in sucrose preference of the n-3-deficient group (**H**; $F_{(\text{dietxtreat})} = 4.306, p < 0.05$). All measures were performed 6 h after JZL184 treatment. Scatter dot plot represents each animal. Error bars indicate SEM. * $p < 0.05$.

natal n-3 PUFA deficiency was instrumental in reducing CB₁R functions in our previous study (Lafourcade et al., 2011).

Dietary n-3 PUFAs protect LTP and cognition from aging and environmental insults (McGahon et al., 1999; Kotani et al., 2006; Lynch et al., 2007; Cao et al., 2010; Kelly et al., 2011; Kavraal et al., 2012; Patten et al., 2013). Consistent with this idea, we report that NMDAR-LTP in the mPFC was absent in the n-3-deficient group. LTP was still expressed in the hippocampus of mice that had received the same dietary treatment (Delpech et al., 2015),

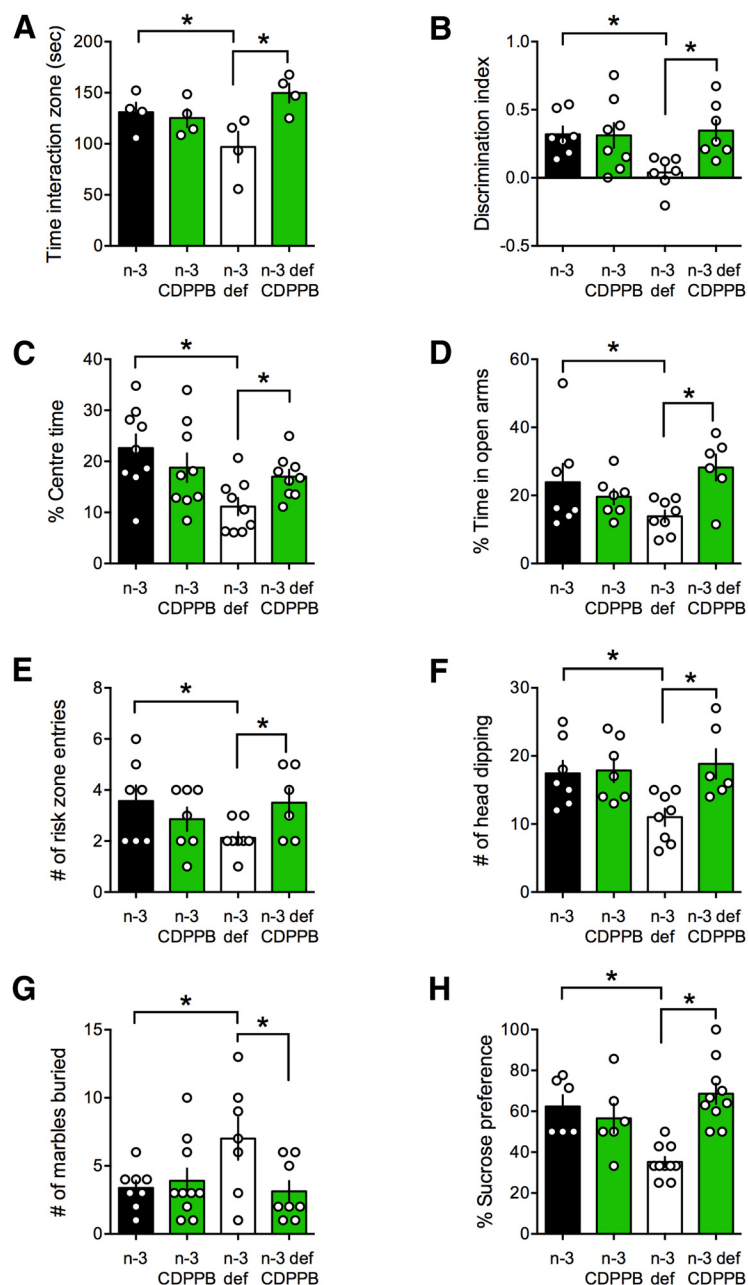


Figure 13. Positive allosteric modulation of mGlu₅ normalized behavioral deficits in n-3-deficient mice. CDPPB rescued cognitive deficits observed in the social interaction (**A**; $F_{(\text{dietxtreat})} = 7.066, p < 0.05$) and temporal order memory (**B**; $F_{(\text{dietxtreat})} = 4.818, p < 0.05$) tasks in n-3 PUFA-deficient mice. CDPPB also normalized anxiety-related behaviors as measured by (**C**; $F_{(\text{dietxtreat})} = 5.496, p < 0.05$) the time spent in the center of the open field; (**D**; $F_{(\text{dietxtreat})} = 6.925, p < 0.05$) the percentage of time spent in the open arm; and (**E**; $F_{(\text{dietxtreat})} = 4.911, p < 0.05$) the frequency of entries in the risk zone in the open arms of the elevated plus maze test as well as the frequency of head dipping (**F**; $F_{(\text{dietxtreat})} = 4.5127, p < 0.05$). CDPPB corrected the deficits in the marble burying test (**G**; $F_{(\text{dietxtreat})} = 5.291, p < 0.05$) and reduced anhedonia in sucrose preference of the n-3-deficient group (**H**; $F_{(\text{dietxtreat})} = 15.360, p < 0.01$). All measures were performed 6 h after CDPPB treatment. Scatter dot plot represents each animal. Error bars indicate SEM. * $p < 0.05$.

suggesting a higher sensitivity of the mPFC to PUFA deficiency. The latter hypothesis is supported by our finding that mPFC-dependent, but not hippocampal-dependent cognition was affected in n-3 PUFA-deficient mice.

The nucleus accumbens, widely implicated in reward and motivation (Carelli, 2002; Carlezon and Thomas, 2009), has also recently drawn attention for its participation in the emotional repertoire, including anxiety and compulsivity (Sturm et al., 2003; Galvan et al., 2006; Millet et al., 2014; Bagot et al., 2015;

Floresco, 2015). While deregulated neuronal and synaptic activity in the accumbens is also observed in naturalistic rodent models of addiction (Kasanetz et al., 2010) or depression (Bosch-Bouju et al., 2016), the accumbens by and large was not identified as a primary target of dietary PUFA deficiency. Here, we found that n-3 deficiency beginning during adolescence is sufficient to abolish eCB-LTD in adult mice. The fact that basic intrinsic and synaptic properties of accumbens medium spiny or mPFC pyramidal neurons were unchanged in n-3-deficient mice made the lack of eCB-mediated LTD even more remarkable. The lack of eCB-LTD cannot be readily explained by a massive reduction of CB₁R functions: accumbens presynaptic CB₁R efficacy and potency were not affected. Thus, it is safe to conclude that reduction of CB₁R potency is not a general response to n-3 PUFA deficiency, an idea supported by our previous report of normal CB₁R presynaptic functions in lifelong n-3-deficient hippocampi (Thomazeau et al., 2017). Moreover, based on our mGlu_{2/3} LTD experiments, the presynaptic LTD machinery is presumed intact. It only makes sense that the central eCB system shows such sensitivity to dietary PUFAs. Not only are PUFAs precursors of eCBs which share common biosynthetic pathways (Katona and Freund, 2012), but nanoscale modifications of the mGlu₅/eCB signalosome in absence of CB₁R desensitization or other gross alterations impede mGlu₅ plasticity (Jung et al., 2012). Further work will be needed to understand how n-3 PUFA deficiency alters eCB-LTD in the mPFC and accumbens.

In the CNS, eCBs are produced on-demand by a multimolecular signaling complex made of a spatially organized network of postsynaptic G-protein-coupled receptors of various neurotransmitters, scaffolding proteins, ion channels, intracellular calcium stores, as well as synthesizing and degrading enzymes (Katona and Freund, 2012). The lack of eCB-LTD in both mPFC and accumbens in n-3-deficient adult mice was reminiscent of what we reported in unrelated synaptopathies: the *fmr1*^{−/y} model of Fragile X (Jung et al., 2012), mice overexpressing the

Down-syndrome gene candidate Dyrk1A (Thomazeau et al., 2014), mice submitted to social stress (Bosch-Bouju et al., 2016), and, more recently, lifelong n-3 deprivation (Thomazeau et al., 2017). In all cases, inhibition of the eCB-deactivating enzyme MGL normalized prefrontal and hippocampal eCB-LTD and in *fmr1*^{−/y} acutely corrected behavioral deficits, including anxiety traits (Jung et al., 2012). Here, we found that JZL184, an MGL inhibitor, restored LTD in n-3-deficient mice. It is important to emphasize that the JZL184 effect on eCB-LTD was mediated by

CB1R, excluding off-target effects of the molecule. Surprisingly, enhancing 2-AG levels also restored LTP. In contrast to LTD, the rescue of LTP did not require CB₁R. It is not clear how 2-AG elevation restores LTP. A rescue of LTP through normalization of heterosynaptic plasticity, as we observed in the hippocampus (Thomazeau et al., 2017), is excluded here where GABA-A receptors were blocked. Another possibility is that 2-AG potentiates NMDAR in a CB₁R-independent manner as recently reported (Yang et al., 2014). MGL inhibition decreases AA levels (Kerr et al., 2013) and AA potentiates NMDAR (Miller et al., 1992). Thus, 2-AG elevation is able to modulate NMDAR independently of CB₁R.

NMDAR and mGlu_{1/5} are the principal postsynaptic effectors of LTD and LTP (Emes et al., 2008; Coba et al., 2009; Melis and Pistis, 2012; Grant, 2016). In the accumbens and mPFC, postsynaptic mGlu₅ receptors are indispensable to eCB-LTD (Robbe et al., 2002; Lafourcade et al., 2007). The pharmacology of the family of mGlu receptors offers great therapeutic promise, notably because of the development of subtype-selective allosteric modulators. We reasoned that amplification of mGlu₅ may enhance eCB-LTD in n-3-deficient mice. We found that a positive allosteric mGlu₅ modulator normalized n-3-deficient LTD in mPFC and accumbens. A possible confound in this interpretation is that mGlu₅ positively interacts with NMDAR (Coba et al., 2009). Indeed, allosteric modulation of mGlu₅ has proven beneficial in restoring NMDAR functions in a mouse model of autism (Won et al., 2012). In contrast, we found here that CB₁R was necessary for the CDPPB effects on LTD, in line with our working hypothesis that the mGlu₅ modulator CDPPB enhances eCB signaling.

We found that acute *in vivo* treatment with either an inhibitor of 2-AG degradation or a positive allosteric modulator of mGlu₅ corrected excessive anxiety/anhedonia and cognitive deficits in adult n-3 PUFA-deficient mice. These results are important for two reasons: (1) they confirm the key roles of the mGlu₅-eCB signaling complex; and (2) they shed light on potential therapeutic routes to compensate for dietary n-3 PUFA deficiency. These new data indicate that an n-3 PUFA balanced diet is necessary throughout life to maintain optimal cognition, emotional control, and synaptic plasticity. By demonstrating that enhancement of mGlu₅ activity or 2-AG levels corrects synaptic and behavioral deficits, this work reveals two new therapeutic routes to treat malnutrition-induced cognitive and emotional impairments. These results warrant a thorough assessment of the behavioral and synaptic consequences of adolescent and adult nutritional deficiencies. In conclusion, these data add to the growing evidence that the mGlu₅-eCB signalosome is a key postsynaptic hub whose functional integrity guarantees a maximal working synaptic range and associated behavioral flexibility.

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