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Deficit in Prepulse Inhibition in Mice Caused by Dietary n-3 Fatty Acid Deficiency

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Abstract

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) may be biosynthesized from a precursor α -linolenic acid (LNA) or obtained preformed in the diet. Dams were fed four diets with different levels of the various n-3 fatty acids during pregnancy and lactation, and their offspring were weaned to the same diets: “n-3 Deficient”, containing (as % total fatty acids) 0.07% of LNA; “Low LNA” (0.4%); “High LNA” (4.8%); and a “DHA+EPA” diet, containing 0.4% of LNA, 2% DHA and 2% EPA. Sensorimotor gating was measured by prepulse inhibition (PPI) of the acoustic startle response in C57Bl6 mice. The n-3 Deficient and Low LNA diets caused a substantial deficit in PPI compared to the DHA+EPA diet, whereas the High LNA diet induced a less pronounced, but significant reduction of PPI. These are the first data that demonstrate a deficit in sensorimotor gating in rodents caused by an inadequate amount of the n-3 fatty acids in the diet. Our results differentiate the effects of a High LNA diet from one with added EPA and DHA even though the difference in brain DHA content is only 12% between these dietary groups.

Keywords

prepulse inhibition; PPI; DHA; n-3 fatty acid deficiency

Introduction

The long-chain polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) can be synthesized from a dietary precursor α -linolenic acid (LNA, 18:3n-3) or obtained directly preformed from the diet. PUFA are important components of membrane phospholipids in neurons, glial and immune cells, and are involved in many functions of the CNS and immune system. DHA is the most abundant PUFA in the brain and plays an important role in learning and memory (summarized in Fedorova & Salem, 2006), and both DHA and EPA provide benefits for the treatment of cardiovascular and inflammatory diseases, and also in mood disorders (Caughey et al., 1996; Freeman et al.,

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2006; Kamphuis et al., 2006; Schwellenbach et al., 2006; Ross et al., 2007, Ryan et al., 2009).

DHA has been known to play a significant role in the brain: incorporation of DHA into brain cell membranes improves membrane fluidity, which may contribute to brain function via their ability to bind ligands and initiate a series of signal transduction processes (Mitchell et al., 2003; Puskas & Kitajka, 2006; Wood, 1990). DHA may also influence brain function by affecting production and function of neurotransmitters such as serotonin and dopamine (for review, see Chalon, 2006), inhibition of phospholipase A₂ (Strokin et al., 2004), and inhibition of protein kinase C (Kim et al., 2001).

In this report, we studied prepulse inhibition (PPI) of the acoustic startle reflex in mice fed diets containing different amount of n-3 fatty acids for two generations. PPI refers to the reduction of a reaction to a startling stimulus when it is preceded by a low-intensity prepulse. The effect of the prepulse upon pulse processing is recognized as an operational measure of sensorimotor gating, and is demonstrable across species from mice to humans. It has been proposed that the mechanism underlying PPI regulates sensory input by filtering out irrelevant or distracting stimuli to prevent sensory information overflow and to allow for selective and efficient processing of relevant information (Swerdlow and Geyer, 1998). PPI can be disrupted in animals by pharmacological or developmental manipulations (for review, see Geyer et al., 2001). PPI is thought to reflect an automatic, involuntary, preattentive inhibitory process that functions to protect the initial processing of the prepulse (Graham and Murray, 1977). Diminished PPI has been consistently demonstrated in patients with a variety of neuropsychiatric disorders such as schizophrenia, schizotypal personality disorder, Huntington's disease, obsessive compulsive disorder, Tourette's syndrome, bipolar disorder and attention deficit disorder (summarized in Swerdlow and Geyer, 1998). These patients are characterized by a general reduction of the ability to gate intrusive sensory, motor or cognitive information, leading to sensory flooding and cognitive fragmentation and, consequently, to a significant deficit in attention and information processing. The experimental diets employed here were designed to provide for varying levels of DHA in the nervous system. It was hypothesized that PPI would vary with nervous system DHA content.

Fatty acids have been the focus of intense study in schizophrenia, one of the most severe mental illnesses. Membrane phospholipid hypothesis (Horrobin, 1998) proposes that vulnerability to schizophrenia is related to a genetically determined abnormality of phospholipid metabolism, which can be modified by nutrition. This is based, in part, on the observation of reduced levels of DHA in cell membrane phospholipids (Assies et al., 2001; Yao et al., 2000), decreased synthesis and an increased breakdown of membrane phospholipids (Fukuzako et al., 1999; Pettegrew et al., 1991; Pettegrew et al., 1993; Stanley et al., 1995), and increased levels of calcium-independent phospholipase A₂ (Gattaz et al., 1995; Ross et al., 1999; Ross, 2003) in brains of schizophrenic patients. Studies have reported a significant correlation between abnormal phospholipid metabolism and schizophrenic symptoms (Fukuzako et al., 1996; Shioiri et al., 1997).

Methods

Animals and Diets

Two day pregnant C57Bl6 mice (Charles River, Portage, MI) were divided into four dietary groups (n=12 per group) on a pseudorandom basis with the constraint that all groups had the same average body weight. All dams were observed for mortality and gross abnormalities twice daily (morning and afternoon). Detailed physical examinations of each animal were made prior to randomization and weekly during the study. Observations included general condition, skin and fur, eyes, nose, oral cavity, abdomen, palpitation for masses, and body weight

measurements. Litters were observed for the number of live and dead pups and for abnormalities as soon as possible after delivery. Thereafter, litters were observed twice daily for irregularities and the presence of nests in cages was noted. Live pups were weighed at birth and at 4, 7, 14 and 21 days of age. On day 4 of lactation, each litter with greater than seven pups was culled to that number while retaining all males where possible. The age at which pups exhibited several physical developmental landmarks (e.g. pinna detachment, eye opening, and incisor eruption) was noted.

Dams were single-housed and fed through gestation and lactation four custom diets based on a modification of the American Institute of Nutrition (AIN-93) diet and prepared commercially (Dyets, Bethlehem, PA). The offspring was weaned to the corresponding diets and behavioral testing started when the offspring were 8 wk old. Only males were tested; each mouse within a dietary group was selected from a different litter; therefore, one animal from each litter was tested in each dietary group. After weaning, the test animals were housed four in a cage; their environment was structurally enriched with nesting material and red plastic tunnels.

All diets (Table 1) had the same basal macronutrients, vitamins, minerals and basal fats, hydrogenated coconut and high oleic safflower oils (Oilseeds Int., San Francisco, CA); the total fat content in all diets was 10g/100g diet. The differences between the diets was solely the amount and type of n-3 fatty acids: n-3 Deficient (n-3 Def) contained 0.07% of LNA; Low LNA contained 0.38% of total fatty acids as LNA; High LNA, 4.8% of LNA; and the DHA +EPA diet contained 0.38% of LNA, 2% DHA and 2% EPA. The differing fatty acid profiles were achieved by adding a small amount of flaxseed oil to low and High LNA diets and menhaden oil to the DHA+EPA diet.

Acoustic Startle Protocol

The acoustic startle measure was based on the reflexive whole-body flinch, or startle response, following exposure to a sudden noise. The timing of appearance of the auditory startle reflex was assessed in pups daily starting from PND 10 through 13 in a startle chamber (SR-LAB, San Diego Instruments, San Diego, CA). A positive auditory startle response was considered to be the sudden, brief extension of hind limbs in response to a 100 dB stimulus. Two consecutive responses were required for the startle reflex to be considered present. Only one randomly chosen male pup from each litter was tested. This pup was not later used for the behavioral testing in the adulthood. When testing prepulse inhibition, mice were placed in a small plexiglas cylinder within a larger, sound-attenuating chamber (Med Associates Inc., St. Albans, VT). The cylinder was seated upon a piezoelectric transducer, which allowed vibrations to be quantified and displayed on a computer. The background sound level (70dB) and calibration of the acoustic stimuli were confirmed with a digital sound level meter (Radio Shack Sound Level Meter). First, mice were placed in the Plexiglas enclosure for a 5 min acclimation period with a 70 dB background noise. Immediately following the acclimation period each mouse was presented with the test session comprised of three blocks of discrete test trials consisting of one or both of two trial types. The trial types included pulse-alone trials and prepulse-*plus*-pulse trials. A pulse-alone trial consisted of a 120 dB, 40 ms noise burst presented alone. The prepulse-*plus*-pulse trials employed prepulses of 75, 80, and 85 dB, which corresponded to 5, 10, and 15 dB above background, respectively, in addition to a pulse stimulus of 120 db. Duration of the prepulse stimuli was 20 ms, and 100 ms after initiating of this prepulse, the 40 ms pulse trial was given. The first block consisted of six trials of pulse-alone trials. Subsequently, the second block consisted of a predetermined pseudo-randomized sequence of 10 trials of each of the following trial types: (1) pulse-alone and (2) prepulse-*plus*-pulse trials of each of the three levels of prepulse. The final block concludes the session with six consecutive pulse-alone trials for a total of 52 trials conducted during the session. The interval between trials averaged 15 s, ranging from 10 to 20 s. In order to gauge startle

habituation, startle responses in the first and third blocks of six consecutive pulse-alone trials were analyzed. Then, the mean startle reactivity of the last block was compared against that of the first block as a ratio of Block3/Block1 mean magnitudes. The reactivity scores following the 120 dB stimulus obtained during only the second block of both the prepulse-*plus*-pulse and pulse-alone trials were utilized to calculate PPI. To measure PPI, reactivity scores from the prepulse-*plus*-pulse trials at different prepulse intensities were averaged and analyzed with respect to the mean reactivity score registered during the pulse-alone trials. PPI is defined as the percent reduction in startle reactivity in the presence of the prepulse stimulus [$100 - (100 \times \text{startle reactivity for prepulse-plus-pulse trial} / \text{startle reactivity for pulse-alone trial})$].

Fatty Acid Analysis

The mice were decapitated at the end of the study (12 wk of age); brains were rapidly removed and subjected to total lipid extraction by a modification of the Folch method (Folch et al., 1957). The total lipid extract was transmethylated with 14% BF₃-methanol at 100°C for 60 min by a modification of the method of Morrison and Smith and the methyl esters analyzed by gas chromatography as previously described (Salem, Reyzer & Karanian, 1996). The fatty acid methyl esters from 10:0 to 24:1n-9 were identified by comparison with the retention times of a quantitative standard mixture (462; Nu-Chek-Prep, Elysian, MN).

Statistical Analysis

The acoustic startle response data were analyzed using Statistica 7 (StatSoft, Inc., Tulsa, OK) to perform one-way (for startle amplitudes) and two-way repeated measures (for prepulse inhibition) ANOVA with Diet as a between-groups factor and Prepulse Intensity (Time Interval) as a within-groups (repeated) factor. Startle habituation was analyzed first using two-way ANOVA for startle response magnitude in the first and last blocks with Diet as a between-groups factor and Block as a within-group (repeated) factor; thereafter one-way ANOVA for the Block3/Block1 ratios was used. Brain concentrations of fatty acids were analyzed separately using one-way ANOVA. Significant effects were analyzed further using post-hoc Tukey HSD test. The data are presented as mean $\pm$ SEM.

Results

No differences were observed between dams from different dietary groups in general health observations, body weight, gestation length, litter size, number of live pups at birth and at 4, 7, 14 and 21 days of age. All dams but one (in the Low LNA group) were able to build nests for their pups. Pup appearance, weight gain, and developmental landmarks were similar in all dietary groups. The auditory startle reflex appeared at about the same age in different experimental groups: on PND 12.2 ± 0.4 , 12.4 ± 0.6 , 12.9 ± 0.8 , and 12.7 ± 0.5 among pups in the n-3 Def, Low LNA, High LNA, and in the DHA+EPA group, respectively.

There were no differences in body weight between dietary groups at any time point (data not shown), and no significant differences between dietary groups with respect to the total brain fatty acid concentration. The different dietary treatments was successful in inducing a loss in brain DHA as mice in the n-3 Def group exhibited a 60, 51 and 46% loss of total brain DHA as compared with the DHA+EPA group, High LNA and Low LNA groups (Fig. 1). The low level of DHA in the n-3 Def group was largely compensated for by a marked increase in brain docosapentaenoic acid (DPAn-6), arachidonic acid (ARA) and docosatetraenoic acid (DTA). The Low and High LNA groups did not differ significantly in their brain DHA level, but the Low LNA group contained significant higher levels of DPAn-6, ARA and DTA levels than the High LNA group. Mice in the High LNA group accumulated about 10% less DHA in the brain than mice in the DHA+EPA group. The DPAn-6 and ARA levels were the same in these groups, but the DTA level was significantly higher in the High LNA group.

Locomotor and anxiety-related behavior in adult animals was not influenced by dietary treatment as there were no differences in the open-field and elevated-plus maze performance between dietary groups.

Analysis of startle response magnitude to 120 dB stimuli in the first block (pulse-alone trials) revealed no differences between dietary groups: n-3 Def mice had an average response amplitude of 591.1 ± 35.9 ; Low LNA, 655.5 ± 57.4 ; High LNA, 560.1 ± 49.0 ; DHA+EPA group, 620.1 ± 52.7 .

We measured prepulse inhibition of acoustic startle response following three different prepulse intensities: 75, 80 and 85 dB (Fig. 2) and found a significant effect of Diet ($F(3,99)=16.72$, $p<0.0001$), of Prepulse Intensity ($F(2,33)=22.19$, $p<0.0001$), but no interaction between Diet $\times$ Prepulse ($F(6, 99)=0.24$, NS). Further analysis using the post-hoc Tukey HSD test demonstrated that the n-3 Def and Low LNA group were not different statistically, but they were different from the High LNA group ($p<0.05$) and DHA+EPA group ($p<0.001$), indicating that the n-3 Def and Low LNA diets produced a significant deficit in PPI compare to the High LNA and DHA+EPA groups. Further, mice on the High LNA diet demonstrated a decrease in the magnitude of PPI compare to mice from the DHA+EPA group ($p<0.01$).

Repeated measures ANOVA of startle response magnitude to 120 dB stimuli in the first and last blocks (pulse-alone trials) did not reveal statistically significant effects of Diet, $F(3,92) = 2.004$, NS, or Block, $F(1,92) = 2.73$, NS, but a significant Diet $\times$ Block interaction: $F(3,92) = 5.91$, $p<0.05$. Further analysis using the post-hoc Tukey HSD test demonstrated that the n-3 Def and Low LNA group were different statistically from the High LNA group and DHA+EPA group ($p<0.05$). Comparison of the ratio of block3 to block1 responses (Fig. 3) showed that mice fed the n-3 Def and the Low LNA diets did not change their startle responses between block 1 and 3 of the test: the ratio of block3/block1 was about 1, while the High LNA and the DHA+EPA groups exhibited diminished responses during the test to 78% and 73% of the initial value, respectively.

Discussion

The present study assessed the effect of different levels of n-3 fatty acids in the diet on PPI when animals were fed throughout gestation, lactation, and into adulthood. The influence of the diet on acoustic startle reflex when intervention was limited to distinct developmental stages (i.e., during prenatal exposure, early postnatal exposure, pubertal exposure, adult exposure) was beyond the scope of the study, as well as changes in startle responses in dams. Previous studies have found that altered postnatal maternal care can produce disruptions of PPI in adulthood. For example, PPI deficits have been found in rats and mice subjected to the stress of preweaning repeated maternal separation by some researchers (Ellenbroek & Cools, 2002; Geyer et al., 1993) but not by others (Lehmann et al., 2000; Millstein et al., 2006). Thus, given the fact that behavioral alterations in the dam might lead to long-term impairment of the offspring, we evaluated dam maternal behavior as evidenced by nest building and pup development and growth. No gross abnormalities were observed in any experimental group; all pups gained weight and developed similarly. These observations make it less likely that the observed PPI deficits in the offspring are related to effects of dam behavior but this possibility cannot be excluded.

Our findings of similar time of appearance of developmental milestones in pups from different dietary groups are in disagreement with the previous study by Haubner et al. (2002) in which dietary supplementation of rat dams with DHA (3% of total fatty acids) during pregnancy and lactation led to a later appearance of acoustic startle response in their pups and longer auditory brainstem conduction times (ABCTs). Because ABCTs are strongly associated with the degree

of myelination of the auditory brainstem, the authors suggested that exposure to high levels of DHA during development may negatively impact myelination of the auditory brainstem. In the present study, however, the timing of appearance of the auditory startle reflex was about the same in all four dietary groups. Moreover, imaging studies of DHA supplementation of children with Zellweger's disease has led to the suggestion that DHA promoted myelination and can even lead to remyelination (Martinez & Vazquez, 1998).

The present results indicate that the depletion of n-3 fatty acids from the diet leads to a pronounced deficit in the prepulse inhibition of the acoustic startle response. Mice fed the n-3 Def and Low LNA diets had lower PPI levels compared to the High LNA and DHA+EPA groups (Fig. 2). The n-3 Def and Low LNA groups behaved similarly in terms of magnitude of the prepulse inhibition and habituation to the startle stimuli, although fatty acid composition of the brain tissues was quite different between these groups. The n-3 Def mice accumulated only half the DHA and significantly more ARA, DPAn-6 and DTA than the Low LNA group (Fig. 1). These results suggest that the amount of DHA in the brain does not solely predict the behavioral outcome; the n-6 fatty acid status also influences the PPI measures. This was confirmed by the differences between the Low LNA and High LNA groups: mice fed the High LNA diet exhibited significantly higher PPI compared to the Low LNA mice, whereas the brain DHA content of these mice was only slightly higher, but levels of all three major n-6 fatty acids (ARA, DPAn-6, and DTA) were much lower in the High LNA mice compared to the mice fed Low LNA diet.

Further, a difference between the High LNA group and mice supplemented with DHA and EPA in their diet was observed: the DHA+EPA group showed higher prepulse inhibition levels but only slightly (about 12%) more DHA in the brain tissues, while the content of ARA and DPAn-6 was the same in these mice. This is the first evidence of the differential effects of High LNA and DHA+EPA diets on behavior. This finding suggests that supplementation with preformed DHA and EPA is beneficial for the sensorimotor gating compared to a diet loaded with a high amount of LNA.

Although these diets were designed principally to manipulate the brain DHA content, these results indicate that this variable does not by itself predict the behavioral outcome. Overall, no correlation between the level of any major long-chain fatty acid (DHA, ARA, DPAn-6 and DTA) and PPI magnitude was observed. Rather the complete fatty acid profile may in some manner determine the PPI response.

There has been a discussion about whether deficits in PPI reflect a sensorimotor gating deficit (leading to compromised processing of prepulse) or an impairment of attention leading to a reduced detectability of the prepulse (summarized in Koch, 1998). It seems likely that both mechanisms play a role, as Swerdlow and co-workers have repeatedly shown that treatments that impair PPI do not affect the reduction in the startle peak latency that occurs concomitant to PPI, indicating that the animals are still able to detect the prepulse under conditions that reduce PPI (Swerdlow et al., 1992). On the other hand, in humans PPI is enhanced if the subjects attended to the prepulse (Jennings et al., 1996). Obviously, there are important attentional components involved in PPI, indicating that the PPI mechanism is more than a pure sensorimotor gate that is a prerequisite for attention. Attentional mechanisms affect PPI at a perceptual level, whereas higher levels of stimulus processing (cognitive processes) are protected by the gating mechanism underlying PPI (Koch, 1998). Both attentional and sensorimotor mechanisms are involved in the learning process and may underline spatial learning and memory deficits that have been repeatedly reported in n-3 deficient animals (for review, see Fedorova & Salem, 2006).

PPI of the acoustic startle reflex is reduced in a variety of neuropsychiatric disorders, for example in schizophrenia, schizotypal personality disorder, Huntington's disease, obsessive compulsive disorder, Tourette's syndrome, bipolar disorder, and attention deficit disorder (summarized in Swerdlow and Geyer, 1998). The PPI deficit observed in patients with schizophrenia is thought to be a measure of the general reduction of the ability to gate intrusive sensory, motor or cognitive information (Geyer et al., 2001; Braff et al., 1992; Kumari et al., 2000; Parwani et al., 2000). Some attempts have been made to treat symptoms of schizophrenia with n-6 and n-3 fatty acids. The results of these clinical trials are summarized elsewhere (Fenton et al., 2000; Peet, 2003; Freeman et al., 2006; Joy et al., 2006; Kidd, 2007; Ross et al., 2007). Double-blind trials of n-6 fatty acid supplementation of neuroleptic medication have yielded negative results (Joy et al., 2006). In contrast, most of n-3 fatty acid supplementation trials report positive results. To date, six double-blind randomized clinical trials with DHA/EPA have been conducted, involving 390 patients with schizophrenia or schizoaffective disorder. Four trials documented clinical benefit from 2 g EPA daily for three months (Emsley et al., 2002; Peet et al., 2001; Peet & Horrobin, 2002). However, the effect sizes were small in most trials, and significant improvement occurred only at one dose in a group of patients having a specific background treatment (Ross et al., 2007). Meta-analyses of treatment studies of schizophrenia found that n-3 PUFA failed to improve schizophrenic symptoms as measured by the PANSS total score (Freeman et al., 2006; Joy et al., 2006).

Habituation of the acoustic startle response as assessed by changes of average startle amplitude in the first and last blocks of the experiment (pulse-only trials) showed differences between the mice fed the n-3 Def and the Low LNA diets, and the ones on the High LNA and DHA +EPA supplemented diets (Fig. 3). The High LNA and the DHA+EPA groups demonstrated diminished startle responses at the end of the experiment compare to the beginning, suggesting an adequate habituation process. Whereas the n-3 Def and the Low LNA groups, on the contrary, did not change their responses during the test, indicating a deficit in short-term habituation. Within-session, or short-term habituation, is the decline of the acoustic startle reflex magnitude following repeated presentation of startling stimuli within a single test session. We have previously reported that n-3 deficient mice showed impairment in the habituation to a novel environment as they did not decrease their locomotor and exploratory activity in the open field test with time to the same extent as n-3 adequate animals (Fedorova and Salem, 2006; Fedorova et al., 2009). In the present study, we observed a habituation deficit in a different paradigm not requiring locomotion or exploration, suggesting that the impaired habituation phenomenon is attributable to the n-3 fatty acid deficiency. Interestingly, schizophrenic patients are also found to be impaired in the habituation of acoustic startle response (Braff et al., 1992; Parwani et al., 2000).

The results of the present study reveal that n-3 fatty acid deficiency induces profound behavioral changes in mice. For the first time, a deficit in sensorimotor gating as assessed by prepulse inhibition of the acoustic startle response was observed in mice fed the n-3 fatty acid deficient and a Low LNA diet. There is a large body of evidence on the importance of n-3 status for learning and memory, but PPI reflects an automatic, involuntary inhibitory process that functions to protect the initial processing of the information. Further, these are the first data that differentiate in rodents the effects of the High LNA diet from one with added EPA/DHA. This technique is objective, readily used in human studies and clearly sensitive to small changes in nervous system DHA content such as may be induced by variations in dietary DHA intake.

Abbreviations used

PPI prepulse inhibition

PUFA	polyunsaturated fatty acids
DHA	docosahexaenoic acid (22:6n-3)
EPA	eicosapentaenoic acid (20:5n-3)
LNA	α -linolenic acid (18:3n-3)
ARA	arachidonic acid (20:4n-6)
n-3 Def	n-3 fatty acid deficient

Reference

- Assies J, Lieverse R, Vreken P, Wanders RJA, Dingemans PM, Linszen DH. Significantly reduced docosahexaenoic acid concentration in erythrocyte membranes from schizophrenic patients compared with a carefully matched control group. *Biological Psychiatry* 2001;49:510–522. [PubMed: 11257236]
- Braff DL, Grillon C, Geyer MA. Gating and habituation of the startle reflex in schizophrenic patients. *Archives of General Psychiatry* 1992;49:206–215. [PubMed: 1567275]
- Caughey GE, Mantzioris E, Gibson RA, Cleland LG, James MJ. The effect on human tumor necrosis factor α and interleukin 1β production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. *American Journal of Clinical Nutrition* 1996;63:116–122. [PubMed: 8604658]
- Chalon S. Omega-3 fatty acids and monoamine neurotransmission. *Prostaglandins, Leukotriens and Essential Fatty Acids* 2006;75:259–269.
- Ellenbroek BA, Cools AR. Early maternal deprivation and prepulse inhibition: the role of the postdeprivation environment. *Pharmacology Biochemistry Behavior* 2002;73:177–184.
- Emsley RA, Myburgh CC, Oosthuizen PP, Van Rensburg SJ. Randomised placebo-controlled study of ethyl-eicosapentaenoic acid as supplementation treatment in schizophrenia. *American Journal of Psychiatry* 2002;159:1596–1598. [PubMed: 12202284]
- Fedorova I, Salem N Jr. Omega-3 fatty acids and rodent behavior. *Prostaglandins, Leukotriens and Essential Fatty Acids* 2006;75:271–289.
- Fedorova I, Hussein N, Baumann M, Di Martino C, Salem N Jr. An n-3 fatty acid deficiency impairs rat spatial learning in the Barnes Maze. *Behavioral Neuroscience* 2009;123:196–205. [PubMed: 19170444]
- Fenton WS, Hibbeln J, Knable M. Essential fatty acids, lipid membrane abnormalities, and the diagnosis and treatment of schizophrenia. *Biological Psychiatry* 2000;47:8–21. [PubMed: 10650444]
- Folch J, Lees M, Sloane-Stanley G. A simple method for the isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry* 1957;226:497–506. [PubMed: 13428781]
- Freeman MP, Hibbeln JR, Wisner KL. Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry. *Journal of Clinical Psychiatry* 2006;67:1954–1967. [PubMed: 17194275]
- Fukuzako H, Fukuzako T, Takeuchi K, Ohbo Y, Ueyama K, Takigawa M, Fujimoto T. Phosphorus magnetic resonance spectroscopy in schizophrenia: correlation between member phospholipid metabolism – the temporal lobe and positive symptoms. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 1996;20:629–640. [PubMed: 8843487]
- Fukuzako H, Fukuzako T, Hashiguchi T, Kodama S, Takigawa M, Fujimoto T. Changes in levels of phosphorus metabolites in temporal lobes of drug naïve schizophrenic patients. *American Journal of Psychiatry* 1999;156:1205–1208. [PubMed: 10450261]
- Gattaz WF, Schmitt A, Maras A. Increased platelet phospholipase A₂ activity in schizophrenia. *Schizophrenia Research* 1995;16:1–6. [PubMed: 7547640]
- Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology* 2001;156:117–154. [PubMed: 11549216]
- Geyer MA, Wilkinson LS, Humby T, Robbins TW. Isolation rearing of rats produces a deficit in prepulse inhibition of acoustic startle similar to that in schizophrenia. *Biological Psychiatry* 1993;34:361–372. [PubMed: 8218603]

- Graham FK, Murray GM. Discordant effects of weak prestimulation on magnitude and latency of the blink reflex. *Physiological Psychology* 1977;5:108–114.
- Haubner LY, Stockard JE, Saste MD, Benford VJ, Phelps CP, Chen LT, Barness L, Wiener D, Carver JD. Maternal dietary docosahexaenoic acid content affects the rat pup auditory system. *Brain Research Bulletin* 2002;58:1–5. [PubMed: 12121805]
- Horrobin DF. The membrane phospholipid hypothesis as a biochemical basis for the neurodevelopmental concept of schizophrenia. *Schizophrenia Research* 1998;30:193–208. [PubMed: 9589514]
- Jennings PD, Schell AM, Filion D, Dawson ME. Tracking early and late stages of information processing: contributions of startle eyeblink reflex modification. *Psychophysiology* 1996;33:148–155. [PubMed: 8851242]
- Joy CB, Mumby-Croft R, Joy LA. Polyunsaturated fatty acid supplementation for schizophrenia. *Cochrane Database of Systematic Reviews* 2006;3:CD001257.
- Kamphuis MH, Geerlings MI, Tijhuis MAR, Kalmijn S, Grobbee DE, Kromhout D. Depression and cardiovascular mortality: A role for n-3 fatty acids? *American Journal of Clinical Nutrition* 2006;84:1513–1517. [PubMed: 17158437]
- Kidd PM. Omega-3 DHA and EPA for cognition, behavior and mood: clinical findings and structural-functional synergies with cell membrane phospholipids. *Alternative Medicine Review* 2007;12:207–227. [PubMed: 18072818]
- Koch M. The neurobiology of startle. *Progress in Neurobiology* 1999;59:107–128. [PubMed: 10463792]
- Kumari V, Soni W, Mathew VM, Sharma T. Prepulse inhibition of the startle response in men with schizophrenia: Effects of age of onset of illness, symptoms, and medication. *Archives of General Psychiatry* 2000;57:609–614. [PubMed: 10839340]
- Lehmann J, Pryce CR, Feldon J. Lack of effect of an early stressful life event on sensorimotor gating in adult rats. *Schizophrenia Research* 2000;41:365–371. [PubMed: 10708346]
- Martinez M, Vazquez E. MRI evidence that docosahexaenoic acid ethyl ester improves myelination in generalized peroxisomal disorders. *Neurology* 1998;51:26–32. [PubMed: 9674774]
- Millstein RA, Ralph RJ, Yang RJ, Holmes A. Effects of repeated maternal separation on prepulse inhibition of startle across inbred mouse strains. *Genes, Brain and Behavior* 2006;5:346–354.
- Mitchell DC, Niu SL, Litman BJ. DHA-rich phospholipids optimize G-protein coupled signaling. *Journal of Pediatrics* 2003;143:S80–S86. [PubMed: 14597917]
- Parwani A, Duncan EJ, Bartlett E, et al. Impaired prepulse inhibition of acoustic startle in schizophrenia. *Biological Psychiatry* 2000;47:662–669. [PubMed: 10745060]
- Peet M, Brind J, Ramchand CN, Shah S, Vankar GK. Two double-blind placebo-controlled pilot studies of eicosapentaenoic acid in the treatment of schizophrenia. *Schizophrenia Research* 2001;49:243–251. [PubMed: 11356585]
- Peet M, Horrobin DF. A dose-ranging exploratory study of the effects of ethyl eicosapentaenoate in patients with persistent schizophrenic symptoms. *Journal of Psychiatric Research* 2002;36:7–18. [PubMed: 11755456]
- Pettegrew JW, Keshavan MS, Panchalingam K, Strychor S, Kaplan DB, Tretta MG. Alterations in brain high-energy phosphate and membrane phospholipid metabolism in first-episode, drug-naïve schizophrenics. A pilot study of the dorsal prefrontal cortex by in vivo phosphorus 31 nuclear magnetic resonance spectroscopy. *Archives of General Psychiatry* 1991;48:563–568. [PubMed: 1898445]
- Pettegrew JW, Keshavan MS, Minshew NJ. ³¹P nuclear magnetic resonance spectroscopy: neurodevelopment and schizophrenia. *Schizophrenia Bulletin* 1993;19:35–53. [PubMed: 8451612]
- Puskas LG, Kitajka K. Nutrigenomic approaches to study the effects of n-3 PUFA diet in the central nervous system. *Nutrition and Health* 2006;18:227–232. [PubMed: 17180868]
- Ross BM, Hudson C, Turennes A, Moszcynska SJ, Warsh JJ, Kish SJ. Differential alteration of phospholipase A₂ activities in brain of patients with schizophrenia. *Brain Research* 1999;821:407–413. [PubMed: 10064828]
- Ross BM. Phospholipid and eicosanoid signaling disturbances in schizophrenia. *Prostaglandins, Leukotriens and Essential Fatty Acids* 2003;69:407–412.
- Ross BM, Seguin J, Sieswerda LE. Omega-3 fatty acids as treatments for mental illness: which disorder and which fatty acid? *Lipids in Health and Disease* 2007;6:21–40. [PubMed: 17877810]

- Ryan AS, Keske MA, Hoffman JP, Nelson EB. Clinical overview of algal-docosahexaenoic acid: effects on triglyceride levels and other cardiovascular risk factors. *American Journal of Therapeutics* 2009;16:183–192. [PubMed: 19145206]
- Salem N Jr. Reyzer M, Karanian J. Losses of arachidonic acid in rat liver after alcohol inhalation. *Lipids* 1996;31:S153–S156. [PubMed: 8729111]
- Schwellenbach LJ, Olson KL, McConnell KJ, Stolcpart RS, Nash JD, Merenich JA, Clinical Pharmacy Cardiac Risk Service Study Group. The triglyceride-lowering effects of a modest dose of docosahexaenoic acid alone versus in combination with low dose eicosapentaenoic acid in patients with coronary artery disease and elevated triglycerides. *Journal of the American College of Nutrition* 2006;25:480–485. [PubMed: 17229894]
- Kim, HF Seung; Weeber, EJ.; Sweatt, JD.; Stoll, AL.; Marangell, LB. Inhibitory effects of omega-3 fatty acids on protein kinase C activity in vitro. *Molecular Psychiatry* 2001;6:246–248. [PubMed: 11317232]
- Shioiri T, Somey T, Murashita J, Kato T, Hamakawa H, Fujii K, Inubushi T. Multiple regression analysis of relationship between frontal lobe phosphorus metabolism and clinical symptoms in patients with schizophrenia. *Psychiatry Research* 1997;76:113–122. [PubMed: 9522403]
- Stanley JA, Williamson PC, Drost DJ, Carr TJ, Rylett RJ, Malla A. An in vivo study of the prefrontal cortex of schizophrenic patients at different stages of illness via phosphorus magnetic resonance spectroscopy. *Archives of General Psychiatry* 1995;52:399–406. [PubMed: 7726721]
- Strokin M, Sergeeva M, Reiser G. Role of Ca²⁺-independent phospholipase A2 and n-3 polyunsaturated fatty acid docosahexaenoic acid in prostanoid production in brain: perspectives for protection in neuroinflammation. *International Journal of Neuroscience* 2004;22:551–557.
- Swerdlow NR, Caine SB, Braff DL, Geyer MA. The neural substrates of sensorimotor gating of the startle reflex: a review of recent findings and their implications. *Journal of Psychopharmacology* 1992;6:176–190.
- Swerdlow NR, Geyer MA. Using an animal model of deficient sensorimotor gating to study the pathophysiology and new treatments of schizophrenia. *Schizophrenia Bulletin* 1998;24:285–301. [PubMed: 9613626]
- Wood JN. Essential fatty acids and their metabolites in signal transduction. *Biochemical Society Transactions* 1990;18:785–786. [PubMed: 1707376]
- Yao JK, Leonard S, Reddy RD. Membrane phospholipid abnormalities in post mortem brains from schizophrenic patients. *Schizophrenia Research* 2000;42:7–17. [PubMed: 10706981]

Fig. 1. Brain fatty acid analysis of 12 wk old mice. Values are given as mean $\pm$ SEM (n=9 in each group). Different letters indicate statistical differences between groups. DHA, docosahexaenoic acid (22:6n-3); DPAn-6, docosapentaenoic acid (22:5n-6); ARA, arachidonic acid (20:4n6); DTA, docosatetraenoic acid (22:4n6).

Fig. 2.

Prepulse inhibition of the acoustic startle response. Data (mean $\pm$ SEM) show percent prepulse inhibition of the startle response following presentation of prepulse-*plus*-pulse acoustic stimuli, $n=12$ in each group. Different letters indicate statistical differences between groups (repeated measures ANOVA followed by Tukey HSD test).

Fig. 3.

Habituation of the acoustic startle response. Data (mean $\pm$ SEM) show ratio of the mean startle reactivity of the last, third block to that of the first block of six consecutive pulse-alone trials. n=12 in each group. * indicates statistical significance, $p < 0.05$ (t-test).

Table 1

Composition of Experimental Diets.

Ingredient	Amount (g/100 g diet)
Alacid 710, acid casein ^a	20
Corn starch	15
Sucrose	10
Dextrose	19.95
Maltose-dextrin	15
Cellulose	5
Salt-mineral mix ^b	3.5
Vitamin mix ^c	1
L-Cystine	0.3
Choline bitartrate	0.25
TBHQ ^{d*}	0.0004
Mixed Tocopherol [*]	0.0019

Fat Sources:	n-3 Def	Low LNA	High LNA	DHA+EPA
Hydrogenated coconut oil	3.25	3.22	3.2	1.68
High Oleic Safflower oil	6.75	6.716	5.85	6.52
Flaxseed oil	-	0.064	0.95	-
Menhaden Oil ^f	-	-	-	1.8

Fatty acid composition (%)	n-3 Def	Low LNA	High LNA	DHA+EPA
Total Saturated	37.8	37.6	37.5	27.1
Mono-unsaturated	51.8	51.7	47.1	53.7
18:2n-6	9.6	9.6	9.8	9.5
18:3n-3	0.07	0.38	4.76	0.38
20:5n-3	-	-	-	1.98
22:6n-3	-	-	-	2.05
Total n-3	0.07	0.38	4.76	4.74
Total n-6	9.6	9.6	9.9	9.9

^a NZMP North America Inc. ALACID casein^b Dyets Inc. catalogue #210025^c Dyets Inc. catalogue #310025^d t-butyl-hydroquinone^{*} -added to all diets except DHA+EPA since tocopherols were already present in the menhaden oil^e Dyets Inc. catalogue #400625, vitamin free casein was used for maternal diet^f - source of menhaden oil was Omega Protein.