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Fatty acid binding proteins and the nervous system: their impact on mental conditions

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Abstract

The brain is rich in lipid and fatty molecules. In this review article, we focus on fatty acid binding proteins (Fabps) that bind to fatty acids such as arachidonic acid and docosahexanoic acid and transfer these lipid ligands within the cytoplasm. Among Fabp family molecules, Fabp3, Fabp5, and Fabp7 are specifically localized in neural stem/progenitor cells, neurons and glia in a cell-type specific manner. Quantitative trait locus analysis has revealed that Fabp7 is related with performance of prepulse inhibition (PPI) that is used as an endophenotype of psychiatric diseases such as schizophrenia. Fabp5 and Fabp7 play important roles on neurogenesis and differentially regulate acoustic startle response and PPI. However, other behavior performances including spatial memory, anxiety-like behavior, and diurnal changes in general activity were not different in mice deficient for Fabp7 or Fabp5. Considering the importance of fatty acids in neurogenesis, we would like to emphasize that lipid nutrition and its dynamism via Fabps play significant roles in mental conditions. This might provide a good example of how nutritional environment can affect psychiatric conditions at the molecular level.

Key words

Fatty acid binding protein; polyunsaturated fatty acids; psychiatric disorders; prepulse inhibition; acoustic startle reflex; neural stem/progenitor cells; neurogenesis

Introduction

Lipids are major components of the brain. This is because cells consisting the brain such as neurons and glia cells have a high cell membrane/cytoplasm ratio due to their complex morphology with many processes. Since the cell membrane is formed as a lipid bilayer, the molecules including saturated and unsaturated fatty acids and cholesterol are the major component of the brain (Bourre et al., 1992; Zerouga et al., 1991). Lipids are also important as nutrients because they have high calorific value, compose biological structures, and produce biologically active substances. Lipids often become more easily deficient than other nutrients such as carbohydrates among various nutritional components in the malnutrition caused by severe famine. Therefore, abnormal diet in terms of excess intake or shortage of lipid nutrients and/or imbalance of the lipid composition may cause malfunctions of the brain. In this manuscript, we focus on fatty acids and fatty acid binding proteins with regard to mental illness.

Players in fatty acid signaling

Fatty acids are not only major components of the cell membranes but also effective energy sources for oxidation in the mitochondria and peroxisomes. Moreover, some fatty acids act as signaling molecules, which lead to a variety of physiological responses. Among polyunsaturated fatty acids (PUFAs), *n*-3 and *n*-6 PUFAs have been most studied on their biological functions. An *n*-3 PUFA, docosahexaenoic acid (DHA), and *n*-6 PUFA, arachidonic acid (ARA), are major components in the brain (Sastry, 1985). In neurons, particularly, the ARA is released from the cell membrane by a membrane-bound enzyme, phospholipase A₂, in the neuronal activity-dependent manner (Bazan et al., 1993; Stella et al., 1995). Released ARA is metabolized into other bioactive substances such as prostagandins by enzymes such as cyclooxygenases, leading to various physiological functions (Oomagari et al., 1991).

Fatty acids also play important roles as signaling molecules in the lipid signaling pathways regulating the gene expression related to lipid and carbohydrate metabolism. Fatty acids taken from diets are present as free fatty acids binding to a carrier protein such as albumin or

triglyceride/phospholipid/lipoprotein complex in blood, and delivered to various tissues including liver, adipocytes, and brain via blood circulation (Jacome-Sosa and Parks, 2014; Picq et al., 2010; Voshol et al., 2009). Since PUFAs cannot be easily soluble within the aqueous conditions, they need to bind to albumin protein within blood plasma (Richieri et al., 1993) and to fatty acid binding proteins (Fabps) within the cytoplasm (Fig. 1). Regarding fatty acid signaling, in which lipid ligands such as PUFAs act its upstream, Fabps play central roles as intracellular carriers to transport the PUFAs to various regions within the cytoplasm (Furuhashi and Hotamisligil, 2008; Liu et al., 2010). In liver and adipocytes, for example, fatty acids are shuttled from the cytosol to the nucleus by Fabps, and modulate gene expression by binding to transcription factors such as peroxisome proliferator-activated receptors (PPARs), a member of nuclear hormone receptor family (Atshaves et al., 2010; Schroeder et al., 2008). Fatty acids, especially ARA and DHA are converted into various eicosanoids and behave as signaling molecules related with inflammation (Tam, 2013).

To date, at least ten genes encoding FABPs have been identified in the human genome (FABP1 to 9, and FABP12) (Fig. 2) (Smathers and Petersen, 2011). FABPs show a wide range of homology (between 20 and 70% in amino acid sequences) but high tertiary structures (Storch and Thumser, 2010). Most of Fabps bind to single long chain fatty acids except for Fabp1, which binds to two fatty acids (Atshaves et al., 2010). Fabp expression is distributed through various tissues in highly specific manners with different levels. In adipocytes, for example, Fabp4 and Fabp5 are expressed at a high level and a trace of amount, respectively, and deficit of Fabp4 resulted in overexpression of Fabp5 to probably compensate the lack of Fabp4 (Coe et al., 1999; Hotamisligil et al., 1996), Fabp3 (H-Fabp), Fabp5 (E-Fabp/PA-Fabp), and Fabp7 (B-Fabp/BLBP) are expressed in the nervous system (Owada et al., 1996a; Veerkamp and Zimmerman, 2001). Therefore, we focus on these brain-specific Fabps in regard with mental illness.

Fabps show a variety of ligand specificity, but many Fabps have a general tendency to bind to long-chain PUFAs more strongly than to short-chain saturated fatty acids (Zimmerman et al., 2001). Fabp7 shows higher specificity to long PUFAs such as eicosapentaenoic acid (EPA; 20:5), DHA (22:6), and ARA (24:4), but low binding affinity to the saturated fatty acids like lauric acid (12:0),

palmitic acid (16:0), and stearic acid (18:0), while Fabp3 has higher affinity to shorter PUFAs, e.g., oleic acid (18:1) and linoleic acid (18:2) than long PUFAs, e.g., EPA and DHA (Balendiran et al., 2000). Fabp5 binds not only to long PUFAs but also to eicosanoids such as 5 (S)- and 15 (S)- hydroperoxyeicosatetraenoic acid (HPETE), which are ARA metabolites, with moderate binding affinities (Kane et al., 1996). Therefore, combinatorial functions of Fabps and their ligands are complex.

Brain Fabps and their implication in mental illness

Fabps play important roles in various aspects of brain functions, as we will discuss below. However, a possibility of involvement of FABPs in regard with mental illness has emerged from a different approach. Yoshikawa and his colleagues have focused on a biological trait called prepulse inhibition (PPI), which is considered to be an endophenotype for psychiatric disorders such as schizophrenia, bipolar disorder, autism, and attention deficit hyperactive disorder (ADHD) (Kohl et al., 2013; Perry et al., 2002). In order to search gene loci responsible for the PPI, they performed quantitative trait locus (QTL) analyses by using different strains of mice. From comparison of C57BL6 (high PPI performer) and C3H (low PPI performer) mice, they have come up with a specific locus with a very high LOD score (Watanabe et al., 2007). This locus contains *Fabp7*, which has further been confirmed by a quantitative complementation test. They have also sequenced *FABP7* gene in Japanese schizophrenic patients, and found significant association of the gene to schizophrenia, especially in males. Moreover, their postmortem study has shown altered expression of *FABP7* mRNA in schizophrenic patients, which is more evident in males when samples are divided by sex. Thus, *FABP7* is a good candidate for a risk gene for mental diseases related with abnormal sensorimotor gating, but there need more studies by other researchers to confirm the responsiveness of *FABP7* gene in schizophrenia and/or other related psychiatric disorders.

The same group have also analyzed association of *FABPs* with other mental disorders, and found positive association of *FABP7* gene and bipolar disorder (Iwayama et al., 2010). In another study, the group has also resequenced *FABP3*, *FABP5*, and *FABP7* genes, and identified 13

polymorphisms, 7 of which are novel, although significant association of the 4 missense SNPs has not been found in autistic and schizophrenic patients (Maekawa et al., 2010). This may be because of a small sample size, and more study using larger samples is needed.

Fabp3 and dopamine signal in neurons

One of the brain Fabps, Fabp3, is originally identified as a heart-specific FABP (Jagschies et al., 1985), but has turned out to be highly expressed in neurons of the postnatal and mature brains (Owada et al., 1996b). Interestingly, Fabp3 interacts with dopamine D2 receptors and is co-localized with the receptors (Shioda et al., 2010). In this study, Fabp3 is shown to be expressed in acetylcholinergic interneurons and glutamatergic terminals but not in dopaminergic terminals in the dorsal striatum. *Fabp3* deficient mice look healthy at a glance, but actually have phenotypes; they show deficits in behavioral responses such as methamphetamine-induced sensitization and haloperidol-induced catalepsy, and exhibit enhanced acetylcholine (ACh) and glutamate (Glu) releases (Shioda et al., 2010). These results suggest an intriguing possibility that Fabp3 can modulate ACh and Glu releases through interaction with D2 receptors. Specific expression of Fabp3 is also observed in neurons in the inner ear (Saino-Saito et al., 2010), although auditory ability of *Fabp3* deficient mice is unexpectedly normal in stages observed (Suzuki et al., 2014). Therefore, roles of Fabp3 in the nervous system may vary in each tissue.

Fabp5 in the brain

Fabp5 (or epidermal fatty acid binding protein, E-FABP) is expressed dominantly in the developing brain (Liu et al., 2000; Owada et al., 1996b). In the adult brain, Fabp5 expression is observed in both in neurons and glia with a region-specific manner with various intensities (Owada, 2008). Fabp5 expression is induced by peripheral nerve injury, suggesting its potential role on neuroregeneration (Owada et al., 1996a). Although significant expression of Fabp5 is observed in the developing brain, *Fabp5*-knockout mice are viable and show no significant deficit in the brain at neither macro- nor microscopic level (Owada et al., 2002). Recently, *Fabp5*-knockout mice have been reported to

show reduced neurogenesis in the hippocampus as described in later sections (Matsumata et al., 2012). Furthermore, it is reported that Fabp5 can regulate cognitive function such as learning and memory through the degradation of endocannabinoid anandamide and the PPARb/d activation (Yu et al., 2014).

Fabp7 in glial cells

Fabp7 is expressed throughout brain development from the early embryonic stage to adult (Kurtz et al., 1994; Owada et al., 1996a). At early stages, intense Fabp7 expression is observed in neural stem/progenitor cells and reaches to maximum on embryonic day 14, gradually becomes decreased after postnatal day 0, and is restricted in the adult brain in some specific areas including the olfactory bulb, hippocampus and cerebellum (Arai et al., 2005; Feng et al., 1994; Kuhar et al., 1993; Owada et al., 2006; Watanabe et al., 2007). Fabp7 also shows astrocytic expression in the amygdala and septal area in adult (Owada et al., 2006). In the rat brain, Fabp7 is abundant especially in astrocytes that are rich in cytoplasmic granules originated from degraded mitochondria (Young et al., 1996). In the stab-injured mouse brain in which reactive astrocytes massively proliferate, Fabp7 shows increased expression in astrocytes as well as in oligodendrocyte precursor cells (Sharifi et al., 2011). In the study, the increase of reactive astrocytes is less in *Fabp7* deficient mice, showing an important role of Fabp7 in proliferation of astrocytes. Recently, It is reported that *Fabp7* mRNA and protein are accumulated at perisynaptic astrocytic processes with diurnal changes (Gerstner et al., 2012). Taking the human genetic studies mentioned above into account, these results suggest that Fabp7 could have unidentified critical functions in astrocytes.

Fabps and neurogenesis

In the last decades, it has been gradually revealed that newborn neurons are continuously produced in specific areas of the brain, including the subventricular zone (SVZ) of the forebrain lateral ventricle walls and the subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) (Ming and Song, 2011; Zhao et al., 2008). The production of neurons and glia is termed “neurogenesis” in which neural stem/progenitor cells (NSPCs) proliferate and

differentiated at multiple steps (see below).

The relationship between newborn granule cells in the DG and mental illnesses of individuals has become paid much attention (Eisch and Petrik, 2012; Osumi and Guo, 2011). In human, MRI images revealed that patients with neurological and/or psychiatric diseases, including Alzheimer's disease, schizophrenia and depression, have a smaller volume of hippocampi than those of control subjects (Francis et al., 2013; Guo et al., 2014; Heckers and Konradi, 2010; Prestia et al., 2014; Reif et al., 2006; Rizos et al., 2014). One possibility for those findings is that neurogenesis may be decreased in those patients. Indeed, a reliable evidence for postnatal neurogenesis in the hippocampus has recently been offered from an analysis using ^{14}C (Spalding et al., 2013). Moreover, a relationship between schizophrenia and postnatal neurogenesis is suggested from a postmortem study showing that proliferation of neural stem/progenitor cells (NSPCs), as detected Ki-67 staining, is reduced in schizophrenic patients (Reif et al., 2006). In an animal model for depression, mice that are continuously exposed to stress hormone (glucocorticoids) or restraint stress show a higher level of anxiety-like behavior and decrease of postnatal neurogenesis, which can be recovered if they are administrated with an antidepressant treatment (David et al., 2009; Snyder et al., 2011). Those data suggest that a disturbance of postnatal neurogenesis in the DG might affect mental conditions of human beings.

As mentioned above, neurogenesis is defined as multiple steps, including proliferation of NSPCs, their differentiation into several neural subtypes (neurons, astrocytes and oligodendrocytes), maturation of neurons and their integration into neural circuits. It is currently revealed that newborn neurons are excessively produced in the proliferation phase but that only the optimized number of newborn neurons survive during the maturation and integration phases (Ming and Song, 2011). We have observed that expression of the brain-specific Fabps (i.e., *Fabp7*, 5 and 3) is sifted from *Fabp7* -> *Fabp5* -> *Fabp3* during hippocampal neurogenesis; *Fabp7* is specifically expressed in NSPCs, *Fabp5* in transit amplifying cells, and *Fabp3* in maturing neurons (Matsumata et al., 2012). We also examined how lack of *Fabp7* and/or *Fabp5* differentially affects hippocampal neurogenesis. In *Fabp7* or *Fabp5* single knockout (KO) mice, proliferation of NSPCs is decreased to about 85% of the wild type mice, and mice

deficient for both genes result in reduced proliferation of NSPCs to 30% of the wild type mice, probably reflecting additive effects of *Fabp7* and *Fabp5* during the proliferation phase. However, survival of newly born cells is affected only in *Fabp7* KO or *Fabp7/5* KO mice but not in *Fabp5* KO mice. In *Fabp7* and *Fabp7/5* KO mice, the survival ratio is about 34% and 49%, respectively, while the ratio is about 27% in wild type or *Fabp5* KO mice (Fig. 3A). These data may suggest that *Fabp7* and *Fabp5* function independently during proliferation of NSPCs, while that lack of *Fabp7* rather plays a pivotal role in survival of neurons.

As previously mentioned, there are many reports showing the shrinkage of the hippocampus in the patients with neurological and/or psychiatric diseases. We did not observe a gross difference in the hippocampal volume despite the increase of survived neurons of the DG in *Fabp7* and *Fabp7/5* KO mice at the stages we examined. There may be some possibilities; for example, these KO mice exhibited poor dendritic expansion due to insufficient maturation of the survived neurons, or glial cells might compensate the hippocampal volume. In any case, we could not simply discuss, in regard with psychiatric disorders, with the relationship of the volume of hippocampi, and postnatal neurogenesis, especially the number of survived cells. In future it should be cleared by more sophisticated analyses using suitable animal models and/or clinical studies.

As noted in a previous section, Fabps work as intracellular cargos of fatty acids, especially of PUFAs such as ARA and DHA, and FAs are ligands of Fabps in the cytoplasm. The effects of PUFAs, especially of ARA and DHA, on neurogenesis have been examined by our group and collaborators. An *in vivo* study using juvenile rats, ARA increases proliferation of NSPCs in the hippocampus (Maekawa et al., 2009). *In vitro* neurosphere assays using cultured NSPCs derived from the embryonic rat cortical primordium, both DHA and ARA added into medium can induce increase in the number of neurospheres at the neurogenic stage (Sakayori et al., 2011b). In the gliogenic stage when the neurospheres are passaged, DHA induces neuronal differentiation, whereas ARA causes astrocytic differentiation (Sakayori et al., 2011b). It would be interesting to know in future how Fabps are related with these differential roles of DHA and ARA in neurogenesis.

Fabps and animal behavior

Behavior studies have intensively been performed by our colleagues. Owada's laboratory has shown that *Fabp7* KO mice need much time to erase fear memory, show more anxiety-like behavior than wild type mice, but are normal in a spatial learning/memory (Owada et al., 2006). We have much interest in sensorimotor gating responses that is scored by PPI test because % PPI are considered, as already mentioned above, as a biological endophenotype of several neuropsychiatric disorders and because PPI is modulated by hippocampal functions. PPI can be measured as the followings. First, acoustic startle reflex (ASR), an innate response to the startle sounds, is measured, and this response is suppressed by a mild stimulus that immediately precedes (Schmajuk and Larrauri, 2005; Swerdlow et al., 1999). Our colleagues have previously reported that the genetic polymorphisms in *Fabp7* locus were related to PPI and that *Fabp7* deficit mice showed 40% decrease in PPI (Watanabe et al., 2007). We thus tried to extend the study by comparing behaviors of *Fabp7* and/or *Fabp5* deficit mice.

To prevent effects of other factors (e.g. mother's care, nutrition of the breast milk etc.) than genotypes of the mice, all mice were fostered by wild type mother mice immediately after birth, and yielded for behavior analysis at 13 weeks or after. At first, we examined ASR, which was significantly increased in *Fabp7/5* KO mice, and the same tendency was observed in *Fabp7* KO mice [one-way factorial ANOVA, $F(3,70)=7.585$, $P=0.0002$, and *Post hoc* Bonferroni's Multiple Comparison Test, WT vs *Fabp7/5* KO; $P<0.01$, *Fabp7* KO vs *Fabp5* KO; $P<0.05$, *Fabp5* KO vs *Fabp7/5* KO; $P<0.001$] (Fig. 3B). We also investigated effects of repeated presentation of startle stimuli on the response. Each addition of the trials (block) or genotypes affected the decrease of ASR, but there was no interactive effects of block x genotype [repeated two-way ANOVA (Block x Genotype), interaction; $F(3, 210)=1.159$, $P=0.3232$, block; $F(3, 210)=47.27$, $P<0.001$, genotype, $F(3, 210)=5.686$, $P=0.0015$, 2nd block; one-way factorial ANOVA, $F(3,70)=6.619$, $P=0.0005$, and *Post hoc* Bonferroni's Multiple Comparison Test, WT vs *Fabp7/5* KO; $P<0.05$, *Fabp7* KO vs *Fabp5* KO; $P<0.05$, *Fabp5* KO vs *Fabp7/5* KO; $P<0.01$, 3rd block; one-way factorial ANOVA, $F(3,70)=7.696$, $P=0.0002$, and *Post hoc* Bonferroni's Multiple Comparison Test,

WT vs *Fabp7/5* KO; $P<0.01$, *Fabp5* KO vs *Fabp7/5* KO; $P<0.001$] (Fig. 3C). Since repeated presentation of startle stimuli decreased ASR amplitude, it is considered that habituation itself was correctly occurred in all genotypes (Fig. 3D). Latency to the response for ASR was not different in any genotypes [one-way factorial ANOVA, $F(3,70)=2.042$, $P=0.1158$]. ASR amplitude is modulated by activities of the caudal pontine reticular nucleus (PnC) and the sensorimotor interfaces of the ASR are believed to locate in the giant neurons (GNs) of PnC (Fendt et al., 2001; Koch, 1999; Schmajuk and Larrauri, 2005). Therefore, it is expected in *Fabp7* and *Fabp7/5* KO mice that activities of GNs of the PnC are higher, but that reduction of activities with repeated presentation of acoustic stimuli is normal. There may be many possibilities for the reason that *Fabp7* KO and *Fabp7/5* KO mice showed higher ASR levels; the number of GNs is increased, the input to GNs is enhanced, post-synaptic transmission and amplitude of GNs is facilitated, or the membrane properties of GNs are sifted thereby firing frequency is higher, and so on. At present, we cannot evaluate which of the reasons might be most likely.

In all the four genotypes, prepulse stimuli decreased ASR response, but the reduction level was significantly smaller in *Fabp7* and *Fabp7/5* KO mice than in wild type and *Fabp5* KO mice [for 68dB, one-way factorial ANOVA, $F(3,70)=0.5330$, $P=0.6612$, 71dB; one-way factorial ANOVA, $F(3,70)=2.041$, $P=0.1160$; for 77dB; one-way factorial ANOVA, $F(3,70)=7.374$, $P=0.0002$, and *Post hoc* Bonferroni's Multiple Comparison Test, WT vs *Fabp7* KO; $P<0.05$, WT vs *Fabp7/5* KO; $P<0.001$, *Fabp5* KO vs *Fabp7/5* KO; $P<0.01$] (Fig. 3A, E). It has been known that the actual role of prepulse for PPI is the repression of the activities of GNs and motor neurons that are downstream of the PnC in the ASR circuit (sensory neurons - PnC - motor neurons) (Fendt et al., 2001; Koch, 1999; Schmajuk and Larrauri, 2005). This circuit is eventually intervened in the PnC by a direct innervation to the pedunclopontine tegmental nucleus (PPTg). Upstream of the circuit related to PPI also modify activity of PnC neurons through the PPTg (Fendt et al., 2001; Schmajuk and Larrauri, 2005). The nucleus accumbens (NAc) is the most well known nucleus to relate with PPI, and the disruption of dopamine concentration from the ventral tegmental area in a synaptic cleft of the NAc presumably affects PPI levels (Bast and Feldon, 2003;

Swerdlow et al., 1999). It is also known that hippocampal activities affect NAc activity and PPI (Bast and Feldon, 2003). As mentioned above, the survival rate of newborn neurons one month after proliferation of NSPCs increased in *Fabp7* and *Fabp7/5* KO mice (Fig. 3A) (Matsumata et al., 2012). Newly born neurons are maturing into granule cells of the DG (David et al., 2009; Ming and Song, 2011) and receive GABA as an excitatory inputs from GABAergic neurons already located within the DG (Tozuka et al., 2005). Combining these results, it is likely that excitatory output from the DG to the NAc through CA3 and CA1 regions of the hippocampus might be increased in *Fabp7* and *Fabp7/5* KO mice (compare Fig. 4A and 4B). On the other hand, we have reported that mice administrated with an anti-proliferative drug show reduction of GABAergic neurons in the DG and decrease in % PPI (Fig. 4C), and that GABA agonists injection into the DG of these mice (i.e., substitution of reduced GABA inputs) recovers their PPI deficits (Fig. 4D) (Guo et al., 2013). Taken altogether, an excessive excitatory activation (excess output of immature newborn neurons and/or less of GABAergic inhibition in the DG) might affect NAc activity and dopamine release, eventually causing disruption of normal PPI in *Fabp7* and *Fabp7/5* KO mice.

Regarding other behavior tests, mice lacking *Fabp7* and/or *Fabp5* showed no significant difference (Fig. 5). Spatial memory, i.e., one of the most famous functions of the hippocampus, was assayed by the radial maze. Basal locomotor activity was unchanged in all mice and they could remember the position of correct arms contained food rewards. Memory retention was not disturbed by three or five day-intervals. All the four genotypes also showed normal reversal learning [for the main effect of genotype in repeated two-way ANOVA (Block $\times$ Genotype), WT, *Fabp7* and *Fabp7/5* KO mice; $F(2,143)=0.5546$, $P=0.5896$, WT and *Fabp5* KO mice; $F(1,165)=1.120$, $P=0.3127$] (Fig. 5A, B). Furthermore, both working memory (as assessment of errors when mice re-entry into the arm that they had already visited on same trial) and reference memory (as assessment of errors when mice visit the empty arms) were at the same level among all the four genotypes. Anxiety-like behavior was unchanged as evaluated by a novelty suppressed feeding test [one-way factorial ANOVA, $F(3,62)=0.7654$, $P=0.5178$] (Fig. 5C). Although *Fabp7*, but not *Fabp5*, shows a diurnal change in its expression level (Gerstner et al., 2008), a circadian rhythm of general behavior

was unaffected in these *Fabp* KO mice, and all the four genotypes showed normal locomotor activity regulated by circadian rhythm [for average of circadian rhythm, WT; 23.95 h, *Fabp7*KO; 24.00 h, *Fabp5*KO; 23.97 h, *Fabp7/5*KO; 24.00 h; as assessed by one-way factorial ANOVA, $F(3,8)=1.833$, $P=0.2192$] (Fig. 5D).

All together, the effect of lacking *Fabp7* and/or *Fabp5* on behaviors related with the principal hippocampal functions was significant for ASR and PPI. We have previously reported that *Fabp3* expression is increased in the hippocampal SGZ of *Fabp7* and/or *Fabp5* KO mice at 4-week, implying a possible compensation of the roles of *Fabp7* and/or *Fabp5* (Matsumata et al., 2012). This might be a reason why no significant difference was observed in basic behavior in these *Fabp* KO mice. On the other hand, deficiency of *Fabp7* was relevant for deficits of ASR and PPI, suggesting a crucial role of *Fabp7* in these natural responses. It would be interesting to know why the lack of *Fabp7* cannot be replaced by other molecules. This is in contrast with the fact that almost all the roles of *Fabp5* on hippocampal related behavior seemed to be compensated, which might be due to compensation of *Fabp5* with *Fabp4* (Furuhashi and Hotamisligil, 2008). In this regard, specific binding of *Fabp7* and its ligand, e.g., DHA, could be a key to elucidate underlying mechanisms.

Conclusion and perspective

Studies on effects of PUFAs repeatedly suggest significant importance of the PUFAs in brain functions. We have shown from neurosphere assays that ARA and DHA have differential roles for proliferation and differentiation of neural stem/progenitor cells (Sakayori et al., 2013; Sakayori et al., 2011a). In the hippocampus of juvenile wild type rats, ARA can increase proliferation of neural stem/progenitor cells (Maekawa et al., 2009). ARA also partially ameliorates PPI deficits in heterozygous rats with a mutation in *Pax6* encoding a neurodevelopmental transcription factor (Maekawa et al., 2009). It is still on debate but if neurogenesis is actually related with hippocampal functions such as memory/learning and mood levels, application of suitable types/amount of PUFAs could be one of therapeutic and/or preventive approaches for patients with mental illness. Malnutrition in terms of the *n-6/n-3* ratio should also be brought under review.

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Figure legends

Fig. 1. Fabp is a cytoplasmic shuttle molecule. Fabp binds to fatty acid and deliver the fatty acid to various organella within a cell, thereby playing pivotal roles in energy production, membrane synthesis, lipid droplet deposition, and gene expression. Illustration is modified from Fig. 1 in ref. (Furuhashi and Hotamisligil, 2008).

Fig. 2. Phylogenetic tree of ten human FABPs. Phylogenetic tree was constructed from the multiple alignment of amino acid sequences by ClustalW.

Fig. 3. Relationship between neurogenesis and PPI (A), ASR amplitude (B-D) and PPI with different prepulse intensities (E) in the wild type (WT; n=24), *Fabp7* KO (7KO; n=14), *Fabp5* KO (5KO; n=17), and *Fabp7/5* KO (7/5 KO; n=19) mice. (A), Table showing the numbers of total BrdU+ cells remaining one day and one month after BrdU injection, the survival rate (the ratio of the number of one month after BrdU+ cells against that of one day after BrdU + cells, for details, see ref. (Matsumata et al., 2012)), and % PPI paired with 77 dB prepulse. The number indicates the mean $\pm$ sem. (B-C), Mice were placed into the isolated startle apparatus and allowed a five min acclimatization period with a 65 dB background white noise. For ASR measurement, 40 ms duration of 120 dB startle stimulus was presented 24 times, and total averages of startle amplitude in digitized units are shown in (B). Repeated presentations of the startle stimuli induced reduction of the ASR magnitude, which is defined as “habituation” as shown in (C). (D) The reduction of ASR magnitude in habituation, calculated as the ratio of the average ASR magnitude of 4th block to that of 1st block. (E), For PPI measurement, three different prepulse intensities were used (3, 6 and 12 dB above background), and 10 trials including startle stimuli following prepulses were randomly presented in each intensity. Prepulse duration was 20 ms and the latency between a prepulse and a startle stimulus was always 100 ms. Percentage PPI induced by each prepulse intensity was calculated as $\{[(\text{ASR amplitude}) - (\text{ASR amplitude with prepulse})]/(\text{ASR amplitude})\} \times 100$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Fig. 4. Summary of relationship between output from the hippocampal dentate

gyrus (DG) and activity of GABAergic projection neurons in the Nucleus accumbens (NAc). (A) In the wild type (WT) mouse, almost all granular neurons are inhibited (blue arrow) by GABAergic interneurons except for activated immature granular neurons (red arrow) in the DG. Activities of GABAergic neurons in the NAc, i.e., a target of hippocampal innervation, are also maintained at the low state (pink arrow). (B) In *Fabp7* and *Fabp7/Fabp5* KO mice, increase of immature granular neurons disturbs the balance between mature and immature granular neurons, and these accumulative neural activities cause an increase of GABAergic neuronal activities in the NAc. (C) In mice administrated with anti-proliferative drug, the number of GABAergic interneurons decreases in the DG and total inhibitory output from the DG is decreased, while GABAergic activities in the NAc relatively increased. (D) In addition to the anti-proliferative drug, administration of a GABA agonist (muscimol) to the DG rescue the level of GABA concentration and inhibition of mature granular neurons in the DG causes to maintain GABAergic neuronal activities to a lower basal level.

Fig. 5. Behavior performance of the wild type (WT), *Fabp7* KO (7KO), *Fabp5* KO (5KO), and *Fabp7/5* KO (7/5 KO) mice. (A, B), Spatial memory task performance in 7KO and 7/5KO mice (A), and in 5KO mice (B) compared with WT mice. A food-deprived mouse was freely allowed to visit arms to obtain the food reward, but only four of eight arms contained a pellet. One trial continues to obtain all four pellets and one block includes three trials. The criterion for successful learning is defined as four correct-arm-entries to obtain all pellets within six-arm-entries in one trial; in other words, entries into wrong arms are only two times in one trial and six times in one block (shown as a dotted line). (C), Latency of the food-deprived mice to bite a food pellet in the center of the novel bright arena. (D), Representative actograms of locomotor activity of all the four genotypes raised under a constant 12 h-light-dark cycle. Room light was turned on at 8:00 am and off at 20:00 pm.

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Fig. 1

Fig. 3

Fig. 4

A WT

B *Fabp7* KO, *Fabp7/5* KO

C MAM-treated

D MAM + GABA agonist

Fig. 5