

January 2013

Reduction of histamine H1 receptor binding induced by high-fat diet can be prevented by DHA and dietary fiber in specific brain areas of male rats

Zhixiang Wu

University of Wollongong, zwu@uow.edu.au

Yinghua Yu

University of Wollongong, yinghua@uow.edu.au

Yizhen Wu

University of Wollongong, yw903@uowmail.edu.au

Craig S. Patch

Nu-Mega Ingredients Pty Ltd, cpatch@uow.edu.au

Alexander Szabo

University of Wollongong, aszabo@uow.edu.au

See next page for additional authors

Follow this and additional works at: <https://ro.uow.edu.au/ihmri>



Part of the [Medicine and Health Sciences Commons](#)

Recommended Citation

Wu, Zhixiang; Yu, Yinghua; Wu, Yizhen; Patch, Craig S.; Szabo, Alexander; and Huang, Xu-Feng, "Reduction of histamine H1 receptor binding induced by high-fat diet can be prevented by DHA and dietary fiber in specific brain areas of male rats" (2013). *Illawarra Health and Medical Research Institute*. 341.
<https://ro.uow.edu.au/ihmri/341>

Reduction of histamine H1 receptor binding induced by high-fat diet can be prevented by DHA and dietary fiber in specific brain areas of male rats

Abstract

High-fat (HF) diet and obesity are risk factors for a number of mental health problems including depression, cognitive dysfunction, dementia, and neurodegenerative diseases. Histamine H1 receptors (H1Rs) are involved in many of these conditions. This study examined H1R receptor binding density in the brain of male rats fed a high-saturated fat (HF) diet, as well as the effect of docosahexaenoic acid (DHA), galacto-oligosaccharide (GOS) and resistant starch (RS) supplementation of HF diet. Alterations of H1R expression in the post-mortem rat brain were detected by [³H]-pyrilamine binding autoradiography. We found that HF diet significantly decreased H1R binding densities in the substantia nigra (SN), caudate putamen (CPu), hypothalamic arcuate nucleus (Arc), ventral tegmental area (VTA), piriform cortex (Pir) and primary motor cortex (M1), compared with low-fat fed rats, and the suppression of receptor binding density ranged from 31% to 48%. Interestingly, supplementing the HF diet with 0.5% n-3 polyunsaturated docosahexaenoic acid (DHA) prevented reduction of H1R binding densities in the SN and CPu. Addition of galacto-oligosaccharide (GOS) and resistant starch (RS) to the diet blunted HF induced reduction of H1R ligand binding in the SN and Pir, respectively. In conclusion this study showed that HF diet can alter H1R binding densities in various brain regions, and many of these changes can be prevented by adding DHA, GOS or RS to the diet.

Keywords

rats, male, areas, brain, specific, fiber, dietary, dha, prevented, histamine, 1, reduction, be, can, diet, fat, high, induced, binding, receptor, h1

Disciplines

Medicine and Health Sciences

Publication Details

Wu, Z., Yu, Y., Wu, Y., Patch, C. S., Szabo, A. & Huang, X. (2013). Reduction of histamine H1 receptor binding induced by high-fat diet can be prevented by DHA and dietary fiber in specific brain areas of male rats. *Brain Research Bulletin*, 97 119-125.

Authors

Zhixiang Wu, Yinghua Yu, Yizhen Wu, Craig S. Patch, Alexander Szabo, and Xu-Feng Huang

1 **Title: Reduction of histamine h1 receptor binding induced by high-fat diet can be**
2 **prevented by DHA and dietary fiber in specific brain areas of male rats**

3
4 **Zhixiang Wu^{a,c}, Yinghua Yu^a, Yizhen Wu^a, Craig Patch^b, Alexander Szabo^{a,d}, Xu-Feng**
5 **Huang^{a*}**

6
7 ^aCenter for Translational Neuroscience, Illawarra Health and Medical Research
8 Institute, School of Health Sciences, University of Wollongong, NSW 2522, Australia;

9 ^bClover Corporation Limited, Nu-Mega Ingredients Pty Ltd, NSW2522,
10 Australia; ^cDepartment of Endocrinology and Metabolism, Affiliated Hospital of
11 Liaoning University of Traditional Chinese Medicine, China

12 ^dANSTO LifeSciences, Australian Nuclear Science and Technology Organisation,
13 Sydney, NSW 2234, Australia

14
15
16
17
18 *Address for correspondence:

19
20 Professor Xu-Feng Huang, MD & PhD
21 32.305 IHMRI, University of Wollongong
22 Northfield Avenue, NSW 2522
23 Australia

24
25 Tel. 61-02-42214300; fax 61-02-42214096

26 E-mail address: xhuang@uow.edu.au

27
28
29 Abbreviated title: Diet influences histamine H1 receptor binding

30
31
32 Total words: 3,372, Words Table: 1 Figure: 4

1 **Abstract-** High-fat (HF) diet and obesity are risk factors for a number of mental health
2 problems including depression, cognitive dysfunction, dementia, and neurodegenerative
3 diseases. Histamine H1 receptors (H1Rs) are involved in many of these conditions. This
4 study examined H1R receptor binding density in the brain of male rats fed a high-saturated
5 fat (HF) diet, as well as the effect of docosahexaenoic acid (DHA), galacto-oligosaccharide
6 (GOS) and resistant starch (RS) supplementation of HF diet. Alterations of H1R expression in
7 the post-mortem rat brain were detected by [³H]-pyrilamine binding autoradiography. We
8 found that HF diet significantly decreased H1R binding densities in the substantia nigra (SN),
9 caudate putamen (CPu), hypothalamic arcuate nucleus (Arc), ventral tegmental area (VTA),
10 piriform cortex (Pir) and primary motor cortex (M1), compared with low-fat fed rats, and the
11 suppression of receptor binding density ranged from 31%-48%. Interestingly, supplementing
12 the HF diet with 0.5% n-3 polyunsaturated docosahexaenoic acid (DHA) prevented reduction
13 of H1R binding densities in the SN and CPu. Addition of galacto-oligosaccharide (GOS) and
14 resistant starch (RS) to the diet blunted HF induced reduction of H1R ligand binding in the
15 SN and Pir, respectively. In conclusion this study showed that HF diet can alter H1R binding
16 densities in various brain regions, and many of these changes can be prevented by adding
17 DHA, GOS or RS to the diet.

18

19

20 **Key words:** histamine H1 receptor; docosahexaenoic acid; galacto-oligosaccharide; resistant
21 starch; receptor autoradiography

22

23

1 1. Introduction

2 Histaminergic neurons are located in the tuberomammillary nucleus of the posterior
3 hypothalamus. These neurons project widely in the brain and are involved in the regulation of
4 homeostasis, arousal, motor behaviour and cognition (Haas and Panula, 2003; Masaki and
5 Yoshimatsu, 2006; Passani et al., 2004). Histamine H1 receptor (H1R) is one of four
6 histamine receptors belonging to the G protein coupled receptor family, and has a broad
7 distribution in the central nervous system (Brown et al., 2001; Martinez-Mir et al., 1990).
8 H1R regulates body energy homeostasis, cognitive function, memory, learning, sleep/wake
9 cycle, feeding rhythms, as well as locomotor activities (Haas et al., 2008; Inoue et al., 1996;
10 Lozeva et al., 2000).

11
12 Certain fatty acids intake can influence neuronal membrane fatty acid composition and in turn
13 affect neurotransmitter interactions with receptors. For example, a high saturated-fat diet
14 reduces dopamine D2 receptor density in the accumbens nucleus, striatum and hypothalamus
15 of mice, when compared to animals fed a low-fat diet (Huang et al., 2006). We have reported
16 that a high n-6 polyunsaturated fatty acid (PUFA) diet reduces muscarinic M2 receptor binding
17 densities in the limbic structures of the rat brain (du Bois et al., 2005); however this is not the
18 case for M1/M4 receptors (du Bois et al., 2006). A decreased docosahexaenoic acid (DHA,
19 22:6 n-3) content in brain tissue induced by α -linolenic acid-deficient diet is associated with
20 decreased brain-derived neurotrophic factor gene expression and increased 5-HT1a receptor
21 densities in the hippocampus of rats (Levant et al., 2008). The effect of a high saturated fat diet

1 and DHA supplementation on the expression of H1R receptor in the brain has not been
2 examined.

3

4 Galacto-oligosaccharide (GOS) and resistant starch (RS) are dietary fibers consumed in human
5 diets. Both GOS and RS are fermentable fibers and can be converted to specific short-chain
6 fatty acids in the colon. These dietary fibers have a number of beneficial health effects
7 including reducing fat absorption, lowering blood cholesterol, stimulating the immune system,
8 and increasing satiety (Davis et al., 2009; Higgins et al., 2001; Higgins et al., 2005). Dietary RS
9 increases mRNA expression of the anorexigenic protein POMC in the hypothalamus of rats
10 (Shen et al., 2009). A recent study of ours has also shown that increasing dietary fiber intake
11 can activate the gut-hypothalamic PYY₃₋₃₆-NPY, axis and increases satiety in diet-induced
12 obese mice (Huang et al., 2011).

13

14 Histamine H1R plays an important role in numerous brain functions. A reduction in the
15 number of H1R binding sites has been reported in elderly individuals, and during
16 Alzheimer's disease, schizophrenia and depression (Higuchi et al., 2000; Yanai et al., 1992).
17 It is known that a chronic high-fat diet can lead to overweight and obesity, which in turn is
18 associated with deficits in brain function including impaired cognitive function, dementia,
19 depression and even neurodegenerative diseases (Park et al., 2010; Winocur and Greenwood,
20 2005). However, the effect of high-fat diet on H1R binding density in the brain has not been
21 examined, and therefore it is of interest to determine whether receptor expression is altered,
22 and more importantly if changes can be prevented by manipulation of macronutrients in the

1 diet. This study examined the effect of a high saturated-fat diet on H1R binding density in
2 various brain regions, and also whether DHA, GOS or RS supplementation prevented
3 high-saturated-fat diet induced alterations in H1R receptor expression in the differing brain
4 regions.

5

6 **2. Materials and methods**

7 2.1. Animals and dietary treatments

8 Twenty five male Wistar rats (300-320g) were obtained from the Animal Resources Centre
9 (Perth, Western Australia, Australia) and housed in environmentally controlled conditions
10 (22°C, 12 hr light–dark cycle with light cycle from 06:00 to 18:00 h and dark cycle from
11 18:00 to 06:00 h) with ad libitum access to standard laboratory chow and water. Rats were
12 allowed 1 week to adapt to their new environment before experiments began. They were
13 randomized into five groups (n=5): (1) standard laboratory chow as low-fat diet (LF, fat
14 content 10% in kcal, saturated fat 1%) control, (2) high-fat (HF, 25% in kcal, saturated fat
15 10%), (3) HF + 5.7% GOS, (4) HF + 5.7% RS and (5) HF + 0.5% DHA. The supplement
16 doses used in this study were based on the doses recommended for humans, including GOS at
17 3g/70kg/day, RS at 2g/70kg/day, and DHA at 250mg/70kg/day (Australian Government Food
18 Agency Recommendations). After four weeks of dietary treatment, rats from each group were
19 weighed and the body weight showed no statistical differences (LF: 442.5g±13.5g, HF:
20 432.8g±11.1g, HF+GOS: 439.4g±7.5g, HF+RS: 453.3g±19.4g, HF+DHA: 426.8g±8.1g;
21 $P=0.599$). There was no statistical difference in average 24 hour energy intake during dietary
22 intervention (LF: 84.69±1.60, HF: 94.38±2.57, HF+GOS: 90.56±2.19, HF+RS: 92.27±2.40,

1 HF+DHA: 90.88 ± 2.78 kcal/day, $P=0.056$). In order to minimize the impact of circadian
2 variation on binding density, the rats were sacrificed between 07:00 and 09:00 hrs by rapid
3 CO₂ asphyxiation, and the brains were immediately removed and frozen in liquid nitrogen.
4 The study was approved by the University of Wollongong Animal Ethics Committee and all
5 animal experiments were conducted in compliance with the National Health and Medical
6 Research Council Australian, Code of Practice for the Care and Use of Animals for Scientific
7 Purposes (2004).

8

9 2.2 Histology and [³H]-pyrilamine binding autoradiography

10 Coronal brain sections (14 μm) were cut at -18°C with a cryotome (Clinicut Cryostat; Bright
11 Instruments), and thaw-mounted onto poly-L-lysine coated microscope slides (Polysine™,
12 Menzel GmbH & Co, KG) (Wang and Huang, 2008). [³H]-pyrilamine autoradiography was
13 performed following procedures as described in previous work from our laboratories and
14 others (Han et al., 2008; Hu et al., 2010). In brief, sections were incubated at room
15 temperature for 15 min, then incubated for 60 min in 50 nM sodium potassium phosphate
16 buffer containing 10 nM [³H]-pyrilamine (specific activity, 25.8 Ci/mmol; Perkin Elmer,
17 Boston, MA) at room temperature. Non-specific binding was determined by addition of 10
18 μM triprolidine to the incubation buffer. Sections were washed in 4 °C buffer (4 × 2 min),
19 dipped in distilled water and dried.

20

21 2.3 Quantification and statistical analysis

22 Images were obtained using high resolution Beta Imager detection (BioSpace, Paris, France)

1 according to the method that we have used previously (Deng and Huang, 2006; Hu et al.,
2 2010; Huang et al., 2008). In brief, sections were placed inside the detection chamber of the
3 Beta Imager and scanned for 3.5 hrs at a high-resolution setting. The levels of bound
4 radioactivity in the brain sections were directly determined by counting the number of
5 β -particles emerging from the tissue sections, which was followed by analysis of activity in
6 the regions of interest using the Beta Vision Plus Program (BioSpace). Radioligand binding
7 signals were expressed in counts per minute per square millimeter (cpm/mm²). For
8 quantification, linearization was done based on standards developed from sections cut from
9 brain pastes containing a series of known amounts of radioligands. This allows the
10 measurement of radioligand binding signals to be converted into nCi [³H] ligand per mg
11 tissue equivalent. The [³H]-pyrilamine binding density in various brain regions was
12 quantified by measuring the average density of each region in three to five adjacent brain
13 sections including both brain hemispheres (Deng and Huang, 2006; Hu et al., 2010; Huang et
14 al., 2008). The specific binding values were obtained by subtracting non-specific binding
15 values from the total binding values. Brain regions were identified with reference to a
16 standard rat brain atlas (Paxinos G, 1997) (Fig 2). The structures of both hemispheres were
17 quantified (n=5 per group). Data were analyzed statistically using the SPSS 17.0 program
18 (SPSS, Chicago, IL, USA). Histamine H1R binding density for each brain area was analyzed
19 by one-way ANOVA followed by a post-hoc Tukey–Kramer–HSD test. Data were expressed
20 as mean±SEM. *P* values less than 0.05 were regarded as statistically significant.

21

22 3. Results

1 Specific binding of the H1R ligand [³H]-pyrilamine was observed in most brain regions
2 examined (Table 1, Fig 1). Among the various brain regions, the highest [³H]-pyrilamine
3 binding densities were observed in the arcuate hypothalamic nucleus (Arc), caudate putamen
4 (CPu), hippocampus (Hip), medial posterodorsal amygdala (MeP), and ventromedial
5 hypothalamic nucleus (VMH). Lower [³H]-pyrilamine binding densities were observed in the
6 substantia nigra (SN), piriform cortex (Pir), primary motor cortex (M1), ventral tegmental
7 area (VTA), and anterior cingulate cortex (ACC). Non-specific binding of [³H]-pyrilamine
8 was less than 5%.

9

10 3.1 Effects of HF diet and GOS, RS, and DHA supplements on H1R binding density in 11 the substantia nigra (SN)

12 There were significant differences in H1R binding densities in the SN among the five diet
13 treatment groups (LF, HF, DHA, GOS and RS) after 4 weeks ($F_{4,20}=14.11$, $P<0.001$) (Table
14 1, Fig 1A, Fig 3). Rats on HF diet had significantly lower H1R binding density in the SN than
15 rats on the LF diet (48%, $P<0.001$). Addition of GOS, RS and DHA to the HF diet
16 significantly increased SN H1R binding densities compared to animals on HF diet alone
17 (GOS: 51%, $P=0.028$; RS: 50%, $P=0.036$; DHA: 105%, $P<0.001$). Furthermore, the DHA
18 group was more potent in increasing H1R density than either the GOS or RS groups (DHA vs.
19 GOS: 36%, $P=0.02$; DHA vs. RS: 37%, $P=0.015$).

20

21 3.2. Effects of HF diet and GOS, RS, and DHA supplements on H1R binding density in 22 the caudate putamen (CPu)

1 Significant differences were observed in H1R binding densities in the CPu among the five
2 treatment groups after 4 weeks of dietary intervention ($F_{4,20}= 8.78$, $P<0.001$) (Table 1, Fig
3 1B, Fig 4). HF diet significantly reduced H1R binding in the CPu (45%, $P<0.001$) in
4 comparison to the LF diet. DHA supplementation significantly increased H1R density
5 compared to HF alone (43%, $P=0.046$), while in the GOS and RS supplement groups binding
6 density was not significantly different from the HF group.

7

8 3.3. Effects of HF diet and GOS, RS, and DHA supplements on H1R binding density in
9 the piriform cortex (Pir)

10 We also found significant differences in H1R binding densities in the Pir among the five
11 groups of rats after 4 weeks of dietary intervention ($F_{4,20}=8.62$, $P<0.001$) (Table 1 and Fig
12 1C). Rats on HF diet had significantly lower H1R binding densities in the Pir than the rats on
13 LF diet (38%, $P<0.001$). In this brain area, GOS supplementation significantly increased
14 H1R binding density compared with the rats on HF diet (35%, $P=0.028$), while DHA and RS
15 supplementation did not increase H1R binding density in comparison with the HF group.

16

17 3.4. Effects of HF diet and GOS, RS, and DHA supplements on H1R binding density in
18 the M1, Arc and VTA

19 In the M1, Arc and VTA regions significant differences were found in H1R binding densities
20 between the five diet treatment groups after 4 weeks of dietary intervention (M1: $F_{4,20}=3.40$,
21 $P=0.028$; Arc: $F_{4,20}=4.51$, $P=0.009$; VTA: $F_{4,20}=3.83$, $P=0.018$) (Table 1 and Fig 1D, E and
22 F). H1R ligand binding in the HF group was significantly lower in the LF group within the

1 M1, Arc, and VTA (M1: 31%, $P=0.032$; Arc: 43%, $P=0.008$; VTA: 41%, $P=0.012$). However,
2 no significant differences were found among the HF, DHA, GOS and RS treatment groups in
3 these brain regions.

4 5 **4. Discussion**

6 Rats fed a high-fat diet had significantly decreased H1R binding densities in a number of
7 brain regions including the SN, CPu, Arc, VTA, Pir and M1, when compared with low-fat
8 diet fed rats, with reductions in binding density ranging from 31-48%. Interestingly, adding
9 DHA to the high-fat diet at least partially prevented a decrease of H1R binding densities in
10 the SN and CPu. Adding GOS in the diet also blunted a decrease of H1R binding in the SN
11 and Pir, while similarly adding RS in the high-fat diet helped maintain H1R binding levels in
12 the SN.

13
14 Using the [³H]-pyrilamine ligand studies have shown that H1R binding sites are extensively
15 distributed in the brain across various species including rodents, cat, guinea pig, primate, and
16 humans (Brabant et al., 2010). A similar distribution of H1R binding was observed in this
17 study as compared to previous studies in rats (Han et al., 2008; Palacios et al., 1981). The
18 present study demonstrated that H1R binding densities were highest in the VMH and MeP,
19 moderate in the SN, CPu, Arc, Pir, M1, ACC and Hip, and low in the VTA. These
20 observations are consistent with the distribution of H1Rs in the brain reported previously
21 (Palacios et al., 1981).

22

1 Previous studies have shown a negative relationship between brain histamine content and
2 fat-intake in rodents (Jorgensen et al., 2006). For example, histidine decarboxylase (a rate
3 limiting enzyme for histamine synthesis) knock-out mice have an increased fat intake and are
4 susceptible to diet-induced obesity compared with wild type mice. Conversely, an increase in
5 brain histamine content suppresses fat intake, but no effect on the intake of either
6 carbohydrate or protein (Lecklin and Tuomisto, 2002). It is known that brain histamine, via
7 H1R, regulates food intake and energy balance (Mercer et al., 1994; Haq et al., 1996). In our
8 study, high-fat diet decreased H1R binding density in most brain regions, especially in
9 nigrostriatal and limbic structures of the brain, suggesting that high-fat diet inhibits the
10 function of H1R. Administration of H1R blocker increases food intake while increasing H1R
11 expression decreases food intake in rats (Haq et al., 1996). Therefore, the decreased H1R
12 binding densities found in this study may further contribute to positive energy balance and
13 the development of obesity when rats are fed a high-fat diet.

14
15 Histaminergic neurons of the hypothalamic tuberomamillary nucleus project to the SN, CPu
16 and VTA (Haas and Panula, 2003) where H1R expression is abundant (Bouthenet et al.,
17 1988). The SN neurons also project to CPu, which is known to be involved in reward-based
18 learning, reinforcement and motor behavior, while the VTA is involved in reward (Rosell and
19 Amaya, 2000; Zangen et al., 2006). The H1R antagonist diphenhydramine enhances
20 membrane potential for additive effects (Wang and Woolverton, 2007), suggesting that a
21 decreased H1R function may contribute to reinforcement. Similarly, the high-fat diet induced
22 reduction of H1R density in the SN, CPu and VTA may also play a role in reinforcement.

1 Therefore, our findings concur with current reports suggesting that the consumption of
2 palatable high-fat diet may drive eating, and highly reinforcing feeding behaviour by
3 suppressing histamine H1R function and contributing to diet induced obesity (Johnson and
4 Kenny, 2010).

5

6 Histamine H1Rs regulate body weight, food intake, adiposity and hypothalamic H1R
7 signalling (Jorgensen et al., 2007; Yoshimatsu, 2008). This study showed that high-fat diet
8 decreased H1R binding density in the Arc region of the hypothalamus. Studies from our
9 laboratory (Han et al., 2008) have shown that olanzapine-induced obesity occurs largely via a
10 down regulation of hypothalamic H1R in the Arc and VMH. The regulation of food intake by
11 the adipose tissue derived hormone leptin is dependent on hypothalamic histamine signalling,
12 as it has been reported that H1R $-/-$ mice are insensitive to leptin induced down regulation of
13 food intake (Fulop et al., 2003). Futhermore, the Arc is more susceptible to the development
14 leptin resistantance, this event occuring after 4 weeks maintenance on a high fat diet in
15 rodents, which is earlier than the development of leptin resistance in other brain regions
16 (Metlakunta et al., 2008; Munzberg et al., 2004). Therefore, a down regulation of Arc H1R
17 binding by high-fat diet may contribute to high-fat diet induced leptin resistance in the Arc.

18

19 H1R binding density in the Pir was significantly lowered (-38%) in rats fed a high fat diet for
20 4 weeks. The Pir is part of the allocortex for primary olfactory function, receives rich afferent
21 fibers from the olfactory bulb, and is thought to serve an important role in olfactory learning
22 tasks (Schoenbaum and Eichenbaum, 1995; Wilson and Stevenson, 2003). Projections

1 between the Pir and orbitofrontal cortex are related to olfactory learning and memory
2 performance (Illig, 2005). Activation of the Pir can lead to recall of odors or odor-related
3 associations in olfactory information processing (Haberly, 2001; Illig, 2005). When rats are
4 fed a high-fat diet they show a reduction in their cognitive ability, and a decline in working
5 memory after nine days (Murray et al., 2009). Studies have also shown that
6 intracerebroventricular injection of histamine facilitates olfactory social memory tasks, while
7 the opposite effect was found by lowering central histamine (Argyriou et al., 1997).
8 Furthermore, activation of H1R has been shown to prevent muscarinic M1 receptor blocker
9 (scopolamine) induced memory deficits (Miyazaki et al., 1995). The finding by this study of
10 reduced H1R binding density in the Pir, suggests a possible deficit or reduced function in
11 olfactory memory tasks caused by high-fat diet, which needs further investigation.

12

13 The function of the primary motor cortex is to control voluntary movements (Sanes and
14 Donoghue, 2000). Mice lacking histamine or the H1R, display reduced exploratory behavior,
15 locomotor activity and time of rearing in a new environment (Inoue et al., 1996). Furthermore,
16 high-fat diet decreases spontaneous motor activity in streptozotocin-treated rats (Ramadan et
17 al., 2006). This study showed that H1R binding density was decreased in the M1 of rats on
18 high-fat diet, indicating that H1Rs in the M1 may be dysregulated by high-fat diet. Decreased
19 physical activity and sedentary lifestyle is one of the features of obese individuals. Therefore,
20 impaired function of histamine - H1R in the M1 may contribute to reduced physical activity
21 occurring during high-fat diet.

22

1 Addition of 0.5% DHA to 25% high-fat diet and fed to rats completely restored H1R binding
2 density in the SN, and partially to LF diet expression levels in the CPu. However this
3 phenomenon was not observed in other brain regions examined during this study, and the
4 precise mechanism of this selective effect is not known. Studies have shown that DHA
5 increases the cell membrane fluidity (Hashimoto et al., 1999; Kidd, 2007), which may affect
6 the interaction between the receptor and its ligand. For example, different types of dietary fats
7 change the receptor-ligand binding of dopaminergic, GABAergic, and cholinergic receptors
8 (Fong and Mcnamee, 1986; Lundbaek and Andersen, 1994; Malnoe et al., 1990; Witt and
9 Nielsen, 1994; Zimmer et al., 2002). Therefore, a potential mechanism of action for DHA is
10 that it decreases membrane viscosity, which in turn influences the H1R receptor/ligand
11 binding.

12
13 This study also demonstrated that both GOS and RS supplementation of high-fat diet restored
14 H1R binding density in the SN to the level of LF diet. It is reported that GOS and RS can be
15 fermented to short-chain fatty acids, such as acetate, propionate, and butyrate in the colon and
16 increase plasma levels of short-chain fatty acids (Smiricky-Tjardes et al., 2003; Topping and
17 Clifton, 2001). Studies have shown that short chain fatty acids can regulate histamine release
18 in vitro. For example, butyric acid markedly increases histamine release from RBL-2H3 cells
19 (Yamada et al., 1996). Short chain fatty acids act as ligands of an orphan G protein-coupled
20 receptor GPR41, stimulating leptin expression in both a mouse adipocyte cell line and mouse
21 adipose tissue in primary culture (Xiong et al., 2004). Oral administration of short-chain fatty
22 acids increase circulating leptin levels in mice (Xiong et al., 2004). Recently, it has been

1 reported that leptin can act in the SN to decrease food intake (Morton et al., 2009).
2 Furthermore, leptin-induced feeding suppression was attenuated in H1R knockout mice,
3 indicating involvement of H1R in feeding regulation downstream of leptin action (Masaki et
4 al., 2001). High-fat diet decreases SN H1R binding density which can be reversed by adding
5 GOS and RS to the diet, suggesting that this may be via an improvement of leptin sensitivity.

6

7 **5. Conclusion**

8 In conclusion, this study showed that a high-fat diet significantly decreases histamine H1R
9 binding density in a number of important brain areas regulating motor, olfactory sensory and
10 limbic functions. Adding dietary fibers, GOS or RS, to a high-fat diet can have significant
11 beneficial effects to the H1R system in terms of preventing the reduction of H1R binding
12 density induced by high-fat diet. More importantly, adding DHA in a high-fat diet can
13 completely prevent high-fat diet induced down-regulation of H1R binding density in the SN
14 as well as significantly restoring expression levels in the CPu. Therefore, a proper use of
15 molecular nutrition and design of functional food formulations may lead to significant
16 benefits via preventing histaminergic H1R changes induced by high-fat diet. Finally, although
17 this study showed that recommended doses of GOS, RS, and DHA are capable of preventing
18 altered H1R changes in the rat brain, this data should not be extrapolated into humans.
19 Further studies will be required to validate these findings in humans, such as using in vivo
20 imaging techniques.

21

22

23

1 REFERENCES

- 2 Argyriou A, Prast H, Philippu A (1997). Olfactory social memory in rats is facilitated by histamine.
3 Inflammation Research 46, 39-40.
- 4 Bouthenet ML, Ruat M, Sales N, Garbarg M, Schwartz JC (1988). A Detailed Mapping of Histamine
5 H1-Receptors in Guinea-Pig Central Nervous-System Established by Autoradiography with [I-125]
6 Iodobolpyramine. Neuroscience 26, 553-600.
- 7 Brabant C, Alleva L, Quertemont E, Tirelli E (2010). Involvement of the brain histaminergic system in addiction
8 and addiction-related behaviors: A comprehensive review with emphasis on the potential therapeutic
9 use of histaminergic compounds in drug dependence. Progress in Neurobiology 92, 421-441.
- 10 Brown RE, Stevens DR, Haas HL (2001). The physiology of brain histamine. Progress in Neurobiology 63,
11 637-672.
- 12 Davis JN, Alexander KE, Ventura EE, Toledo-Corral CM, Goran MI (2009). Inverse relation between dietary
13 fiber intake and visceral adiposity in overweight Latino youth. The American Journal of Clinical
14 Nutrition 90, 1160-1166.
- 15 Deng C, Huang XF (2006). Increased density of GABAA receptors in the superior temporal gyrus in
16 schizophrenia. Experimental Brain Research 168, 587-590.
- 17 du Bois TM, Bell W, Deng C, Huang X-F (2005). A high n-6 polyunsaturated fatty acid diet reduces muscarinic
18 M2/M4 receptor binding in the rat brain. Journal of Chemical Neuroanatomy 29, 282-288.
- 19 du Bois TM, Deng C, Bell W, Huang XF (2006). Fatty acids differentially affect serotonin receptor and
20 transporter binding in the rat brain. Neuroscience 139, 1397-1403.
- 21 Fong TM, Mcnamee MG (1986). Correlation between Acetylcholine-Receptor Function and
22 Structural-Properties of Membranes. Biochemistry 25, 830-840.
- 23 Haas H, Panula P (2003). The role of histamine and the tuberomamillary nucleus in the nervous system. Nature
24 Reviews Neuroscience 4, 121-130.
- 25 Haas HL, Sergeeva OA, Selbach O (2008). Histamine in the nervous system. Physiol Rev 88, 1183-1241.
- 26 Haberly LB (2001). Parallel-distributed Processing in Olfactory Cortex: New Insights from Morphological and
27 Physiological Analysis of Neuronal Circuitry. Chemical Senses 26, 551-576.
- 28 Han M, Deng C, Burne THJ, Newell KA, Huang X-F (2008). Short- and long-term effects of antipsychotic drug
29 treatment on weight gain and H1 receptor expression. Psychoneuroendocrinology 33, 569-580.
- 30 Haq AU, Bundrant HM, Mercer LP (1996). Food intake is inversely correlated with central nervous system
31 histamine receptor (H-1) concentrations in male Sprague-Dawley rats fed normal, low protein, low
32 energy or poor quality protein diets. Journal of Nutrition 126, 3083-3089.
- 33 Hashimoto M, Hossain S, Yamasaki H, Yazawa K, Masumura S (1999). Effects of eicosapentaenoic acid and
34 docosahexaenoic acid on plasma membrane fluidity of aortic endothelial cells. Lipids 34, 1297-1304.
- 35 Higgins J, Donahoo T, Higbee D, Hill J, Bessesen D (2001). Consumption of resistant starch promotes
36 postprandial lipid oxidation. Obesity Research 9, 182s-182s.
- 37 Higgins J, Donahoo W, Higbee D, Bing K, Brown I, Bessesen D (2005). Chronic consumption of resistant
38 starch promotes postprandial lipid oxidation. Obesity Research 13, A4-A4.
- 39 Higuchi M, Yanai K, Okamura N, Meguro K, Arai H, Itoh M, Iwata R, Ido T, Watanabe T, Sasaki H (2000).
40 Histamine H1 receptors in patients with Alzheimer's disease assessed by positron emission tomography.
41 Neuroscience 99, 721-729.
- 42 Hu C-H, Deng C, Mackovski N, Long L, Zhu C, Yang Y, Wang Y, Chen J, Huang X-F, Wang Q (2010). Effects
43 of simvastatin and 6-hydroxydopamine on histaminergic H1 receptor binding density in rat brains.
44 Progress in Neuro-Psychopharmacology and Biological Psychiatry 34, 1419-1425.

- 1 Huang XF, Yu Y, Li Y, Tim S, Deng C, Wang Q (2008). Ventromedial hypothalamic NPY Y2 receptor in the
2 maintenance of body weight in diet-induced obesity in mice. *Neurochem Res* 33, 1881-1888.
- 3 Huang XF, Zavitsanou K, Huang X, Yu Y, Wang H, Chen F, Lawrence AJ, Deng C (2006). Dopamine
4 transporter and D2 receptor binding densities in mice prone or resistant to chronic high fat diet-induced
5 obesity. *Behav Brain Res* 175, 415-419.
- 6 Illig KR (2005). Projections from orbitofrontal cortex to anterior piriform cortex in the rat suggest a role in
7 olfactory information processing. *The Journal of Comparative Neurology* 488, 224-231.
- 8 Inoue I, Yanai K, Kitamura D, Taniuchi I, Kobayashi T, Niimura K, Watanabe T, Watanabe T (1996). Impaired
9 locomotor activity and exploratory behavior in mice lacking histamine H-1 receptors. *Proceedings of*
10 *the National Academy of Sciences of the United States of America* 93, 13316-13320.
- 11 Johnson PM, Kenny PJ (2010). Dopamine D2 receptors in addiction-like reward dysfunction and compulsive
12 eating in obese rats. *Nat Neurosci* 13, 635-641.
- 13 Jorgensen EA, Vogelsang TW, Knigge U, Watanabe T, Warberg J, Kjaer A (2006). Increased susceptibility to
14 diet-induced obesity in histamine-deficient mice. *Neuroendocrinology* 83, 289-294.
- 15 Kidd PM (2007). Omega-3 DHA and EPA for Cognition, Behavior, and Mood: Clinical Findings and
16 Structural-Functional Synergies with Cell Membrane Phospholipids. *Alternative Medicine Review* 12,
17 207-228.
- 18 Lecklin A, Tuomisto L (2002). Inhibition of histamine catabolism suppresses fat intake but not the intake of
19 carbohydrates and protein. *Inflammation Research* 51, S53-S54.
- 20 Levant B, Ozias MK, Davis PF, Winter M, Russell KL, Carlson SE, Reed GA, McCarson KE (2008). Decreased
21 brain docosahexaenoic acid content produces neurobiological effects associated with depression:
22 Interactions with reproductive status in female rats. *Psychoneuroendocrinology* 33, 1279-1292.
- 23 Lozeva V, Valjakka A, Lecklin A, Olkkonen H, Hippelainen M, Itkonen M, Plumed C, Tuomisto L (2000).
24 Effects of the histamine H(1) receptor blocker, pyrilamine, on spontaneous locomotor activity of rats
25 with long-term portacaval anastomosis. *Hepatology* 31, 336-344.
- 26 Lundbaek JA, Andersen OS (1994). Lysophospholipids Modulate Channel Function by Altering the
27 Mechanical-Properties of Lipid Bilayers. *Journal of General Physiology* 104, 645-673.
- 28 Malnoe A, Milon H, Reme C (1990). Effect of in vivo modulation of membrane docosahexaenoic acid levels on
29 the dopamine-dependent adenylate cyclase activity in the rat retina. *J Neurochem* 55, 1480-1485.
- 30 Martinez-Mir MI, Pollard H, Moreau J, Arrang JM, Ruat M, Traiffort E, Schwartz JC, Palacios JM (1990).
31 Three histamine receptors (H1, H2 and H3) visualized in the brain of human and non-human primates.
32 *Brain Research* 526, 322-327.
- 33 Masaki T, Yoshimatsu H (2006). The hypothalamic H1 receptor: a novel therapeutic target for disrupting diurnal
34 feeding rhythm and obesity. *Trends in Pharmacological Sciences* 27, 279-284.
- 35 Masaki T, Yoshimatsu H, Chiba S, Watanabe T, Sakata T (2001). Targeted Disruption of Histamine H1-Receptor
36 Attenuates Regulatory Effects of Leptin on Feeding, Adiposity, and UCP Family in Mice. *Diabetes* 50,
37 385-391.
- 38 Mercer LP, Kelley DS, Humphries LL, Dunn JD (1994). Manipulation of Central-Nervous-System Histamine or
39 Histaminergic Receptors (H-1) Affects Food-Intake in Rats. *Journal of Nutrition* 124, 1029-1036.
- 40 Metlakunta AS, Sahu M, Sahu A (2008). Hypothalamic Phosphatidylinositol 3-Kinase Pathway of Leptin
41 Signaling Is Impaired during the Development of Diet-Induced Obesity in FVB/N Mice.
42 *Endocrinology* 149, 1121-1128.
- 43 Miyazaki S, Imaizumi M, Onodera K (1995). Effects of thioperamide, a histamine H3-receptor antagonist, on a
44 scopolamine-induced learning deficit using an elevated plus-maze test in mice. *Life Sci* 57, 2137-2144.

1 Morton GJ, Blevins JE, Kim F, Matsen M, Figlewicz DP (2009). The action of leptin in the ventral tegmental
2 area to decrease food intake is dependent on Jak-2 signaling. *American Journal of Physiology -*
3 *Endocrinology And Metabolism* 297, E202-E210.

4 Munzberg H, Flier JS, Bjorbaek C (2004). Region-Specific Leptin Resistance within the Hypothalamus of
5 Diet-Induced Obese Mice. *Endocrinology* 145, 4880-4889.

6 Murray AJ, Knight NS, Cochlin LE, McAleese S, Deacon RM, Rawlins JN, Clarke K(2009). Deterioration of
7 physical performance and cognitive function in rats with short-term high-fat feeding. *The FASEB*
8 *Journal*, 23(12): p. 4353-4360.

9 Palacios JM, Wamsley JK, Kuhar MJ (1981). The distribution of histamine H1-receptors in the rat brain: an
10 autoradiographic study. *Neuroscience* 6, 15-37.

11 Park HR, Park M, Choi J, Park K-Y, Chung HY, Lee J (2010). A high-fat diet impairs neurogenesis: Involvement
12 of lipid peroxidation and brain-derived neurotrophic factor. *Neuroscience Letters* 482, 235-239.

13 Passani MB, Lin JS, Hancock A, Crochet S, Blandina P (2004). The histamine H3 receptor as a novel
14 therapeutic target for cognitive and sleep disorders. *Trends Pharmacol Sci* 25, 618-625.

15 Paxinos G WC (1997) *The rat brain in stereotaxic coordinates.*: San Diego: Academic Press.

16 Ramadan W, Dewasmes G, Petitjean M, Loos N, Delanaud S, Geloën A, Libert J-P (2006). Spontaneous motor
17 activity in fat-fed, streptozotocin-treated rats: A nonobese model of type 2 diabetes. *Physiology &*
18 *Behavior* 87, 765-772.

19 Rosell A, Amaya JMG (2000). Considerations upon the anatomical model of reward-based learning in the basal
20 ganglia. *Medical Hypotheses* 54, 397-399.

21 Sanes JN, Donoghue JP (2000). Plasticity and primary motor cortex. *Annu Rev Neurosci* 23, 393-415.

22 Schoenbaum G, Eichenbaum H (1995). Information coding in the rodent prefrontal cortex. I. Single-neuron
23 activity in orbitofrontal cortex compared with that in pyriform cortex. *Journal of Neurophysiology* 74,
24 733-750.

25 Shen L, Keenan MJ, Martin RJ, Tulley RT, Raggio AM, McCutcheon KL, Zhou J (2009). Dietary resistant
26 starch increases hypothalamic POMC expression in rats. *Obesity (Silver Spring)* 17, 40-45.

27 Smiricky-Tjardes MR, Grieshop CM, Flickinger EA, Bauer LL, Fahey GC, Jr. (2003). Dietary
28 galactooligosaccharides affect ileal and total-tract nutrient digestibility, ileal and fecal bacterial
29 concentrations, and ileal fermentative characteristics of growing pigs. *J Anim Sci* 81, 2535-2545.

30 Topping DL, Clifton PM (2001). Short-Chain Fatty Acids and Human Colonic Function: Roles of Resistant
31 Starch and Nonstarch Polysaccharides. *Physiological Reviews* 81, 1031-1064.

32 Wang Q, Huang XF (2008). Effects of chronic treatment of olanzapine and haloperidol on peptide YY binding
33 densities in the rat brain. *Experimental Neurology* 209, 261-267.

34 Wang Z, Woolverton WL (2007). Self-administration of cocaine-antihistamine combinations: Super-additive
35 reinforcing effects. *European Journal of Pharmacology* 557, 159-160.

36 Wilson DA, Stevenson RJ (2003). The fundamental role of memory in olfactory perception. *Trends in*
37 *Neurosciences* 26, 243-247.

38 Winocur G, Greenwood CE (2005). Studies of the effects of high fat diets on cognitive function in a rat model.
39 *Neurobiology of Aging* 26, 46-49.

40 Witt MR, Nielsen M (1994). Characterization of the Influence of Unsaturated Free Fatty-Acids on Brain
41 Gaba/Benzodiazepine Receptor-Binding in-Vitro. *Journal of Neurochemistry* 62, 1432-1439.

42 Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedzierski RM, Yanagisawa M (2004). Short-chain
43 fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41.
44 *Proceedings of the National Academy of Sciences of the United States of America* 101, 1045-1050.

1 Yamada K, Mori M, Matsuo N, Shoji K, Ueyama T, Sugano M (1996). Effects of fatty acids on accumulation
2 and secretion of histamine in RBL-2H3 cells and leukotriene release from peritoneal exudate cells
3 isolated from Wistar rats. *J Nutr Sci Vitaminol (Tokyo)* 42, 301-311.

4 Yanai K, Watanabe T, Meguro K, Yokoyama H, Sato I, Sasano H, Itoh M, Iwata R, Takahashi T, Ido T (1992).
5 Age-dependent decrease in histamine H1 receptor in human brains revealed by PET. *Neuroreport* 3,
6 433-436.

7 Zangen A, Solinas M, Ikemoto S, Goldberg SR, Wise RA (2006). Two Brain Sites for Cannabinoid Reward. *The*
8 *Journal of Neuroscience* 26, 4901-4907.

9 Zimmer L, Vancassel S, Cantagrel S, Breton P, Delamanche S, Guilloteau D, Durand G, Chalon S (2002). The
10 dopamine mesocorticolimbic pathway is affected by deficiency in n-3 polyunsaturated fatty acids. *Am J*
11 *Clin Nutr* 75, 662-667.

12

FIGURE LEGENDS

Fig 1. The effects of dietary intervention on [³H]-pyrilamine binding (nCi/mg tissue) in rat brain. Data are expressed as mean ± SEM. Abbreviations: SN, substantia nigra; CPu, Caudate putamen; Arc, hypothalamic arcuate nucleus; Pir, Piriform cortex; VTA, Ventral tegmental area; M1, Primary motor cortex. HF, high-fat diet; LF, low-fat diet; GOS, galacto-oligosaccharide; DHA, n-3 polyunsaturated docosahexaenoic acid; RS, resistant starch. ^a*P* < 0.05 vs. HF, ^b*P* < 0.05 vs. GOS and RS.

Fig 2. Representative autoradiographs of coronal brain sections illustrating total [³H]-pyrilamine binding density (A', B', and C'), and nonspecific [³H]-pyrilamine binding density (A'', B'', and C''). The maps of A, B, C were adopted from a rat brain atlas (Paxinos G, 1997) indicating the brain levels where the [³H]-pyrilamine binding density was measured. Abbreviations: CPu, caudate putamen; M1, primary motor cortex; ACC, anterior cingulate cortex; Hip, hippocampus; VMH, ventromedial hypothalamic nucleus; Arc, hypothalamic arcuate nucleus; MeP, medial posterodorsal amygdala; Pir, piriform cortex; VTA, ventral tegmental area; SN, substantia nigra; HF, high-fat diet; LF, low-fat diet; GOS, galacto-oligosaccharide; DHA, n-3 polyunsaturated docosahexaenoic acid; RS, resistant starch.

Fig 3. Autoradiograph showing [³H]-pyrilamine binding in the brain of rats fed LF diet (A), HF diet (B), HF + GOS diet (C), HF + RS diet (D), and HF + DHA diet (E). Abbreviations: HF, high-fat diet; LF, low-fat diet; GOS, galacto-oligosaccharide; DHA, n-3 polyunsaturated docosahexaenoic acid; RS, resistant starch.

Fig 4. Autoradiograph depicts the expression of [³H]-pyrilamine binding as A, LF diet; B, HF diet; C, HF+DHA diet. The density of [³H]-pyrilamine binding was significantly decreased in the CPu induced by HF diet whereas the reduction was prevented by supplementations of DHA (C). HF, high-fat diet; LF, low-fat diet; DHA, n-3 polyunsaturated docosahexaenoic acid.

Table 1. Specific [³H]-pyrilamine 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 (n=5)	HF (n=5)	GOS (n=5)	RS (n=5)	DHA (n=5)	F (4,20)	P value	HF vs. LF	HF vs. GOS	HF vs. RS	HF vs. DHA
SN	2.08±0.08	1.08±0.06	1.63±0.13	1.61±0.12	2.21±0.17	14.110	<0.001	<0.001	0.028	0.036	<0.001
CPu	2.56±0.11	1.42±0.09	1.72±0.17	1.95±0.18	2.03±0.15	8.780	<0.001	<0.001	0.585	0.104	0.046
Arc	2.75±0.10	1.57±0.20	1.71±0.24	2.11±0.28	2.11±0.21	4.510	0.009	0.008	0.989	0.417	0.421
VTA	1.58±0.04	0.93±0.13	1.30±0.13	1.13±0.18	1.35±0.09	3.830	0.018	0.012	0.258	0.793	0.155
Pir	2.17±0.10	1.35±0.12	1.82±0.10	1.66±0.10	1.64±0.07	8.620	<0.001	<0.001	0.028	0.251	0.309
M1	2.25±0.22	1.55±0.19	1.94±0.07	2.20±0.12	2.13±0.12	3.400	0.028	0.032	0.410	0.052	0.097
VMH	3.21±0.14	2.93±0.29	2.59±0.19	3.26±0.32	2.94±0.33	1.010	0.425	—	—	—	—
MeP	3.21±0.13	2.50±0.22	2.75±0.24	3.01±0.31	2.72±0.18	1.870	0.155	—	—	—	—
ACC	2.08±0.25	1.53±0.11	1.78±0.04	2.01±0.20	1.74±0.11	1.940	0.143	—	—	—	—
Hip	2.69±0.17	2.28±0.12	2.30±0.12	2.48±0.14	2.26±0.09	1.540	0.230	—	—	—	—

Abbreviations: SN, Substantia nigra; CPu, Caudate putamen; Arc, hypothalamic arcuate nucleus; Hip, Hippocampus; ACC, anterior cingulate cortex; Pir, Piriform cortex; VTA, Ventral tegmental area; VMH, Ventromedial hypothalamus; Mep, Medial posterodorsal amygdala; M1, Primary motor cortex; HF, high-fat diet; LF, low-fat diet; GOS, galacto-oligosaccharide; DHA, n-3 polyunsaturated docosahexaenoic acid; RS, resistant starch.

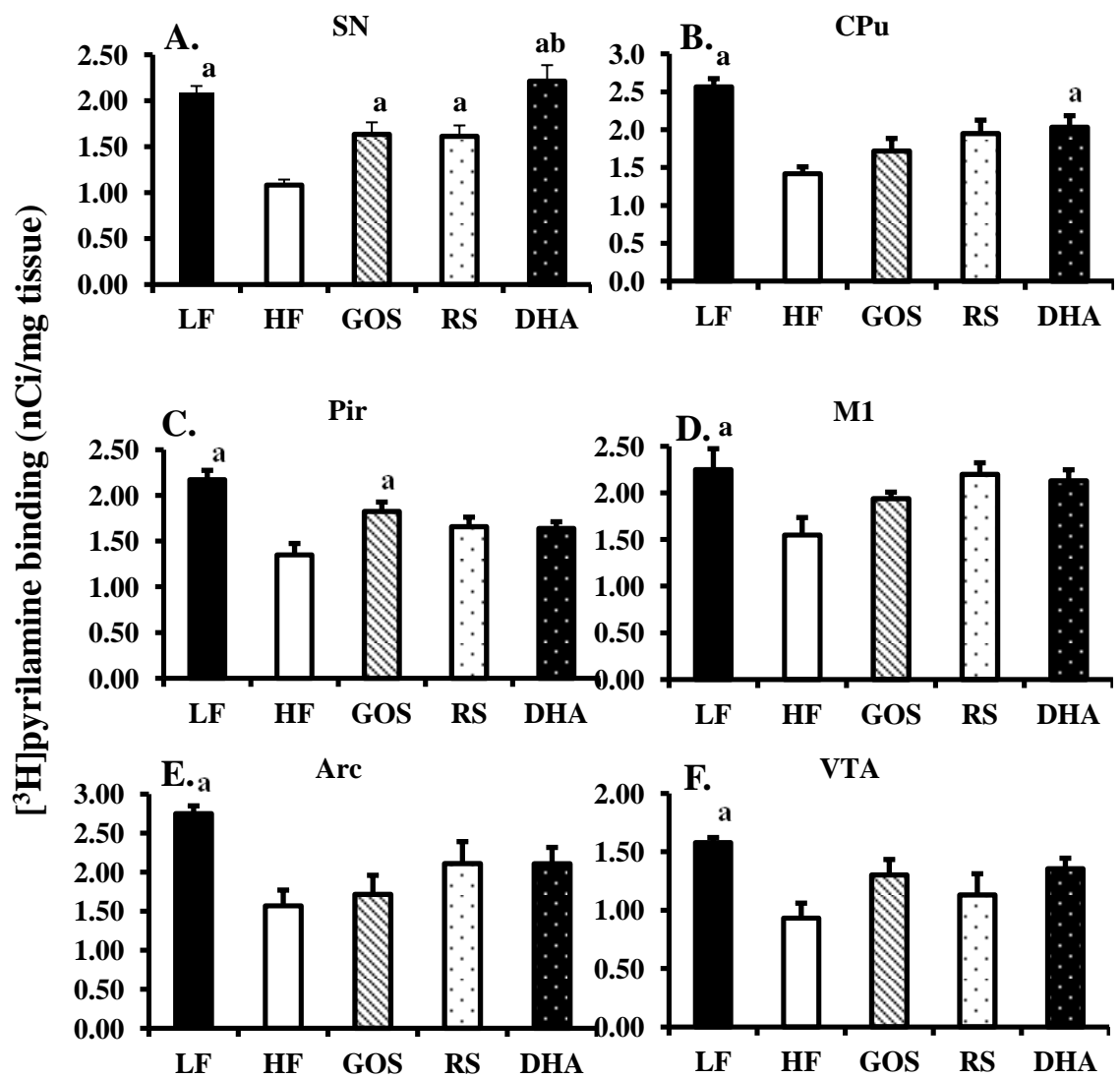


Fig 1.

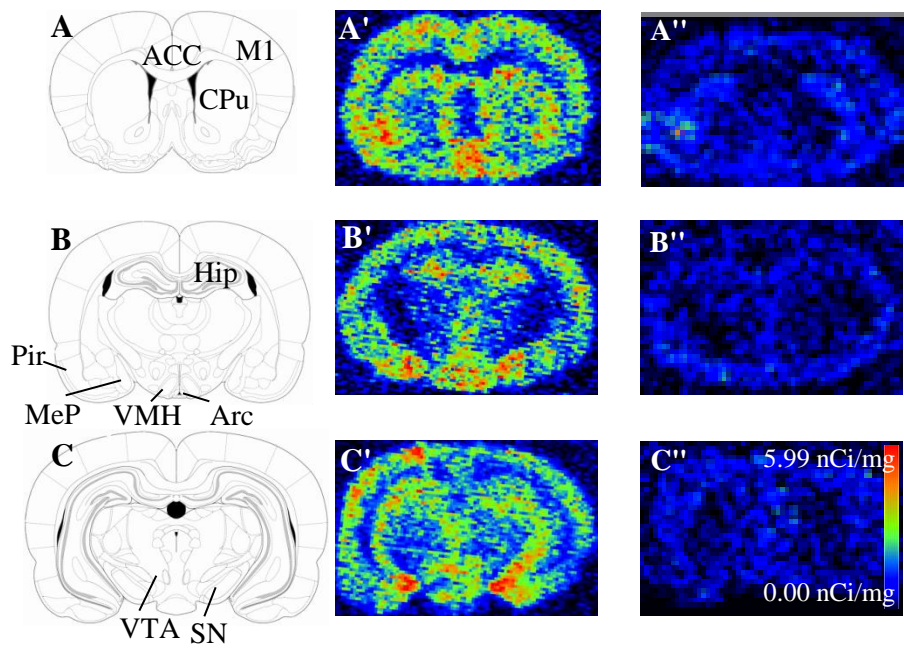


Fig 2.

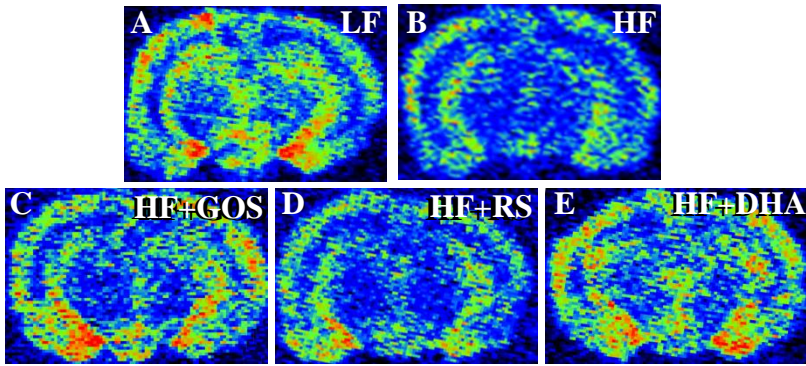


Fig 3.

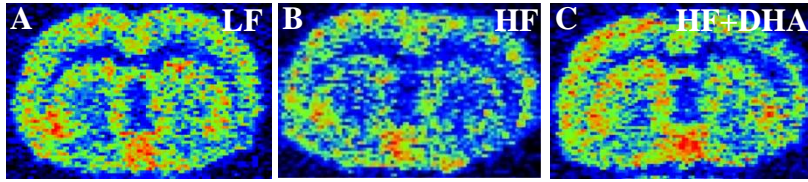


Fig 4.