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## DHA prevents altered 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, CB<sub>1</sub> and GABAA receptor binding densities in the brain of male rats fed a high-saturated-fat diet

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## DHA prevents altered 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, CB1 and GABA<sub>A</sub> receptor binding densities in the brain of male rats fed a high-saturated-fat diet

### Abstract

Low levels of docosahexaenoic acid (DHA) have been linked to a number of mental illnesses such as memory loss, depression and schizophrenia. While supplementation of DHA is beneficial in improving memory and cognition, the influence of dietary fats on the neurotransmitters and receptors involved in cognitive function is still not known. The aim of this study was to investigate serotonin receptor (5-HT<sub>1A</sub> and 5-HT<sub>2A</sub>), cannabinoid receptor (CB1) and gamma-aminobutyric acid type A (GABA<sub>A</sub>) receptor binding densities in the brain of male rats fed a high-saturated-fat (HF) diet, as well as the effect of DHA supplementation on HF diet. Alterations of these receptors in the post-mortem rat brain were detected by [<sup>3</sup>H]-WAY-100635, [<sup>3</sup>H]-ketanserin, [<sup>3</sup>H]-CP-55,940 and [<sup>3</sup>H]-muscimol binding autoradiography, respectively. In the hippocampus, the 5-HT<sub>1A</sub>, CB1 and GABA<sub>A</sub> receptor binding densities significantly increased in response to an HF diet, while in the hypothalamus, 5-HT<sub>1A</sub> and CB1 binding densities significantly increased in HF-fed rats. Importantly, DHA supplementation prevented the HF-induced increase of receptors binding density in the hippocampus and hypothalamus. Furthermore, DHA supplementation attenuated 5-HT<sub>2A</sub> receptor binding density in the caudate putamen, anterior cingulate cortex and medial mammillary nucleus, which was also increased in HF group. This study showed that an HF diet increased 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, CB1 and GABA<sub>A</sub> receptor binding densities in the brain regions involved in cognitive function and that dietary DHA can attenuate such alterations. These findings provide insight into the mechanism by which DHA supplementation ameliorates reduced cognitive function associated with an HF diet.

### Keywords

fat, saturated, high, fed, rats, male, brain, densities, diet, binding, dha, receptor, gabaa, cb1, ht2a, ht1a, 5, prevents, altered

### Disciplines

Medicine and Health Sciences

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1 **Title: DHA prevents altered 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, CB1 and GABA<sub>A</sub> receptor binding**  
2 **densities in the brain of male rats fed a high-saturated fat diet**

3

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34

**Abstract**

Low levels of docosahexaenoic acid (DHA) have been linked to a number of mental illnesses such as memory loss, depression and schizophrenia. While supplementation of DHA is beneficial in improving memory and cognition, the influence of dietary fats on the neurotransmitters and receptors involved cognitive function is still not known. The aim of this study was to investigate serotonin receptor (5-HT<sub>1A</sub> and 5-HT<sub>2A</sub>), cannabinoid receptor (CB1) and gamma-aminobutyric acid type A (GABA<sub>A</sub>) receptor binding densities in the brain of male rats fed a high-saturated fat (HF) diet, as well as the effect of DHA supplementation on HF diet. Alterations of these receptors in the post-mortem rat brain were detected by [<sup>3</sup>H]-WAY-100635, [<sup>3</sup>H]-Ketanserin, [<sup>3</sup>H]-CP-55,940 and [<sup>3</sup>H]-Muscimol binding autoradiography, respectively. In the hippocampus, the 5-HT<sub>1A</sub>, CB1 and GABA<sub>A</sub> receptor binding densities significantly increased in response to a HF fat diet. While in the hypothalamus, 5-HT<sub>1A</sub> and CB1 binding densities significantly increased in HF fed rats. Importantly, DHA supplementation prevented the HF induced increase of receptors binding density in the hippocampus and hypothalamus. Furthermore, DHA supplementation attenuated 5-HT<sub>2A</sub> receptor binding density in the caudate-putamen, anterior cingulate cortex and medial mammillary nucleus, which was also increased in HF group. This study showed that a high-saturated fat diet increased 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, CB1 and GABA<sub>A</sub> receptor binding densities in the brain regions involved in cognitive function, and that dietary DHA can attenuate such alterations. These findings provide insight into the mechanism by which DHA supplementation ameliorates reduced cognitive function associated with a high-saturated fat diet.

Keywords: DHA, high-saturated fat, serotonin receptor, CB1 receptor, GABA<sub>A</sub> receptor

58

## 59 **Introduction**

60 Different types of dietary fats affect body metabolism and cognitive function differently [1].  
61 Studies have shown that a diet high in saturated fat promotes fat deposition and impairs  
62 memory and learning, and even contributes to the development of depression [2-4].  
63 Conversely, a diet high in n-3 polyunsaturated fat, especially docosahexaenoic acid (DHA),  
64 can have the opposite effect [2-4]. A growing body of clinical findings implicates low DHA  
65 status with being overweight [5], impaired cognitive function, and depression [6-8]. Plasma  
66 DHA was lowered in elderly subjects with depressive disorders compared to individuals  
67 without depression [8]. The tissue DHA content of the orbitofrontal cortex and cingulate  
68 cortex was also found to be lower in individuals with major depression [6, 7]. Beneficial  
69 effects of DHA by improving cognition and anti-depressive effects have been described in  
70 clinical trials and animal studies. There is evidence that DHA supplementation improves  
71 cognition [9], enhances memory [10] and induces an anti-stress response [11], however, the  
72 underlying mechanisms remain unclear. Certain brain areas such as the hippocampus and  
73 cingulate cortex are important for cognitive function. However, there is little information on  
74 how dietary fat influences key receptors in these brain regions, which are important in the  
75 regulation of cognitive and metabolic function.

76

77 The neurotransmitter serotonin (5-HT) acts via 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors and has an  
78 important role in various central functions including control of energy intake, obesity,  
79 memory and learning [12-14]. 5-HT<sub>1A</sub> receptors are distributed throughout the brain and are  
80 located either pre or post-synaptically, where they regulate various brain functions [12, 15].  
81 As presynaptic autoreceptors, the 5-HT<sub>1A</sub> receptors are found in dorsal and median raphe  
82 nuclei and negatively regulate 5-HT synthesis. A highly palatable diet in rats increases the  
83 density of 5-HT<sub>1A</sub> pre-synaptic receptor in these regions, suggesting a decrease in synthesis

84 and consequently a decreased release of 5-HT [16]. 5-HT<sub>1A</sub> receptors as post-synaptic  
85 receptors have a wide distribution in the brain with high density in the cortical and limbic  
86 areas, especially in the hippocampus and cortex, and low expression in other brain regions  
87 such as the hypothalamus, striatum and amygdala [17]. Clinical studies have shown that 5-  
88 HT<sub>1A</sub> receptor expression is negatively associated with memory function [18]. Postsynaptic  
89 5-HT<sub>2A</sub> receptors can be found in high levels in cerebral cortical areas and at intermediate  
90 levels in the hypothalamus, striatum and hippocampus [19, 20]. Using [<sup>125</sup>I] DOI binding  
91 autoradiography, a high-saturated fat diet increased 5-HT<sub>2A</sub> binding density in the  
92 ventromedial hypothalamic nucleus and anterior olfactory nucleus in diet induced obese mice,  
93 but not in mice resistant to obesity development [21]. Furthermore, using [<sup>3</sup>H]-Ketanserin  
94 autoradiography, 5-HT<sub>2A</sub> receptor binding densities were significantly increased in post-  
95 mortem tissue from the temporal cortex of patients with dementia [22]. Based on the  
96 accumulated evidence of clinical trials, blockade of 5-HT<sub>2A</sub> receptor ameliorates both the  
97 positive and negative symptoms, and to some extent the cognitive deficits in schizophrenia  
98 [23, 24]. The highly selective 5-HT<sub>2A</sub> antagonists MDL 100907 and EMD 281014, both  
99 developed as anti-psychotics, have also been shown to enhance cognitive function in animal  
100 models [25, 26].

101

102 The cannabinoid CB1 receptor plays an important role in various aspects of neural functions  
103 including learning and memory, anxiety, depression, addiction, appetite and feeding  
104 behaviour. Both CB1 knockout mice and CB1 antagonist (SR141716)-treated wild-type mice  
105 exhibited deficits in extinction of spatial memory [27, 28]. The systemic administration of the  
106 CB1 agonist WIN55,212-2 in rats impaired the acquisition of contextual fear conditioning  
107 [29], which is known to depend on the hippocampus [30]. GABA is the major inhibitory  
108 neurotransmitter in the brain. There are two receptors that mediate GABA neurotransmission

109 in the brain; GABA<sub>A</sub> and GABA<sub>B</sub>. The inhibitory function of GABA<sub>A</sub> is increasingly being  
110 recognised as important in the regulation of cognition, emotion, memory and obesity. It has  
111 been reported that the density of GABA<sub>A</sub> receptors was increased in the cortex of  
112 schizophrenia patients in order to compensate for the lowered levels of GABA [31, 32].  
113 Allelic variants in the GABA<sub>A</sub>α6 receptor subunit gene (GABRA6) were also associated with  
114 abdominal obesity [33]. Furthermore, the majority of leptin's antiobesity effects were  
115 mediated by GABAergic neurons reducing inhibitory tone to postsynaptic anorexigenic  
116 POMC neurons in the hypothalamus [34].

117

118 The effect of a DHA supplemented high-saturated fat diet on these receptor binding densities  
119 in brain regions associated with cognition has not been thoroughly investigated. To address  
120 this issue, we have used multiple ligands including [<sup>3</sup>H]-WAY-100635, [<sup>3</sup>H]-Ketanserin, [<sup>3</sup>H]-  
121 CP-55,940 and [<sup>3</sup>H]-Muscimol to examine the regional changes of 5-HT<sub>1A</sub>, 5-HT<sub>2A</sub>, CB1 and  
122 GABA<sub>A</sub> receptor in the rat brain. Rats were fed either high-saturated fat diet, DHA  
123 supplement in high-saturated fat diet or low-fat diet for 4 weeks. We examined alterations in  
124 receptor expression in response to a high-saturated fat diet, and if these alterations could be  
125 prevented by a supplementation of dietary DHA.

126

## 127 **Experimental procedure**

### 128 **Animals and dietary treatments**

129 Thirty male Wistar rats (300-320g) were obtained from the Animal Resources Centre (Perth,  
130 Western Australia, Australia) and housed in environmentally controlled conditions (22°C, 12  
131 hr light–dark cycle with light cycle from 06:00 to 18:00 h and dark cycle from 18:00 to 06:00  
132 h) with *ad libitum* access to standard laboratory chow and water. Rats were allowed 1 week to  
133 adapt to their new environment before experiments began. They were randomized into three

134 groups with different diets: (1) standard laboratory chow as the low-fat control (LF, fat  
135 content 10% in kcal, saturated fat 1%), (2) high-fat diet (HF, 25% in kcal, saturated fat 10%),  
136 (3) high-fat diet + 0.5% DHA. The dose of DHA supplementation used in this study was  
137 based on the dose recommended for humans at 250mg/70kg/day (European Food Safety  
138 Authority) [35]. After four weeks of dietary treatment, rats were sacrificed by rapid CO<sub>2</sub>  
139 asphyxiation between 07:00 and 09:00 hrs in order to minimize the impact of circadian  
140 variation, and the brains were immediately removed and frozen in liquid nitrogen. Five rats  
141 per group were used to examine [<sup>3</sup>H]-WAY-100635, [<sup>3</sup>H]-Ketanserin, [<sup>3</sup>H]-CP-55,940 and  
142 [<sup>3</sup>H]-Muscimol binding in the brain. The study was approved by the University of  
143 Wollongong Animal Ethics Committee and all animal experiments were conducted in  
144 compliance with the National Health and Medical Research Council Australian, Code of  
145 Practice for the Care and Use of Animals for Scientific Purposes (2004).

146

#### 147 **Histological procedures**

148 Coronal brain sections (14 μm) were cut in a cryostat at -18 °C from the level of Bregma -  
149 0.24mm to -5.16mm [36], thaw-mounted onto poly-L-lysine coated microscope slides  
150 (Polysine™, Menzel GmbH & Co, KG) [37] and stored at -20 °C.

151

#### 152 **[<sup>3</sup>H]-WAY-100635, [<sup>3</sup>H]-Ketanserin, [<sup>3</sup>H]-CP-55,940 and [<sup>3</sup>H]-Muscimol binding** 153 **autoradiography**

154 [<sup>3</sup>H]-WAY-100635 autoradiography was performed to examine 5-HT<sub>1A</sub> receptor binding  
155 density following procedures as described in previous work from our laboratories [38]. Brain  
156 sections were warmed to room-temperature and pre-incubated in 50 nM Tris-HCl buffer (pH  
157 7.4) for 30 min. The sections were then incubated with 5 nM [<sup>3</sup>H]-WAY-100635 (specific  
158 activity 83.0 Ci/mmol, Amersham Biosciences, UK Limited) at room temperature for 2.5 hrs



159 in 50 mM Tris–HCl (pH 7.4) containing 10  $\mu$ M pargyline (Sigma). Non-specific binding was  
160 determined by incubating consecutive sections exposed to 10  $\mu$ M 5-HT. All sections were  
161 washed for 2 min and then 3 min in ice-cold 50 mM Tris–HCl buffer.

162  
163 [ $^3$ H]-Ketanserin autoradiography was performed as described previously [19]. Binding of  
164 [ $^3$ H]-Ketanserin (67.0Ci/mmol; PerkinElmer Life Sciences, Boston, MA, USA) to 5-HT<sub>2A</sub>  
165 receptors was measured by preincubating sections in 170 mM Tris-HCl buffer (pH 7.4) for 15  
166 min at room temperature. Sections were then incubated for 120 min at room temperature in  
167 buffer containing 2 nM [ $^3$ H]-Ketanserin. Nonspecific binding was determined by the addition  
168 of 2  $\mu$ M spiperone to consecutive sections. Sections were washed in ice-cold buffer (2  $\times$  10  
169 min), dipped in distilled water and dried.

170  
171 Binding of [ $^3$ H]-CP-55,940 was used to assess binding density of CB1 receptor [39]. Sections  
172 were allowed to defrost and then preincubated for 30 min in Tris-HCl buffer (5% bovine  
173 serum albumin (BSA), 50 mM Tris-HCl, pH 7.4) at room temperature. The binding sites of  
174 CB1 receptor were defined by incubation with 10 nM [ $^3$ H]-CP-55,940. Nonspecific binding  
175 was determined in the presence of 10  $\mu$ M CP-55,940. Following incubation for 2 hrs at room  
176 temperature, slides were washed firstly for 1 hr and then 3 hrs in ice-cold buffer (1% BSA, 50  
177 mM Tris–HCl, pH 7.4), and then finally washed for a further 5 min in buffer containing no  
178 BSA. Slides were then dipped briefly in ice-cold distilled water and dried under a gentle  
179 stream of cool air.

180  
181 [ $^3$ H]-Muscimol binding was performed to examine GABA<sub>A</sub> receptor binding density based  
182 on the method described in previous work from our laboratories [31]. Briefly, all sections  
183 underwent three 5 min pre-incubations at 4 °C in 50 mM Tris-citrate (pH 7.0). Sections were

184 then incubated for 45 min at 4 °C in the same buffer containing 3 nM [<sup>3</sup>H]-Muscimol  
185 (specific activity 29.5 Ci/mmol, PerkinElmer, USA). Non-specific binding was determined  
186 by incubating adjacent sections in [<sup>3</sup>H]-Muscimol plus 100 μM GABA. Following incubation,  
187 sections were rinsed four times for 2s each in 4 °C buffer.

188

### 189 **Quantification and statistical analysis**

190 Quantification of binding sites was performed on a high-resolution Beta Imager (BioSpace,  
191 Paris, France) according to our previous study [40]. Briefly, sections were placed in a sample  
192 holder inside the detection chamber of the Beta Imager. The levels of bound radioactivity in  
193 the brain sections were directly determined by counting the number of β-particles emerging  
194 from the tissue sections. The Beta Vision Plus program (BioSpace, France) was used to  
195 measure the activities in the regions of interest. Radioligand binding signal was expressed in  
196 counts per minute per square millimetre (cpm/mm<sup>2</sup>), and with the use of standards was  
197 converted to fmol/mg tissue equivalents. The receptor density in various brain regions was  
198 quantified by measuring the average density of each region in three to five adjacent brain  
199 sections. Different brain regions were identified by reference to a standard rat brain atlas [36].  
200 Data was expressed as mean ± SEM. [<sup>3</sup>H]-WAY-100635, [<sup>3</sup>H]-Ketanserin, [<sup>3</sup>H]-CP-55,940  
201 and [<sup>3</sup>H]-Muscimol binding densities for each brain region were analyzed using a one-way  
202 ANOVA followed by a post-hoc Tukey–Kramer–HSD test using the SPSS 15.0 program  
203 (Chicago, IL). *P* values of less than 0.05 were regarded as statistically significant, and *P*  
204 values of less than 0.10 as a statistically significant trend.

205

## 206 **RESULTS**

### 207 **5-HT<sub>1A</sub> receptor binding**

208 The 5-HT<sub>1A</sub> receptor was widely distributed throughout the rat brain (Table 1). High 5-HT<sub>1A</sub>

209 receptor density was observed in the hippocampus, anterior cingulate cortex (ACC), lateral  
210 septal nucleus, primary motor cortex, and medial posterodorsal amygdala. Binding to 5-HT<sub>1A</sub>  
211 receptor was also observed in the ventromedial hypothalamus (VMH) and piriform cortex in  
212 lower levels.

213 Within the hippocampus there was a significant effect of dietary intervention on 5-HT<sub>1A</sub>  
214 receptor density ( $F_{(2, 12)}=11.641$ ,  $P=0.002$ ) (Table 1). The rats on HF diet had significantly  
215 higher 5-HT<sub>1A</sub> binding density (+54%,  $P=0.006$ ), compared to rats on LF diet. For the DHA  
216 supplemented group, 5-HT<sub>1A</sub> binding density was significantly lower than the HF group (-  
217 40%,  $P=0.002$ ), but there was no significant difference in 5-HT<sub>1A</sub> binding density in the  
218 hippocampus between DHA group and LF group (Fig 1A, Fig 2).

219 A dietary effect was also observed on 5-HT<sub>1A</sub> receptor density within the VMH ( $F_{(2, 12)}=8.222$ ,  
220  $P=0.006$ ) (Table 1). Rats maintained on HF diet had significantly higher 5-HT<sub>1A</sub>  
221 receptor expression in VMH than rats on LF diet (+58%,  $P=0.007$ ). In addition, dietary  
222 intervention by the addition of DHA to the HF diet significantly decreased receptor densities  
223 compared to the rats on HF diet (-31% decrease,  $P=0.022$ ), but there was no significant  
224 difference in 5-HT<sub>1A</sub> receptor expression in the VMH between the DHA and LF group (Fig  
225 3A, Fig 2).

### 226 **5-HT<sub>2A</sub> binding density**

227 There was abundant binding of [<sup>3</sup>H]-Ketanserin to 5-HT<sub>2A</sub> receptors in the ACC, caudate  
228 putamen, medial mammillary nucleus (MM), primary motor cortex, piriform cortex, medial  
229 posterodorsal amygdala and VMH. 5-HT<sub>2A</sub> receptor expression was also observed at lower  
230 levels in the hippocampus (Table 2).

231 5-HT<sub>2A</sub> binding density in the ACC differed between the various diet treatment groups in this  
232 study ( $F_{(2, 12)}=12.474$ ,  $P=0.001$ ) (Table 2). The 5-HT<sub>2A</sub> binding density was significantly  
233 higher in the HF group than the LF or HF + 0.5% DHA group (+71%,  $P=0.003$  and +75%,  $P$

234 =0.002 respectively) (Fig 4A and Fig 5). There was no significant difference between DHA  
235 and LF group.

236 Within the caudate putamen dietary intervention had a significant effect on 5-HT<sub>2A</sub> binding  
237 density ( $F_{(2, 12)}=11.179$ ,  $P =0.002$ ) (Table 2). Rats fed the HF diet had significantly higher 5-  
238 HT<sub>2A</sub> binding density (+43%,  $P =0.001$ ) compared to rats on the LF diet. The DHA  
239 supplemented group had significantly lower 5-HT<sub>2A</sub> binding density compared with the HF  
240 group (-19% lower,  $P =0.026$ ), while there was no significant difference between DHA group  
241 and LF group (Fig 4B and Fig 5).

242 This study also demonstrated differences between diet treatment groups in 5-HT<sub>2A</sub> receptor  
243 density in the MM ( $F_{(2, 12)}=6.857$ ,  $P =0.010$ ) (Table 2). In the HF group 5-HT<sub>2A</sub> binding  
244 density was 47% higher than the LF group ( $P =0.026$ ) and 55% higher than the DHA  
245 supplemented group ( $P =0.015$ ). No difference was observed between LF and DHA groups  
246 (Fig 4C and Fig 6). A similar pattern of receptor expression in response to diet treatment was  
247 also observed in the anterior amygdaloid area.

#### 248 **CB1 receptor binding density**

249 Diet affected the expression of CB1 receptor within the hippocampus ( $F_{(2, 12)}=2.960$ ,  $P$   
250 =0.048) (Table 3). The rats on HF diet had 43% elevated CB1 receptor density compared with  
251 rats on LF diet ( $P =0.007$ ) (Fig 1B, Fig 7). DHA supplementation significantly lowered CB1  
252 receptor binding density compared with the HF group (-22%,  $P =0.041$ ), but there was no  
253 significant difference in hippocampal CB1 receptor density between the DHA and LF groups.

254 There was also a significant effect by dietary intervention on CB1 receptor density in the Arc  
255 ( $F_{(2, 12)}=37.138$ ,  $P <0.001$ ) (Table 3). In this region, rats on the HF diet had significantly  
256 higher CB1 receptor density than the rats on LF diet (+64%,  $P <0.001$ ) (Fig 3B, Fig 7). The  
257 supplementation of DHA in the HF diet significantly decreased receptor expression compared  
258 to the rats on HF diet (-39%,  $P <0.001$ ), but no difference was observed between DHA and

259 LF groups.

260 Furthermore, HF diet significantly increased CB1 receptor density in the substantia nigra  
261 (SN), ventral tegmental area (VTA), and amygdala compared with LF diet (SN: +37%,  $P$   
262 =0.003; VTA: +15%,  $P$  =0.020; amygdala: +20%,  $P$  =0.045) (Table 3). CB1 receptor binding  
263 density was decreased with DHA supplementation compared with the HF group in these brain  
264 areas. There was no effect of dietary intervention on CB1 in the VMH, caudate putamen,  
265 piriform cortex, primary motor cortex and ACC.

### 266 **GABA<sub>A</sub> binding density**

267 GABA<sub>A</sub> receptor binding density in the hippocampus was affected by the different diets  
268 utilised in this study ( $F_{(2, 12)}$ =4.386,  $P$  =0.040) (Table 4). Hippocampal GABA<sub>A</sub> receptor  
269 density was increased 53% in the HF group compared to the LF group ( $P$  =0.021) (Fig 1C,  
270 Fig 8), while DHA supplementation significant lowered the HF induced elevation in GABA<sub>A</sub>  
271 receptor binding density by 42% ( $P$  =0.038). There was also a positive correlation between  
272 CB1 and GABA<sub>A</sub> receptor binding density in the hippocampus ( $R$ =0.593,  $P$  =0.025) (Fig 9).

273 In the thalamus and posterior cingulate cortex (PCC), HF diet significantly decreased  
274 GABA<sub>A</sub> receptor density compared with LF diet (thalamus, -41%,  $P$  =0.020; PCC -60%,  $P$   
275 =0.011) (Table 4). While GABA<sub>A</sub> receptor density was significantly increased by DHA  
276 supplementation compared with HF group in these brain areas (thalamus, +77%,  $P$  =0.011;  
277 +PCC 154%,  $P$  =0.009). There was no significant effect of dietary intervention on GABA<sub>A</sub>  
278 receptor density in the ACC.

### 279 **Energy intake, body weight, and plasma leptin level of rats with dietary intervention**

280 The average of energy intake during the dietary treatment was significantly different among  
281 the three groups ( $P$  =0.010, HF: 94.38±2.69 kcal/24hours; LF: 84.69±1.56 kcal/24hours; HF  
282 + 0.5% DHA: 90.81±1.86 kcal/24hours), in which HF group was significantly higher than LF  
283 group ( $P$  =0.007). No significant difference was found between other groups. The four week

284 accumulative energy intake was also significantly higher in HF group than the LF group  
285 (11.44%,  $P = 0.012$ ). There was no significant difference in body weight changes among  
286 three groups ( $P = 0.503$ , HF:  $84.80 \pm 5.48$ g; LF:  $81.78 \pm 6.05$ g; HF + 0.5% DHA:  $83.00 \pm 6.04$ g).  
287 The plasma level of leptin in HF diet fed rats ( $11.47 \pm 2.17$ ng/ml) was significantly higher than  
288 that of the LF group ( $4.72 \pm 0.73$ ng/ml) ( $P = 0.005$ ). DHA supplementation decreased the  
289 plasma leptin level ( $7.21 \pm 1.01$ ng/ml) of rats compared with HF group in statistically  
290 significant trend ( $P = 0.070$ ), while there was no significant difference in plasma leptin  
291 between DHA and LF group ( $P = 0.290$ ).

292

293

294

## DISCUSSION

295 Serotonin, cannabinoids and GABA systems play an important role in cognitive function [14,  
296 29, 31], and a chronic high-saturated fat diet has been shown to affect memory and learning  
297 [2]. Therefore, the effects of high-saturated fat diets on these neurotransmitter systems are of  
298 interest. This study showed that a high-saturated fat diet increased the density of 5-HT<sub>1A</sub>  
299 receptor in the hippocampus and VMH, 5-HT<sub>2A</sub> receptor in the ACC, caudate putamen and  
300 MM, CB1 receptor in the hippocampus, Arc, SN, VTA and amygdale, and GABA<sub>A</sub> receptor  
301 in the hippocampus. These regions are primarily limbic structures associated with the  
302 regulation of cognition. In addition, these HF diet induced changes in receptor density can be  
303 prevented by dietary supplementation of 0.5% DHA.

304

305 A number of changes in receptor expression have been observed in the brain of individuals  
306 with abnormal cognitive function. It has been reported that 5-HT<sub>1A</sub> receptor binding density  
307 in the human hippocampus is negatively correlated with memory [18]. Furthermore, 5-HT<sub>1A</sub>  
308 and 5-HT<sub>2A</sub> receptor binding densities are significantly increased in the temporal cortex of

309 patients with dementia [22]. Both GABA<sub>A</sub> and CB1 receptor densities are increased in the  
310 posterior cingulate cortex of schizophrenia [41, 42]. This study similarly found alterations in  
311 receptor density in response to a high-saturated fat diet, specifically increased 5-HT<sub>1A</sub>, 5-  
312 HT<sub>2A</sub>, GABA<sub>A</sub> and CB1 receptor densities in a number of brain regions, particularly in the  
313 limbic structures. Although the mechanism for the alteration of receptor binding densities is  
314 unclear, such effects could be due to the high-saturated fat diet decreasing the level of the  
315 respective neurotransmitters in the limbic regions. This is supported by a study showing that  
316 a high-fat diet (20% corn oil) for six weeks significantly decreased 5-HT levels in the  
317 brainstem of rats [43]. In addition, maternal high-fat consumption results in a significant  
318 decrease in CSF 5-HT content leading to 55% of offspring with increased anxiety as assessed  
319 by the novel object tests, and 78% with aberrant behavior (anxious and/or aggressive) [44].

320

321 We found that hippocampal 5-HT<sub>1A</sub> binding density was increased in rats fed a high-saturated  
322 fat diet. Hippocampal circuits play an important role in learning and memory, but also in the  
323 hedonic aspects of eating [18, 45]. 5-HT<sub>1A</sub> receptors in the hippocampus are negatively  
324 associated with memory function in clinical and animal studies [18, 46]. Using positron  
325 emission tomography (PET), a significant negative correlation was found between explicit  
326 memory function and 5-HT<sub>1A</sub> receptor expression localized in the bilateral hippocampus of  
327 healthy subjects. Furthermore, administration of the 5-HT<sub>1A</sub> agonist tandospirone dose-  
328 dependently impaired explicit verbal memory [18]. In a rat study, injection of the 5-HT<sub>1A</sub>  
329 agonist 8-OH-DPAT into hippocampus resulted in memory and learning impairment [46].  
330 Conversely, administration of WAY 100635, a 5-HT<sub>1A</sub> antagonist, into the hippocampus of  
331 rats prevented the deficit of spatial learning induced by administration of CPP, a NMDA  
332 receptor antagonist [47]. Recent findings indicate that dietary factors which promote  
333 excessive food intake and weight gain can also interfere with hippocampal functioning. For

334 example, epidemiological and animal studies show that intake of diets high in saturated fat  
335 are associated with memory deficits and microglial activation (indicating inflammation  
336 and/or gliosis) in the hippocampus [2, 3]. Therefore, the high-saturated fat diet induced  
337 increase in hippocampal 5-HT<sub>1A</sub> receptor expression observed in this study may be involved  
338 in impairment of hippocampus function associated with learning and memory which in turn  
339 contributes to an increased energy intake.

340

341 Furthermore, we found that both CB1 and GABA<sub>A</sub> receptor density were increased in the  
342 hippocampus of rats fed high-saturated fat diet. It is known that CB1 receptors are highly  
343 expressed in the hippocampus and are involved in memory function in this brain region. An  
344 intrahippocampal administration of rimonabant, a CB1 antagonist, completely attenuated the  
345 memory disruptive effects of cannabinoid induced memory impairment [48]. Systemic and  
346 intrahippocampal administration of cannabinoid agonists have been shown to impair  
347 hippocampal-dependent memory tasks [48, 49]. Oral administration of a CB1 inverse agonist,  
348 SLV319, inhibits the CB1 receptor-mediated catalepsy induced by HU-210 ip injection in  
349 mice [50]. In the present study, the elevated CB1 receptor binding density suggests that  
350 activation of CB1 in the hippocampus may contribute to high-saturated fat associated  
351 memory deficits. Endocannabinoid (eCB) ligands have been shown to act on the CB1  
352 receptor to inhibit the release of GABA in the rat hippocampus [51]. In this study the  
353 increased GABA<sub>A</sub> receptor expression in response to high-fat diet may reflect decreased  
354 GABA as a consequence of CB1 receptor activation in the hippocampus. This is supported by  
355 our observation that CB1 receptor density is positively correlated with GABA<sub>A</sub> receptor  
356 density. Furthermore, a previous study showed that in high-fat diet induced obese mice CB1  
357 receptor immunoreactivity and the eCBs, anandamide and 2-arachidonoyl glycerol (2-AG)  
358 were increased in the hippocampus [52]. In this study CB1 receptor binding density in the



359 hippocampus was increased even without any changes in body weight. This suggests that  
360 high-fat diet alone rather than obesity increases CB1 binding.

361

362 Both clinical trials and animal studies have shown that DHA supplementation can improve  
363 learning and memory [53, 54]. Conversely, depletion of DHA in rat brain was found to  
364 increase 5-HT<sub>1A</sub> expression in the hippocampus and was associated with impairment of  
365 spatial learning and memory [55, 56]. In our study, addition of DHA to the diet prevented the  
366 increase of hippocampal 5-HT<sub>1A</sub> density in rats induced by a high-saturated-fat diet. DHA  
367 supplementation is also able to prevent increased CB1 and GABA<sub>A</sub> receptor densities  
368 induced by high-fat diet, as shown in this study. These findings suggest the effect of DHA  
369 supplementation on improving learning and memory may be via its influence on hippocampal  
370 5-HT<sub>1A</sub>, CB1 and GABA<sub>A</sub> systems.

371

372 The hypothalamus is well recognised as a critical centre in the regulation of energy balance.  
373 Hypothalamic 5-HT<sub>1A</sub> receptors are involved in the control of negative energy balance. A  
374 negative relationship has been reported between the 5-HT content in the hypothalamus and  
375 amount of fat and food intake in rodents. For example, an infusion of 5-HT into the  
376 hypothalamus can lead to a dose-related decrease in the amount of fat intake in either fat- or  
377 carbohydrate- preferring rats [57]. The intrahypothalamic injection of a 5-HT<sub>1A</sub> agonist, 8-  
378 OH-DPAT, decreases food intake and promotes satiety [58]. Conversely, intra-hypothalamic  
379 injection of WAY-100635, a 5-HT<sub>1A</sub> antagonist, blocks the anorexic effect induced by 5-HT  
380 [59]. The present study showed that rats fed a high-fat diet had increased 5-HT<sub>1A</sub> receptor  
381 expression in the ventromedial hypothalamus (VMH). This finding supports the assertion that  
382 a high-fat diet significantly decreases central 5-HT levels in rats [43]. Moreover, in the  
383 present study DHA supplementation prevented the increase in VMH 5-HT<sub>1A</sub> receptor density

384 induced by a high-saturated fat diet, which is in agreement with various reports in the  
385 literature. Previous studies have shown that n-3 PUFA/DHA intake influences 5-HT levels in  
386 the brain. A positive association has been reported between the amount of dietary DHA and  
387 brain 5-HT in piglets [60]. While rats maintained on a n-3 deficient diet have a low response  
388 to fenfluramine induced 5-HT stimulation [61]. Finally, n-3 PUFA supplementation in mice  
389 reverses the stress-induced reduction in 5-HT levels [62].

390

391 CB1 receptor expression was also increased in the Arc of the hypothalamus as a result of 4  
392 weeks of high-saturated fat diet, and this was prevented by dietary DHA supplementation.  
393 Hypothalamic eCBs and the CB1 receptor are involved in food intake and the response to  
394 peripheral feeding signals. Intravenous injection of leptin reduces the levels of the eCBs  
395 anandamide and 2-AG in the hypothalamus of normal rats and ob/ob mice [63]. High-  
396 saturated fat diets increase plasma leptin thereby downregulating eCBs in the Arc, which may  
397 have led to the upregulation of Arc CB1 receptor density observed in this study. Moreover,  
398 the prevention of hyperleptinemia in high-saturated fat fed rats supplemented with DHA may  
399 have played a role in maintaining CB1 receptor binding density at levels similar to LF rats.

400

401 In the present study a high-saturated fat diet increased 5-HT<sub>2A</sub> receptor binding density in the  
402 caudate putamen (striatum), ACC and MM of rats. The striatal serotonergic (5-HT) system is  
403 involved in reward behaviour; elevated 5-HT neurotransmission increases reward (positive  
404 feedback) sensitivity and decreases negative feedback sensitivity in rats [64, 65]. Rats fed a  
405 high-saturated fat diet have lowered levels of 5-HT release from striatal slices compared to  
406 rats fed a low-fat diet [64]. High saturated-fat diet induced obesity has been considered as a  
407 compulsive disorder reflecting a “reward deficiency syndrome” [66]. Therefore, the increase  
408 in striatal 5-HT<sub>2A</sub> receptor binding density observed in this study may contribute to deficits in

409 the reward system. The ACC and MM are involved in cognitive and memory function [67,  
410 68]. Studies with functional neuroimaging techniques, including PET and functional  
411 magnetic resonance imaging (fMRI), have ascribed the ACC with cognitive function and  
412 working memory [69]. Rodents with lesions of the MM are impaired on tests of spatial  
413 memory tasks and working memory [70, 71]. When rats are fed a high-fat diet they show a  
414 reduction in their cognitive ability and a decline in working memory after just nine days [72].  
415 The 5-HT<sub>2A</sub> receptor plays an important role in cognitive abilities and working memory  
416 process [13, 73]. In the present study, 5-HT<sub>2A</sub> receptor binding density increased in brain  
417 regions related to cognition and memory (ACC and MM).

418

419 Decreased DHA content in the brain is associated with increased density of cortical 5-HT<sub>2A</sub>  
420 receptors and altered serotonergic neurotransmission [74, 75]. Perinatal DHA-deficient rats  
421 have significantly lowered 5-HT content in the prefrontal cortex [74]. Moreover, a n-3  
422 PUFA-supplemented diet reverses decreased brain 5-HT levels in mice subjected to chronic  
423 mild stress [76]. In the present study adding DHA into the high-saturated fat diet of rats  
424 prevents increased levels of 5-HT<sub>2A</sub> binding density in the striatum, ACC and MM. The  
425 previously discussed ability of DHA supplementation to maintain central 5-HT levels is a  
426 potential mechanism by which DHA prevents 5-HT<sub>2A</sub> receptor upregulation. In addition,  
427 DHA content influences the physicochemical properties of neuronal membranes, and thus  
428 modulates the function of membrane bound proteins, such as receptors [77, 78]. Alterations  
429 in the fatty acid composition of neural membranes with DHA supplementation may result in  
430 changes in the affinity of neuronal receptors towards their neurotransmitter [77]. Therefore it  
431 is also possible that DHA directly affects the 5-HT<sub>2A</sub> receptor by increasing affinity to its  
432 neurotransmitter, negating the need for an increase in expression to cope with reduced 5-HT  
433 levels. DHA can affect gene expression as well as mRNA stability [77]. It is therefore also

434 possible that DHA exerts its effects on the 5-HT<sub>2A</sub> receptor at a transcriptional level.  
435 However, the exact mechanism by which DHA influences this receptor requires further  
436 research.

437

438 In summary, we found that a high-saturated fat diet significantly increased 5-HT<sub>1A</sub>, CB1 and  
439 GABA<sub>A</sub> receptor binding densities in various rat brain regions, especially in limbic structures  
440 such as hippocampus and hypothalamus, which are important in the regulation of energy  
441 balance, learning, memory and cognitive functions. Furthermore, 5-HT<sub>2A</sub> receptor binding  
442 was increased in the caudate putamen, anterior cingulate cortex and medial mammillary  
443 nucleus of rats fed a high-saturated fat diet. The anatomical distributions of these receptor  
444 alterations suggest serotonin, cannabinoid and GABA receptor contribute at least partially to  
445 cognitive dysfunctions and abnormal energy balance induced by high-saturated fat diet,  
446 which is well supported by current literature. Importantly, the addition of dietary DHA  
447 prevented alteration of these receptor binding densities in rats induced by high-fat diet. The  
448 present findings point to DHA acting on numerous receptor systems in various areas of the  
449 brain. Furthermore, our results support the assertion that DHA supplements have beneficial  
450 effects on improving memory and cognition. Therefore, potential strategies to improve  
451 mental function against the adverse effects of high-saturated fat diets include targeting the  
452 serotonin, CB1 and GABA receptor systems, as well the proper application of molecular  
453 nutrition using supplements such as DHA.

454

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460

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673 **Figure Legends:**

674

675 Fig 1. The effect of dietary intervention on [<sup>3</sup>H]-WAY-100635 (A), [<sup>3</sup>H]-CP55940 (B) and  
676 [<sup>3</sup>H]-Muscimol (C) binding (nCi/mg tissue) in the hippocampus of the rat brain. Data are  
677 expressed as mean ± SEM. Abbreviations: LF, low-fat diet; HF, high-saturated fat diet; DHA,  
678 n-3 polyunsaturated docosahexaenoic acid; Hip: hippocampus. \*P <0.05 vs. HF.

679

680 Fig 2. Autoradiograph depicting [<sup>3</sup>H]-WAY-100635 binding in the hippocampus and  
681 ventromedial hypothalamus (VMH) of rats fed a LF (B), HF (C) and HF+DHA diet (D).  
682 Panel A is from a rat brain atlas. The density of [<sup>3</sup>H]-WAY-100635 binding was significantly  
683 increased in the hippocampus and VMH by HF diet, whereas the DHA supplement prevented  
684 the increase of [<sup>3</sup>H]-WAY-100635 binding by HF diet. LF, low-fat diet; HF, high-saturated  
685 fat diet; DHA, n-3 polyunsaturated docosahexaenoic acid.

686

687 Fig 3. The effect of dietary intervention on [<sup>3</sup>H]-WAY-100635 (A) and [<sup>3</sup>H]-CP55940 (B)  
688 binding (nCi/mg tissue) in the hypothalamus of the rat brain. Data are expressed as mean ±  
689 SEM. Abbreviations: LF, low-fat diet; HF, high-saturated fat diet; DHA, n-3 polyunsaturated  
690 docosahexaenoic acid; VMH, ventromedial hypothalamus; Arc, hypothalamic arcuate  
691 nucleus. \*P <0.05 vs. HF.

692 Fig 4. The effect of dietary intervention on [<sup>3</sup>H]-Ketanserin binding density (nCi/mg tissue)  
693 in the rat brain. Data are expressed as mean ± SEM. Abbreviations: MM, medial mammillary  
694 nucleus; CPu, caudate putamen; ACC, anterior cingulate cortex; LF, low-fat diet; HF, high-  
695 saturated fat diet; DHA, n-3 polyunsaturated docosahexaenoic acid. \*P <0.05 vs. HF.

696

697 Fig 5. Autoradiograph depicting [<sup>3</sup>H]-Ketanserin binding in the anterior cingulae cortex and  
698 caudate putamen of rats on LF (B), HF (C) and HF+DHA diet (D). Panel (A) is from a rat

699 brain atlas. The density of [<sup>3</sup>H]-Ketanserin binding was significantly increased in the anterior  
700 cingulae cortex and caudate putamen by HF diet whereas the DHA supplement prevented the  
701 increase of [<sup>3</sup>H]-Ketanserin binding by HF diet. LF, low-fat diet; HF, high-saturated fat diet;  
702 DHA, n-3 polyunsaturated docosahexaenoic acid.

703

704 Fig 6. Autoradiograph depicting [<sup>3</sup>H]-Ketanserin binding in the medial mammillary nucleus  
705 of rats on LF (B), HF (C) and HF+DHA diet (D). Panel (A) is from a rat brain atlas. The  
706 density of [<sup>3</sup>H]-Ketanserin binding was significantly increased in the medial mammillary  
707 nucleus induced by HF diet, whereas the DHA supplement prevented the increase of [<sup>3</sup>H]-  
708 Ketanserin binding by HF diet. LF, low-fat diet; HF, high-saturated fat diet; DHA, n-3  
709 polyunsaturated docosahexaenoic acid.

710

711 Fig 7. Autoradiograph depicting [<sup>3</sup>H]-CP-55,940 binding in the hippocampus (A-C) and  
712 hypothalamic arcuate nucleus (D-F) of rats on LF (A and D), HF (B and E) and HF+DHA  
713 diet (C and F). The density of [<sup>3</sup>H]-CP-55,940 binding was significantly increased in the  
714 hippocampus and hypothalamic arcuate nucleus by HF diet, whereas the DHA supplement  
715 prevented the increase of [<sup>3</sup>H]-CP-55,940 binding by HF diet. LF, low-fat diet; HF, high-  
716 saturated fat diet; DHA, n-3 polyunsaturated docosahexaenoic acid.

717

718 Fig 8. Autoradiograph depicting [<sup>3</sup>H]-Muscimol binding in the hippocampus of rats on LF  
719 (A), HF (B) and HF+DHA diet (C). The density of [<sup>3</sup>H]-Muscimol binding was significantly  
720 increased in the hippocampus by HF diet, whereas the DHA supplement prevented the  
721 increase of [<sup>3</sup>H]-Muscimol binding by HF diet. LF, low-fat diet; HF, high-saturated fat diet;  
722 DHA, n-3 polyunsaturated docosahexaenoic acid.

723

724

725 Fig 9. There was significant correlation between [<sup>3</sup>H]-CP55940 and [<sup>3</sup>H]-Muscimol binding

726 (nCi/mg tissue) in the hippocampus of rat brain.

727

728

Table 1. Specific [<sup>3</sup>H]-WAY-100635 binding (nCi/mg tissue; mean ± SEM) in different brain regions following 4 weeks of dietary intervention

	Mean ± SEM.			One-way ANOVA		<i>P</i> value, Tukey's HSD post hoc		
	LF (n=5)	HF (n=5)	HF+DHA (n=5)	<i>F</i> (2, 12)	<i>P</i> value	HF vs. LF	HF vs. DHA	DHA vs. LF
Hip	2.14±0.21	3.29±0.16	1.97±0.24	11.641	<b>0.002</b>	<b>0.006</b>	<b>0.002</b>	0.827
VMH	0.74±0.06	1.17±0.10	0.81±0.08	8.222	<b>0.006</b>	<b>0.007</b>	<b>0.022</b>	0.790
M1	1.66±0.09	1.58±0.09	2.05±0.07	1.167	0.344	–	–	–
ACC	1.64±0.12	1.41±0.15	1.48±0.17	0.635	0.547	–	–	–
LSD	2.89±0.32	2.65±0.24	3.43±0.42	0.043	0.958	–	–	–
MeP	1.46±0.07	1.67±0.22	1.69±0.13	0.654	0.538	–	–	–
Pir	1.09±0.08	1.04±0.05	1.11±0.10	0.192	0.828	–	–	–

Abbreviations: VMH, Ventromedial hypothalamus; Hip, Hippocampus; M1, primary motor cortex; ACC, anterior cingulate cortex; LSD, lateral septal nucleus; MeP, Medial posterodorsal amygdala; Pir, Piriform cortex; LF, low-fat diet; HF, high-fat diet; DHA, n-3 polyunsaturated docosahexaenoic acid.

Table 2. Specific [<sup>3</sup>H]-Ketanserin binding (nCi/mg tissue; mean ± SEM) in different brain regions following 4 weeks of dietary intervention

	Mean ± SEM.			One-way ANOVA		<i>P</i> value, Tukey's HSD post hoc		
	LF (n=5)	HF (n=5)	HF+DHA (n=5)	<i>F</i> (2, 12)	<i>P</i> value	HF vs. LF	HF vs. DHA	DHA vs. LF
ACC	1.92±0.25	3.29±0.21	1.88±0.21	12.474	<b>0.001</b>	<b>0.003</b>	<b>0.002</b>	0.99
CPu	2.13±0.20	3.04±0.07	2.45±0.10	11.179	<b>0.002</b>	<b>0.001</b>	<b>0.026</b>	0.276
MM	2.44±0.39	3.58±0.20	2.31±0.14	6.857	<b>0.010</b>	<b>0.026</b>	<b>0.015</b>	0.943
AA	2.50±0.19	3.28±0.14	2.39±0.13	9.660	<b>0.003</b>	<b>0.006</b>	<b>0.002</b>	0.888
Hip	0.98±0.05	0.97±0.06	0.89±0.04	0.916	0.426	–	–	–
VMH	1.36±0.07	1.35±0.14	1.34±0.09	0.016	0.984	–	–	–
MeP	1.82±0.05	1.76±0.11	1.82±0.16	0.116	0.891	–	–	–
Pir	3.20±0.22	3.48±0.30	3.73±0.39	0.698	0.517	–	–	–
M1	4.45±0.50	4.81±0.43	4.92±0.39	0.303	0.744	–	–	–

Abbreviations: MM, Medial mammillary nucleus; ACC, Anterior cingulated cortex; AA, Anterior amygdaloid area; CPu, Caudate putamen; Hip, hippocampus; M1, primary motor cortex; MeP, Medial posterodorsal amygdala; Pir, Piriform cortex; VMH, Ventromedial hypothalamus; LF, low-fat diet; HF, high-fat diet; DHA, n-3 polyunsaturated docosahexaenoic acid.

Table 3. Specific [<sup>3</sup>H]-CP55940 binding (nCi/mg tissue; Mean±SEM) in different brain regions following 4 weeks of dietary intervention

	Mean±SEM			One-way ANOVA		P value, Tukey's HSD post hoc		
	LF	HF	HF+DHA	F (2, 12)	p- value	HF vs. LF	HF vs. DHA	DHA vs. LF
Hip	82.92±7.39	118.49±14.70	92.47±4.83	2.960	<b>0.048</b>	<b>0.007</b>	<b>0.041</b>	0.778
Arc	37.15±1.72	61.01±3.01	37.27±1.62	37.138	<b>&lt;0.001</b>	<b>&lt;0.001</b>	<b>&lt;0.001</b>	0.999
VMH	65.99±4.41	75.06±4.06	61.45±3.02	3.202	0.077	–	–	–
Amg	47.89±1.32	57.55±2.56	45.38±3.25	6.559	<b>0.012</b>	<b>0.045</b>	<b>0.013</b>	0.764
SN	37.68±1.81	51.73±4.61	34.75±2.93	7.465	<b>0.008</b>	<b>0.003</b>	<b>0.001</b>	0.810
VTA	40.12±1.55	47.34±1.57	38.30±1.10	11.260	<b>0.002</b>	<b>0.020</b>	<b>0.005</b>	0.651
CPu	54.10±6.20	56.78±4.10	51.53±2.66	0.331	0.725	–	–	–
Pir	56.13±3.20	65.76±7.12	54.34±2.87	1.635	0.236	–	–	–
M1	65.14±6.95	63.59±5.15	61.87±6.21	0.071	0.932	–	–	–
ACC	57.30±6.91	60.84±6.21	55.27±3.41	0.244	0.787	–	–	–

Abbreviations: Arc, hypothalamic arcuate nucleus; SN, Substantia nigra; VTA, Ventral tegmental area; Hip, hippocampus; VMH, Ventromedial hypothalamus; Amg, Amygdala; CPu, Caudate putamen; Pir, Piriform cortex; M1, Primary motor cortex; ACC, anterior cingulate cortex; HF, high-fat diet; LF, low-fat diet; DHA, n-3 polyunsaturated docosahexaenoic acid.



Table 4. Specific [ $^3\text{H}$ ]-Muscimol binding (nCi/mg tissue; mean  $\pm$  SEM) in different brain regions following 4 weeks of dietary intervention

	Mean $\pm$ SEM.			One-way ANOVA		<i>P</i> value, Tukey's HSD post hoc		
	LF (n=5)	HF (n=5)	DHA (n=5)	<i>F</i> (2, 12)	<i>P</i> value	HF vs. LF	HF vs. DHA	DHA vs. LF
Hip	3.43 $\pm$ 0.47	5.25 $\pm$ 0.57	3.05 $\pm$ 0.67	4.386	<b>0.040</b>	<b>0.021</b>	<b>0.038</b>	0.656
PCC	3.23 $\pm$ 0.68	1.23 $\pm$ 0.34	3.12 $\pm$ 0.32	6.923	<b>0.011</b>	<b>0.012</b>	<b>0.009</b>	0.878
Thalamus	5.10 $\pm$ 0.58	3.01 $\pm$ 0.60	5.35 $\pm$ 0.47	5.375	<b>0.022</b>	<b>0.020</b>	<b>0.011</b>	0.760
ACC	2.23 $\pm$ 0.21	1.96 $\pm$ 0.34	2.59 $\pm$ 0.47	0.733	0.502	–	–	–

Abbreviations: ACC, Anterior cingulate cortex; Hip, hippocampus; PCC, posterior cingulate cortex; LF, low-fat diet; HF, high-fat diet; DHA, n-3 polyunsaturated docosahexaenoic acid.

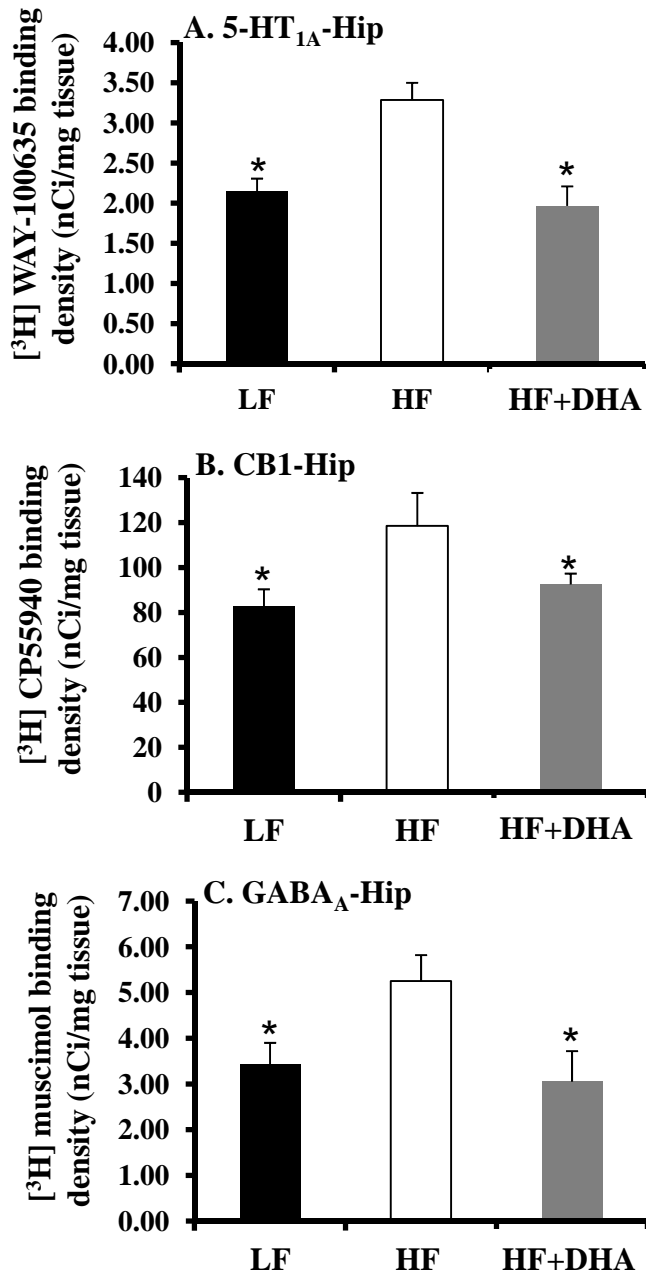


Fig 1.

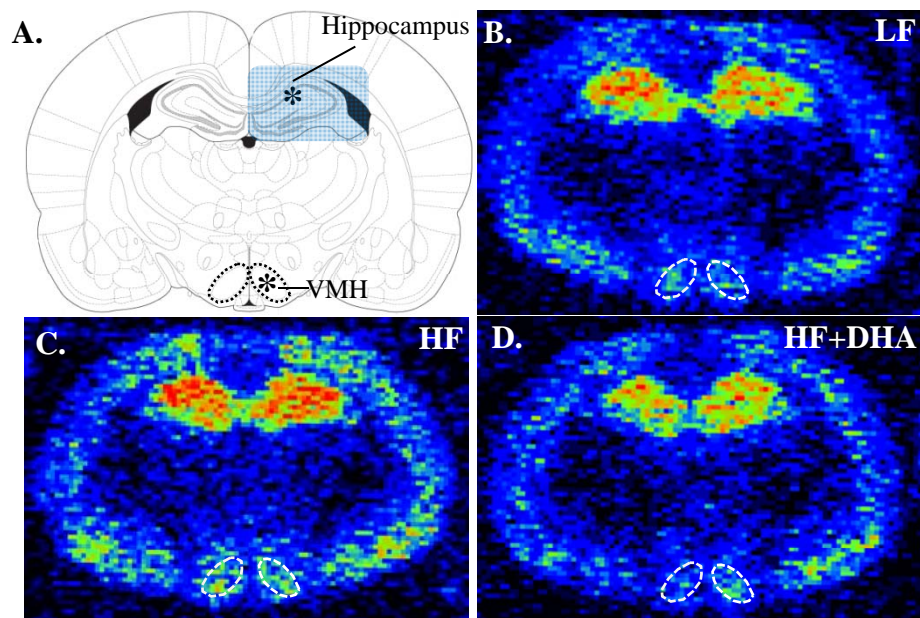


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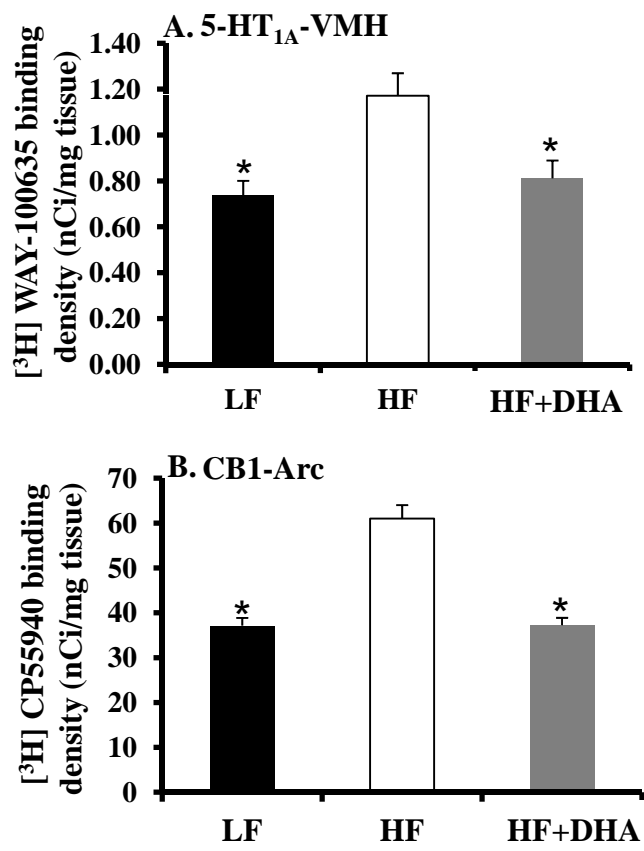


Fig 3.

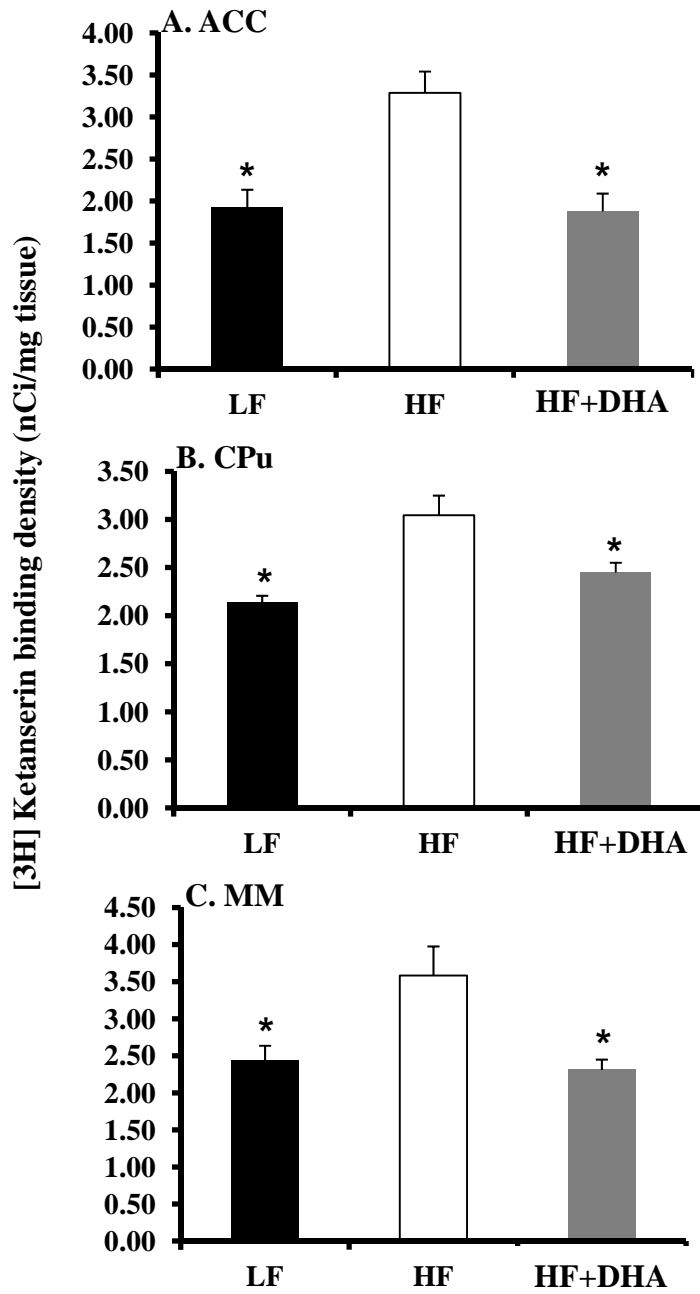


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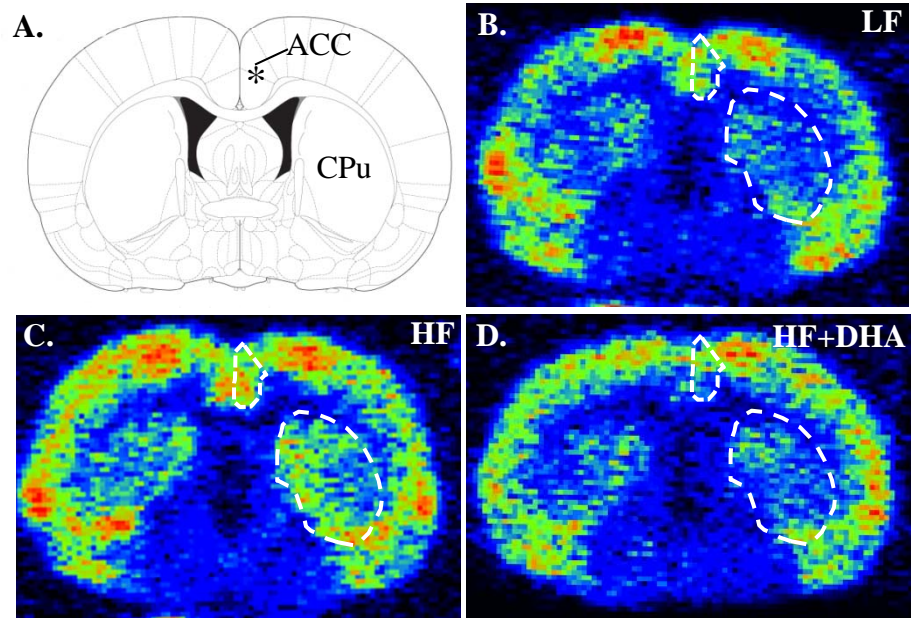


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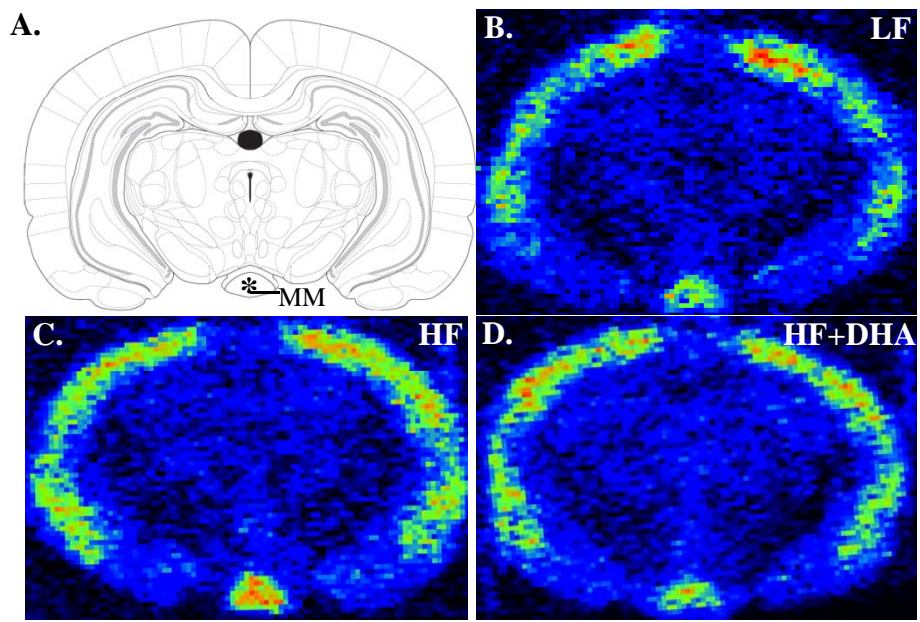


Fig 6.

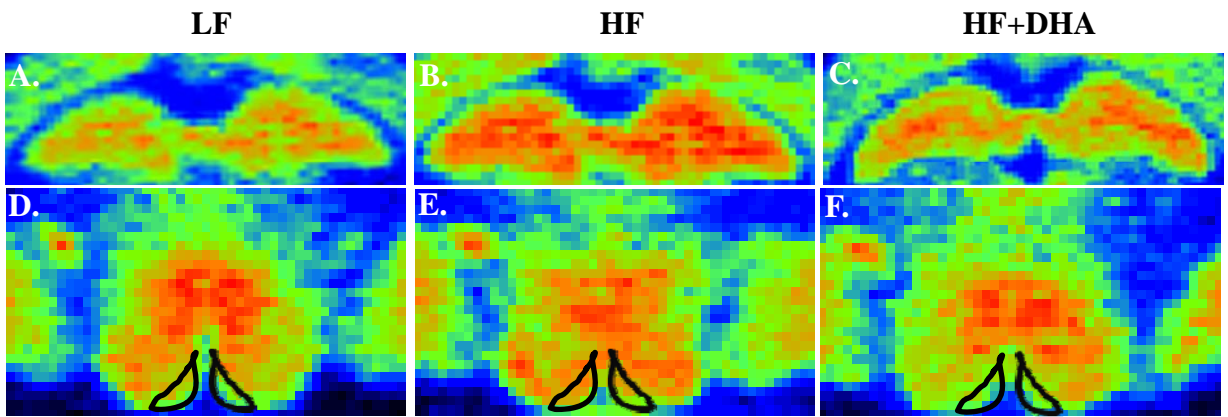


Fig 7.



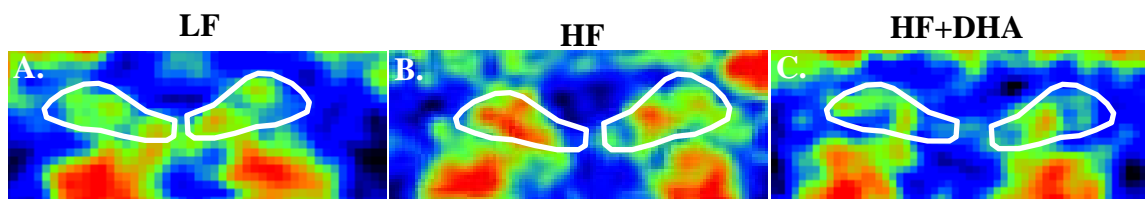


Fig 8.

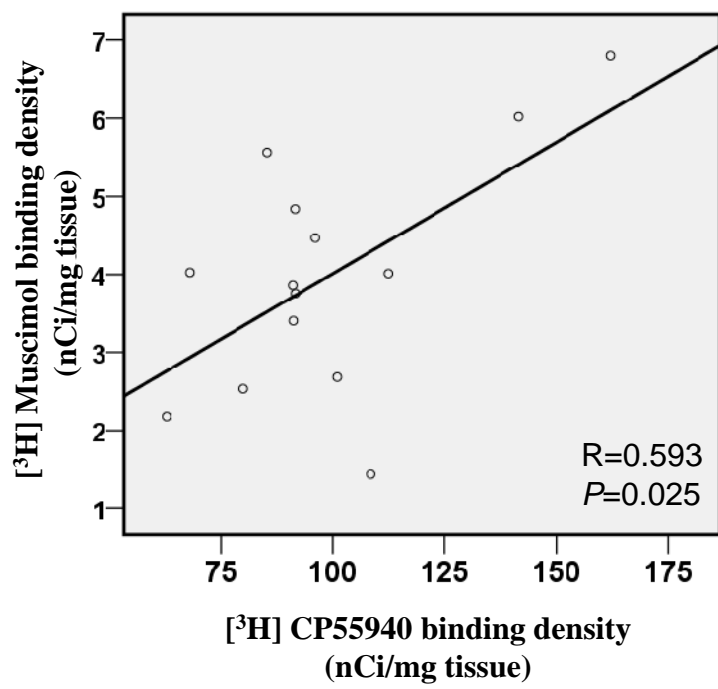


Fig 9.