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Dietary Galacto-Oligosaccharides and Resistant Starch Protect Against Altered CB1 and 5-HT1A and 2A Receptor Densities in Rat Brain: Implications for Preventing Cognitive and Appetite Dysfunction During a High-Fat Diet

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Abstract

Scope: A high-fat, but low-fiber, diet is associated with obesity and cognitive dysfunction, while dietary fiber supplementation can improve cognition. Methods and results: This study examines whether dietary fibers, galacto-oligosaccharides (GOS) and resistant starch (RS), could prevent high-fat (HF)-diet-induced alterations in neurotransmitter receptor densities in brain regions associated with cognition and appetite. Rats are fed a HF diet, HF diet with GOS, HF diet with RS, or a low-fat (LF, control) diet for 4 weeks. Cannabinoid CB1 (CB1R) and $5HT_{1A}$($5HT_{1A}R$) and 5-HT_{2A}($5HT_{2A}R$) receptor binding densities are examined. In the hippocampus and hypothalamus, a HF diet significantly increases CB1R binding, while HF + GOS and HF + RS diets prevented this increase. HF diet also increases hippocampal and hypothalamic 5-$HT_{1A}R$ binding, while HF + GOS and HF + RS prevented the alterations. Increased 5-HT_{2A} binding is prevented by HF + GOS and HF + RS in the medial mammillary nucleus.

Conclusions: These results demonstrate that increased CB1R, 5-$HT_{1A}R$ and 5-$HT_{2A}R$ induced by a HF diet can be prevented by GOS and RS supplementation in brain regions involved in cognition and appetite. Therefore, increased fiber intake may have beneficial effects on improving learning and memory, as well as reducing excessive appetite, during the chronic consumption of a HF (standard Western) diet.

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Dietary galacto-oligosaccharides and resistant starch protect against altered CB1 and 5-HT1A and 2A receptor densities in rat brain: Implications for preventing cognitive and appetite dysfunction during a high-fat diet

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Abstract

Scope: A high fat, but low fiber, diet is associated with obesity and cognitive dysfunction, while dietary fiber supplementation can improve cognition.

Methods and results: This study examined whether dietary fibers, galacto-oligosaccharides (GOS) and resistant starch (RS), could prevent high-fat (HF) diet-induced alterations in neurotransmitter receptor densities in brain regions associated with cognition and appetite. Rats were fed a HF diet, HF diet with GOS, HF diet with RS, or a low-fat (LF, control) diet for 4 weeks. Cannabinoid CB1 (CB1R) and $5HT_{1A}$ ($5HT_{1A}R$) and 5-HT_{2A} ($5HT_{2A}R$) receptor binding densities were examined. In the hippocampus and hypothalamus, HF diet significantly increased CB1R binding, while HF+GOS and HF+RS diets prevented this increase. HF diet also increased hippocampal and hypothalamic 5-$HT_{1A}R$ binding, while HF+GOS and HF+RS prevented the alterations. Increased 5-HT_{2A} binding was prevented by HF+GOS and HF+RS in the medial mammillary nucleus.

Conclusions: These results demonstrate that increased CB1R, 5-$HT_{1A}R$ and 5-$HT_{2A}R$ induced by HF diet can be prevented by GOS and RS supplementation in brain regions involved in cognition and appetite. Therefore, increased fiber intake may have beneficial effects on improving learning and memory, as well as reducing excessive appetite, during the chronic consumption of a HF (standard Western) diet.

Keywords: galacto-oligosaccharides, resistant starch, high-fat diet, CB1 receptor, serotonin receptor

1. Introduction

A high fat, but low fiber, diet is associated with obesity and a decline in cognitive performance,[1-5] with one study demonstrating impaired cognitive ability and a decline in working memory in rats after consuming high-fat (HF) diet for only 9-days.[6] Both human and rodent studies have shown that a HF diet promotes fat deposition, impairs memory and learning, and even contributes to the development of depression.[1, 7, 8] On the other hand, dietary fiber intake is positively correlated with improved cognition in pre-pubertal children[2] and elderly people,[2, 3] while oral administration of dietary fiber derived from wheat effectively reversed scopolamine-induced learning and memory impairment in rats.[9] The fact that diets high in saturated fats or dietary fiber can alter brain function suggests that they may alter neurotransmitter systems. Indeed, some evidence suggests that saturated fat and dietary fiber can differentially regulate key neurotransmitter systems that are involved in regulating learning, memory and energy metabolism in animal models.[10-12] For example, serotonin (5-HT) was decreased by dietary saturated fat in the brainstem of rats, while plasma 5-HT levels were increased in horses fed a high-fiber diet.[10-12] We have previously shown that a HF diet alters serotonergic and cannabinoid receptor densities in the rat brain.[13] However, it is unclear if increased dietary fiber intake affects key neurotransmitter systems in the brain regions regulating cognitive function and appetite, such as the hippocampus, cortex and hypothalamus, in rats on HF diet.

The endogenous cannabinoid system is implicated in normal human functioning, including learning and memory, emotion, addiction, appetite and feeding behavior.[14] The CB1 receptor (CB1R) is highly expressed in brain regions implicated in learning and memory, particularly the hippocampus, anterior cingulate cortex and midbrain (ventral tegmental area (VTA) and substantia nigra (SN)), as well as the hypothalamus where it regulates appetite.[15-18] A study

by Wise et al[19] reported that hippocampal CB1R activation by delta 9-tetrahydrocannabinol (Δ9-THC) impaired memory in rats, while intrahippocampal administration of a CB1R antagonist (rimonabant) restored cognitive deficits. In addition, central CB1R knockdown transgenic mice exhibit reduced body weight, appetite and adiposity compared to wild-type littermates.[18] Interestingly, we found that a chronic HF diet increased $5HT_{1A}R$ and CB1R binding density in the hippocampus and hypothalamus of rats.[13] Another report showed that CB1R immunoreactivity and levels of the endogenous cannabinoids, anandamide and 2-arachidonoyl glycerol (2-AG), were increased in the hippocampus of mice fed a chronic HF diet.[20] Therefore, cannabinoid signaling in the brain can be altered by diet, and may contribute to body weight and cognitive function; however, whether dietary fiber can restore normal cannabinoid signalling in regions of the brain implicated in learning, memory and appetite during a HF diet is unknown.

The serotonergic (5-HT) system has long been implicated in the process of learning and memory formation, as well as energy balance. $5\text{-}HT_{1A}$ and $5\text{-}HT_{2A}$ receptors ($5\text{-}HT_{1A}R$ and $5\text{-}HT_{2A}R$) play important roles in the control of energy intake, obesity, memory and learning.[21-23] The $5\text{-}HT_{1A}R$ is widely distributed throughout the brain, with a high density in the cortical and limbic areas, especially the hippocampus and cortex, but low expression in other brain regions, such as the hypothalamus, striatum and amygdala.[24] Clinical studies have shown that $5\text{-}HT_{1A}R$ expression is negatively associated with memory function,[25] while $5\text{-}HT_{1A}R$ antagonists enhance cognition in humans and rodents.[26] Similarly, evidence suggests a role for the $5\text{-}HT_{2A}R$ sub-type in cognition as binding density is significantly increased in the temporal cortex of patients with dementia.[27] The $5\text{-}HT_{2A}R$ sub-type is highly expressed in the cortex, striatum and medial mammillary nucleus (MM) of rats and humans, where it plays an important role in learning by modulating cortical neuronal excitability.[28, 29] There is

limited evidence to suggest that dietary saturated fat and fiber can alter 5-HT differentially;[10-12] however, further studies are required to investigate whether dietary fiber can restore homeostasis to 5-HT_{1A}R and 5-HT_{2A}R signaling in regions of the brain implicated in cognition and body weight during a HF diet.

Galacto-oligosaccharides (GOS) and resistant starch (RS) are important dietary fibers consumed by humans that act as prebiotics, i.e. regulate the gut microbiota in a manner that benefits the host.[30] GOS are produced through the enzymatic conversion of lactose, a component of bovine milk, and are not digestible by humans; instead they are fermented by gut microbiota.[30, 31] RS is the fraction of dietary starch that escapes digestion in the small intestine and passes into the colon, where it is fermented by the microbiota producing short-chain fatty acids.[32] Evidence shows a number of health benefits from GOS and RS-rich diets, including reduced food intake, circulating triglyceride levels and blood pressure, and improved insulin sensitivity in overweight and obese people.[33] Furthermore, dietary fiber can improve cognition in humans[2, 3] and rats,[31] and has the ability to modulate cannabinoid and serotoninergic tone.[11, 12, 34] This study examined whether dietary fibers, GOS and RS, could prevent imbalances in CB1R, 5-HT_{1A}R and 5-HT_{2A}R densities in the rat brain during a chronic HF diet.

2. Experimental procedure

2.1 Ethics Statement

The study was approved by the University of Wollongong Animal Ethics Committee (AE 09/22) and all animal experiments were conducted in compliance with the National Health and Medical Research Council Australian, Code of Practice for the Care and Use of Animals for Scientific Purposes.[35]

2.2 Animals and dietary treatments

Forty male Wistar rats (300-320g) were obtained from the Animal Resources Centre (Perth, Western Australia, Australia) and housed in environmentally controlled conditions (22°C, 12 hr light–dark cycle with light cycle from 06:00 to 18:00 h and dark cycle from 18:00 to 06:00 h) with *ad libitum* access to standard laboratory chow and water. Rats were allowed 1 week to habituate to the new environment, then randomized into four dietary groups: (1) standard laboratory chow as the low-fat (LF) control (fat content of 10% in kcal and 1% saturated fat), (2) high-fat (HF) diet (fat content of 25% in kcal with 10% saturated fat), (3) HF diet + 5% GOS, (4) HF diet + 5% RS. The dose of dietary fiber supplementation used in this study was based on previous studies.[36, 37] Energy intake and body weight were measured twice per week. After four weeks of dietary intervention, rats were sacrificed by rapid CO_2 asphyxiation between 07:00 and 09:00 hrs in order to minimize the impact of circadian variation. Brain tissues were immediately removed and frozen in liquid nitrogen, then stored at -80 °C until further analysis. Plasma levels of the chemokine, monocyte chemoattractant protein (MCP-1), were examined due to its role in obesity and cognitive impairment,[38, 39] using a mouse metabolic magnetic bead panel kit (Merck Millipore, MA, USA).

2.3 Histological procedures

Five rats per group were used to examine [^{3}H]-CP-55,940, [^{3}H]-WAY-100635, and [^{3}H]-ketanserin binding densities in the brain. Coronal brain sections (14 μm) were sectioned using a cryostat (-18 °C) from the level of Bregma -0.24mm to -5.16mm,[40] then thaw-mounted onto poly-L-lysine coated microscope slides (Polysine™, Menzel GmbH & Co, KG) and stored at -20 °C.

2.4 [^{3}H]-CP-55,940, [^{3}H]-WAY-100635 and [^{3}H]-Ketanserin autoradiography

[^{3}H]-CP-55,940 was used to assess CB1R binding density, as previously described.[13] Briefly, sections were allowed to defrost and then pre-incubated for 30 min in Tris-HCl buffer (5% bovine serum albumin, BSA, 50 mM Tris-HCl, pH 7.4) at room temperature. The binding sites of CB1R were defined by incubation with 10 nM [^{3}H]-CP-55,940. Non-specific binding was determined in the presence of 10 μM CP-55,940. Following incubation for 2 hours at room temperature, slides were washed firstly for 1 hour and then 3 hours in ice-cold buffer (1% BSA, 50 mM Tris–HCl, pH 7.4), and then finally washed for a further 5 min in the buffer containing no BSA. Slides were then rinsed briefly in ice-cold distilled water and air dried.

[^{3}H]-WAY-100635 was used to assess 5-HT$_{1A}$R binding density, as previously described.[13] Brain sections were pre-incubated in 50 nM Tris–HCl buffer (pH 7.4, room temperature) for 30 min. Sections were then incubated with 5 nM [^{3}H]-WAY-100635 (Amersham Biosciences, UK Limited) in 50 mM Tris–HCl (pH 7.4) containing 10 μM pargyline (Sigma) at room temperature for 2.5 hours. Non-specific binding was determined by incubating consecutive sections exposed to 10 μM 5-HT. All sections were washed in ice-cold buffer for 2 min and then 3 min, rinsed in distilled water and dried.

[^{3}H]-Ketanserin was used to assess 5-HT$_{2A}$R binding density, as previously described.[13, 28] Briefly, sections were preincubated with [^{3}H]-Ketanserin (PerkinElmer Life Sciences, Boston, MA, USA) in 170 mM Tris-HCl buffer (pH 7.4) for 15 min at room temperature. Nonspecific binding was determined by the addition of 2 μM spiperone (Sigma) to consecutive sections. Sections were washed in ice-cold buffer (2 × 10 min), rinsed in distilled water and dried.

2.5 Quantification and statistical analysis

Quantification of binding sites was performed using a high-resolution Beta Imager (BioSpace, Paris, France) according to our previous studies.[41, 42] Briefly, sections were placed in a sample holder inside the detection chamber of the Beta Imager. The levels of bound radioactivity in the brain sections were directly determined by counting the number of β-particles emerging from the tissue sections. The Beta Vision Plus program (BioSpace, France) was used to measure the activities in the regions of interest. Radioligand binding signal was expressed in counts per minute per square millimetre (cpm/mm^2), and was converted to fmol/mg tissue equivalents with the use of standards. Specific binding was determined by subtracting non-specific binding from total binding density (example binding shown in Fig 1, 3 and 5, panels A''-C''and A'-C', respectively). The receptor density in various brain regions was quantified by measuring the average density of each region in three to five adjacent brain sections. Different brain regions were identified by reference to a standard rat brain atlas.[40] Data was expressed as mean ± SEM. [^{3}H]-CP-55,940, [^{3}H]-WAY-100635 and [^{3}H]-Ketanserin binding densities for each brain region were analyzed using a one-way ANOVA followed by a post-hoc Tukey–Kramer–HSD test using SPSS 19.0 (Chicago, IL, USA). *P* values of less than 0.05 were regarded as statistically significant, and *P* values of less than 0.10 as a statistical trend.

3. RESULTS

Effect of GOS and RS supplementation on CB1R binding density during a HF diet

[^{3}H]-CP55940 binding sites were detected in some cortical and mesolimbic brain regions (Fig 1 and Table 1). There was a significant effect of diet on CB1R binding density in the hippocampus ($F_{(3, 19)}$=4.763, *P*=0.015) (Fig 2A). The HF diet significantly increased CB1R

density compared with rats on the LF diet (P=0.003), while both GOS and RS supplementation in the HF diet prevented this increase, with significantly lowered CB1R binding density compared to the HF diet group (P=0.016 and P=0.011, respectively) and no significant difference between the fiber supplement and LF groups (Fig 2A).

In the mediobasal hypothalamus (MBH), there was also a significant effect of dietary intervention on CB1R density ($F_{(3, 19)}$=11.860, P<0.001) (Fig 2B). The CB1R density in rats on HF diet significantly increased compared to that of rats on LF diet (P<0.001), while the supplementation of GOS and RS in the HF diet prevented this imbalance, with a significant decrease in CB1R binding density compared to the HF diet group (both P <0.001) that did not differ to the LF controls (Fig 2B). Furthermore, dietary intervention significantly influenced CB1R binding density in the substantia nigra and ventral tegmental area ($F_{(3, 19)}$=5.644, P =0.008 and $F_{(3, 19)}$=4.981, P =0.013, respectively) (Fig 2C and D), where GOS and RS supplementation prevented the HF diet-induced increases in CB1R binding density in these two brain areas (all P<0.05). There was no effect of dietary intervention on CB1R binding density in the caudate putamen (CPu), piriform cortex (Pir), primary motor cortex (M1), amygdala (Amg) or anterior cingulate cortex (ACC) (Table 1).

Effect of GOS and RS supplementation on 5-HT$_{1A}$R binding density during a HF diet

[^{3}H]-WAY-100635 binding sites were detected in the hippocampus, MBH, Amg, lateral septal nucleus (LS), ACC and M1 (Fig 3 and Table 2). Diet significantly affected 5-HT$_{1A}$R binding density in the hippocampus ($F_{(3, 19)}$=6.888, P=0.003) (Fig 4A), with a significant increase in 5-HT$_{1A}$R binding density in the HF group compared to the LF diet group (P<0.001). Dietary intervention by the addition of GOS to the HF diet significantly decreased receptor density compared to the rats on HF diet (P=0.042), but was still significantly greater than the LF

group (P=0.037). RS supplementation in the HF diet did not significantly decrease 5-HT_{1A}R binding density compared to the HF diet (P>0.050).

A dietary effect was also observed on 5-HT_{1A}R density within the MBH ($F_{(3, 19)}$=6.001, P =0.006) (Fig 4B), with a significantly higher density in the HF group compared to the LF diet group (P=0.001). For the RS-supplemented group, the 5-HT_{1A}R density was significantly lower than that of rats on HF diet (P=0.005), but GOS supplementation did not significantly alter 5-HT_{1A} receptor binding in the MBH compared to the HF diet (P>0.050). On the other hand, 5-HT_{1A}R binding densities in the Amg, LS, ACC and M1 were not influenced by diet (Table 2).

Effect of GOS and RS supplementation on 5-HT_{2A} binding density during a HF diet

[^{3}H] Ketanserin binding sites were detected in the MM, ACC, CPu, M1 and Pir (Fig 5 and Table 3). There was a significant effect of diet on 5-HT_{2A}R density in the MM ($F_{(3, 19)}$=3.803, P=0.033) (Fig 6A). In the HF diet group, 5-HT_{2A}R binding density was significantly higher than the LF (P=0.012), GOS-supplemented HF (P=0.012) and RS-supplemented HF (P=0.029) diet groups. Dietary fiber supplementation during a HF diet prevented increased 5-HT_{2A}R binding as these groups did not differ to the LF controls (both P>0.050).

In the ACC, there was a significant diet effect on 5-HT_{2A}R binding density ($F_{(3, 19)}$=6.571, P=0.004) (Fig 6B), with an increase in the HF group compared to the LF diet group (P=0.001) and a significant decrease in the GOS-supplemented HF diet group compared to the HF diet group (P=0.006) that did not differ to the controls (P=0.438 *vs* LF). RS supplementation did not significantly alter 5-HT_{2A}R binding density compared to the HF group (P=0.273). A similar trend was observed in the CPu, with an overall significant effect

of diet on 5-HT_{2A}R binding density ($F_{(3, 19)}$=9.476, P =0.001) and increased binding in the HF group compared to the LF diet group (P =0.001) (Fig 6C). However, GOS and RS supplementation in the HF diet group did not significantly alter the 5-HT_{2A} binding density in the CPu compared to HF diet group (both P >0.050). Diet did not significantly affect 5-HT_{2A} binding density in the M1 or Pir (Table 3).

Body weight, energy intake, and plasma MCP-1 level of rats with dietary intervention

There was no significant difference in body weight gain among the four groups (LF: 82.33±6.37g; HF: 86.89±5.66g; HF + GOS: 86.67±3.54g; HF + RS: 87.78±6.52g, P=0.905). The average energy intake during the dietary treatment was significantly different among the four groups (LF: 84.02±1.57 kcal/24hours; HF: 95.89±2.49 kcal/24hours; HF + GOS: 89.19±1.91 kcal/24hours; HF + RS: 90.69±2.01 kcal/24hours, P=0.003). Further analysis revealed that the average energy intake in HF diet group was significantly higher than the LF diet group (P<0.001), while the average energy intake in the fiber supplement groups, HF + GOS or HF + RS, were significantly lower (P=0.026) or trended towards significantly lower (P=0.078) than the HF diet group, respectively. The average plasma concentrations of the chemokine MCP-1 was significantly higher in the HF group compared to the LF diet group (251.38±38.76pg/ml *vs* 183.40±8.76pg/ml, P=0.016), while supplementation with GOS or RS during a HF diet significantly reduced plasma MCP-1 concentrations compared to a HF diet alone (153.94±6.21pg/ml, P=0.001 and 160.83±10.31pg/ml, P=0.002, respectively).

Discussion

This study showed that supplementation of 5% dietary fiber on HF diet can prevent altered cannabinoid CB1R and serotoninergic 5-HT_{1A}R and 5-HT_{2A}R signaling pathways in the brain implicated in the regulations of cognition and body weight. Our results showed that a chronic

HF diet upregulated CB1R, 5-HT_{1A}R and 5-HT_{2A}R binding density in the rat brain compared to the LF diet group, while densities in both the GOS and RS supplementation groups largely resembled levels that were in line with the LF control group. These findings may have important implications in preventing the weight gain/obesity and cognitive decline associated with the chronic consumption of a diet high in saturated fats and low in dietary fiber,[43] through application of dietary fibre supplementation.

A chronic HF diet can affect memory and learning,[1] while dietary fiber intake is positively correlated with cognitive performance;[2, 3] however, the mechanisms underlying these behavioral changes remain unclear. This study showed that dietary fiber prevented a HF diet-induced increase in CB1R density in the hippocampus, SN, and VTA. Cannabinoid CB1Rs are highly expressed in the hippocampus where they play an important role in regulating cognitive function.[44, 45] In addition, the SN and VTA of the midbrain house dopaminergic neurons and form a synaptic loop with the hippocampus that regulates long-term memory.[46, 47] Indeed, the increase in CB1R binding density observed in the HF diet group in the present study coincides with previous reports of CB1R upregulation in states of cognitive impairment. For example, it has been reported that CB1R binding densities are increased in the posterior cingulate cortex of people with schizophrenia,[48] a psychiatric illness associated with cognitive impairment.[14] In addition, rats with a schizophrenia-like phenotype show improved learning and memory following treatment with cannabidiol,[49] a CB1R negative allosteric modulator.[50] Furthermore, a HF diet has been reported to increase CB1R immunoreactivity and levels of the endogenous cannabinoids, anandamide and 2-arachidonoyl glycerol, in the hippocampus of mice.[20] The increase in CB1R binding density in the hippocampus of the HF diet in the present study suggests that activation of CB1R may contribute to HF diet-associated memory deficits. Importantly, our study found that dietary

fiber supplementation prevented the elevation in CB1R binding density.

Previous reports suggest that HF diets can induce a pro-inflammatory response in the body, while dietary fermentable fiber decreases the inflammatory response.[51, 52] This anti-inflammatory state is associated with improved balance in the gut microbiota and reduced plasma levels of lipopolysaccharide (LPS), an endotoxin and major component of the outer membrane in Gram-negative bacteria that elicits an immune response in humans.[51, 52] Other studies concur that while a HF diet can increase plasma and fecal LPS levels and result in dysregulation of the gut microbiota in rodents,[53] dietary fiber can be fermented in the intestine to regulate gut microbiota and decrease LPS.[54] Interestingly, obese mice exhibit increased intestinal endogenous cannabinoid content and CB1 receptor mRNA expression associated with altered gut microbiota.[34] Furthermore, systemic administration of LPS can increase CB1R mRNA and protein levels in the hippocampus of mice.[55] In the present study, we found that both GOS and RS supplementation significantly prevented the HF diet-induced increase in plasma levels of MCP-1, a key chemokine that responds to LPS,[56] demonstrating an anti-inflammatory effect of these two dietary fibers. Overall, in the context of the existing literature, the results of the current study suggest that a HF diet increases levels of plasma MCP-1, an indicator of LPS-induced inflammation, which may lead to increased CB1R expression in the hippocampus and other brain regions observed in this study. Moreover, GOS or RS supplementation can reduce plasma MCP-1, thereby preventing the up-regulation of the CB1R in the brain caused by a high fat diet. Further research is required to confirm this potential mechanism.

In the present study, dietary fiber supplementation with GOS during a HF diet showed some beneficial effects in preventing HF diet-induced increases in 5-HT_{1A}R density in the

hippocampus. A HF diet also increased 5-HT_{2A}R binding density in the MM, ACC and CPu (striatum) of rats, while dietary fiber supplementation exhibited preventative effects in the MM and ACC, but not the CPu. In addition to the hippocampus, the ACC and MM are important brain regions for cognitive and memory function. For example, ACC activity is related to cognitive control such as error detection, conflict monitoring, task difficulty, and/or task switching,[57] while the MM is involved in episodic memory[58] and studies show that lesion of the MM impairs the rodent in the performance in spatial working memory tasks.[59, 60] It is well known that the 5-HT_{1A}R and 5-HT_{2A}R play important roles in cognitive abilities and working memory processes.[22, 23, 61] The increases in 5-HT_{1A}R and 5-HT_{2A}R binding densities in HF diet group in the present study are consistent with existing evidence showing a negative effect of these receptors on memory function in clinical and animal studies. For example, 5-HT_{1A}R and 5-HT_{2A}R densities were increased in the temporal cortex of dementia patients[27] and verbal memory was impaired following administration of a 5-HT_{1A}R agonist to humans.[25] In rats, 5-HT_{1A}R agonist (8-OH-DPAT) injected into the hippocampus induced cognitive impairment,[62] while intrahippocampal administration of a 5-HT_{1A}R antagonist improved spatial learning.[63] In addition, using positron emission tomography (PET), a significant negative correlation was found between 5-HT_{1A}R expression localized in the bilateral hippocampus and explicit memory function of healthy subjects.[25] While mechanisms underlying the diet-induced changes in 5-HT_{1A}R and 5-HT_{2A}R binding densities observed in the present study are unknown, evidence suggests that diet can alter serotonin levels. For example, one study showed that a chronic HF diet (20% corn oil) decreased serotonin levels in the brainstem of rats,[10] while other studies have reported that dietary fiber increases circulating serotonin levels and its secretion in the colon.[11, 12] Altered serotonin levels could lead to altered receptor expression in the brain regions. Therefore, further studies investigating the effects of dietary fiber on serotonin and influences on the 5-HT_{1A}R and 5-

$HT_{2A}R$ in the brain are required.

The MBH is important in the regulation of food intake and energy balance.[64] Within the hypothalamus, endogenous cannabinoids and the CB1R are critically involved in the regulation of food intake.[65] Indeed, the anorectic effects of the CB1R antagonist, SR141716, in humans and animal models are well-documented.[18, 66-68] Therefore, the results of the present study coincide with the literature, as CB1R binding density was increased in the MBH after a HF diet and this increase was prevented by dietary GOS and RS supplementation. In addition, the changes in CB1R binding density in the MBH were mirrored by the expected changes in food intake. The present study also revealed alterations in hypothalamic binding density of serotonergic 5-$HT_{1A}R$, which are considered to be involved in the control of negative energy balance. Indeed, the intra-hypothalamic injection of the 5-$HT_{1A}R$ agonist, 8-OH-DPAT, decreases food intake and promotes satiety in rats,[69] while the same administration of a 5-$HT_{1A}R$ antagonist, WAY-100635, blocks the anorexic effect induced by 5-HT.[70] Importantly, a HF diet significantly decreases central 5-HT levels in rats.[10] Consistent with those reports, our data showed that a HF diet increased 5-$HT_{1A}R$ binding density in the MBH, which may be compensatory response to decreased 5-HT. Overall, the prevention of increased CB1R and 5-$HT_{1A}R$ binding density in the MBH in rats fed a HF diet supplemented with dietary fiber may contribute to the suppressed food intake observed in these groups, particularly GOS-supplemented rats.

In summary, the results of this study demonstrate that a HF diet supplemented with 5% dietary fiber (GOS and RS) can prevent hyperphagia and imbalances in cannabinoid CB1R and serotonergic 5-$HT_{1A}R$ and 5-$HT_{2A}R$ binding densities in regions of the rat brain involved in cognition and food intake, Limitations of this study include the use of only male rats and

further studies in females are required. In addition, future rodent studies may benefit from examining key learning and memory domains that are influenced by diet. Overall, the data from this study imply that dietary fiber may have beneficial effects on improving learning and memory, as well as reducing excessive appetite, during the chronic consumption of a HF (standard Western) diet and further investigation is warranted.

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Conflict of Interest Statement

The authors declare that they have no financial/commercial conflicts of interest.

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Figure Legends:

Fig 1 The schematic drawings shown in A-C were adapted from a rat brain atlas (Paxinos & Watson, 1997), indicating the Bregma levels used to measure [^{3}H]-CP55940 binding density. Autoradiographs A'-C' and A''-C'' depict the expression of total [^{3}H]-CP55940 binding and non-specific [^{3}H]-CP55940 binding, respectively, at different rostrocaudal levels of the rat brain. Abbreviations: Hip, hippocampus; MBH, mediobasal hypothalamus; SN, substantia nigra; VTA, ventral tegmental area; CPu, caudate putamen; Pir, piriform cortex; M1, primary motor cortex; Amg, amygdala; ACC, anterior cingulate cortex.

Fig 2. CB1 receptor binding densities (using [^{3}H]-CP55940), in the brains of rats fed either a low or high fat (LF, HF) diet, or a HF diet supplemented with dietary fibers, galacto-oligosaccharide (GOS) (HF+GOS) or resistant starch (RS) (HF+RS), for 4 weeks. Abbreviations: Hip, hippocampus; MBH, mediobasal hypothalamus; SN, substantia nigra; VTA, ventral tegmental area. Data are expressed as mean+S.E.M. Means without a common letter are significantly different, $P<0.05$.

Fig 3. The schematic drawings shown in A-C were adapted from a rat brain atlas (Paxinos & Watson, 1997), indicating the Bregma levels used to measure [^{3}H]-WAY-100635 binding density. Autoradiographs A', B' and A'', B'' depict the expression of total [^{3}H]-WAY-100635 binding and non-specific [^{3}H]-WAY-100635 binding, respectively, at different rostrocaudal levels of the rat brain. Abbreviations: Hip, hippocampus; MBH, mediobasal hypothalamus; Amg: amygdala; LS, lateral septal nucleus; ACC, anterior cingulate cortex; M1, primary motor cortex.

Fig 4. 5-HT$_{1A}$ receptor binding densities (using [^{3}H]-WAY-100635), in the brains of rats fed

either a low or high fat (LF, HF) diet, or a HF diet supplemented with dietary fibers, galacto-oligosaccharide (GOS) (HF+GOS) or resistant starch (RS) (HF+RS), for 4 weeks. Abbreviations: Hip, hippocampus; MBH, mediobasal hypothalamus. Data are expressed as mean+S.E.M. Means without a common letter are significantly different, $P<0.05$.

Fig 5. The schematic drawings shown in A and B were adapted from a rat brain atlas (Paxinos & Watson, 1997) indicating the Bregma levels used to measure [^{3}H]-Ketanserin binding density. Autoradiographs A', B' and A'', B'' depict the expression of total [^{3}H]-Ketanserin binding and non-specific [^{3}H]-Ketanserin binding, respectively, at different rostrocaudal levels of the rat brain. Abbreviations: MM, medial mammillary nucleus; ACC, anterior cingulate cortex; CPu, caudate putamen; M1, primary motor cortex; Pir, piriform cortex.

Fig 6. 5-HT$_{2A}$ receptor binding densities (using [^{3}H]-Ketanserin), in the brains of rats fed either a low or high fat (LF, HF) diet, or a HF diet supplemented with dietary fibers, galacto-oligosaccharide (GOS) (HF+GOS) or resistant starch (RS) (HF+RS), for 4 weeks. Abbreviations: MM, medial mammillary nucleus; ACC, anterior cingulate cortex; CPu, caudate putamen. Data are expressed as mean+S.E.M. Means without a common letter are significantly different, $P<0.05$.

Fig 1.

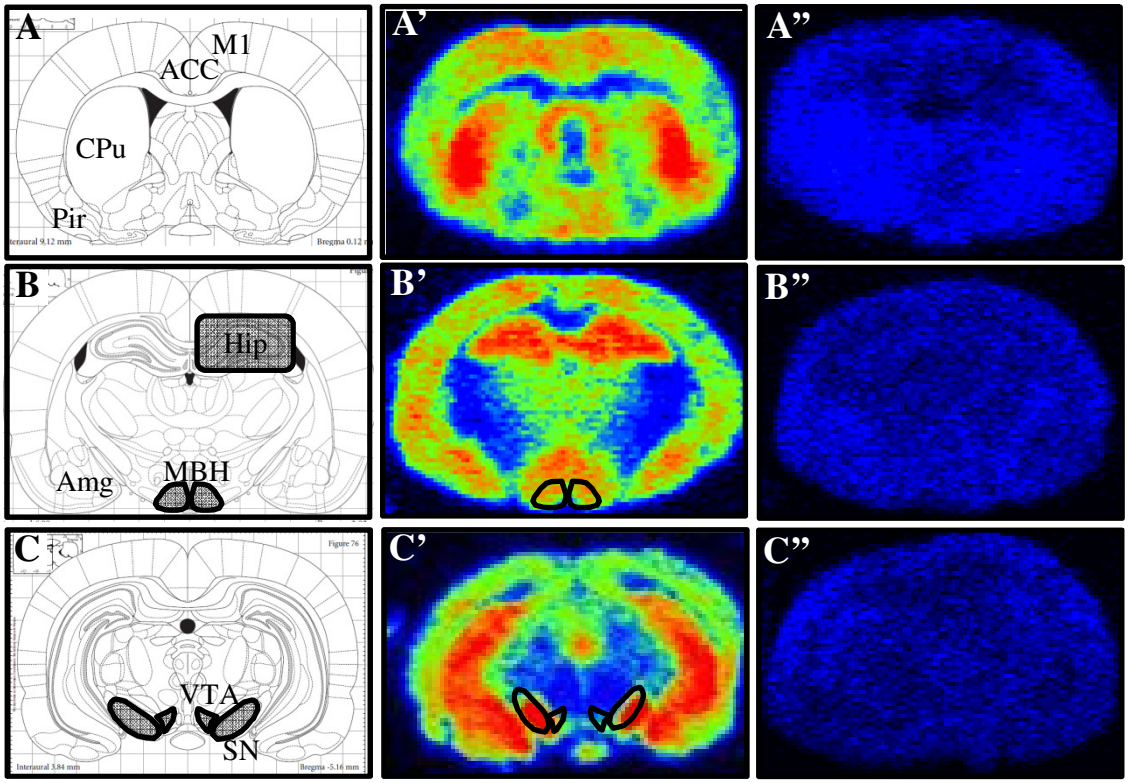


Fig 2.

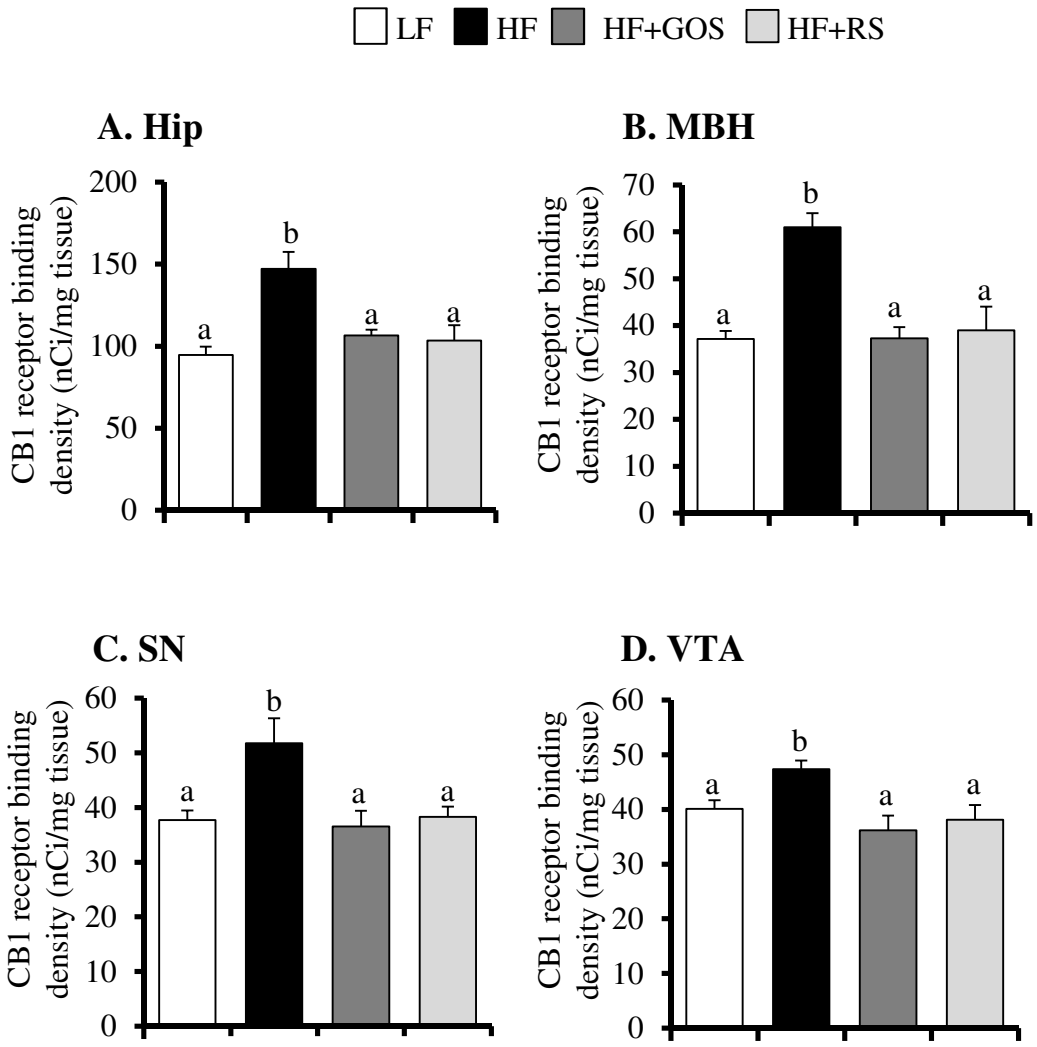

LF
HF
HF+GOS
HF+RS
A. Hip
B. MBH
C. SN
D. VTA
CB1 receptor binding density (nCi/mg tissue)
a
b
a
a

Fig 3.

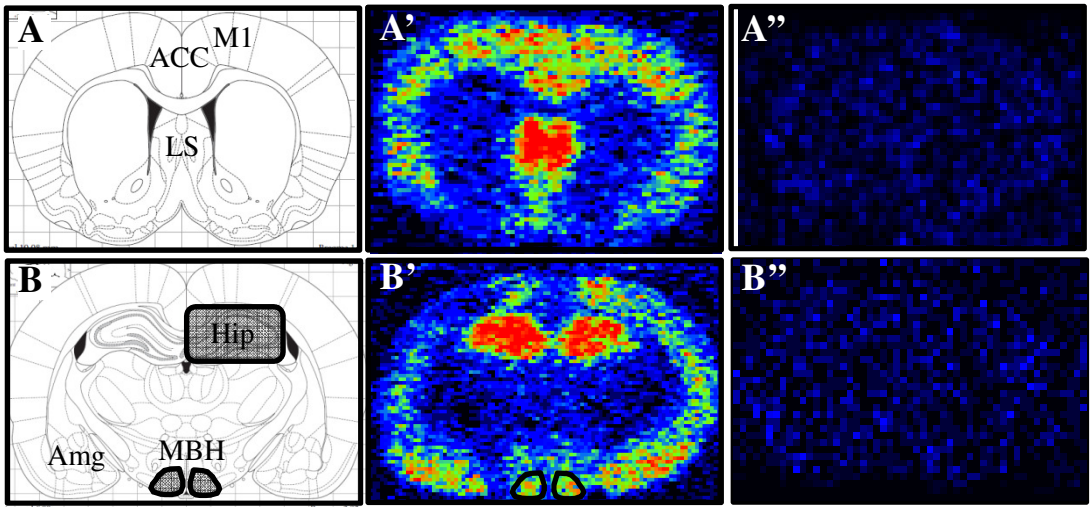


Fig 4.

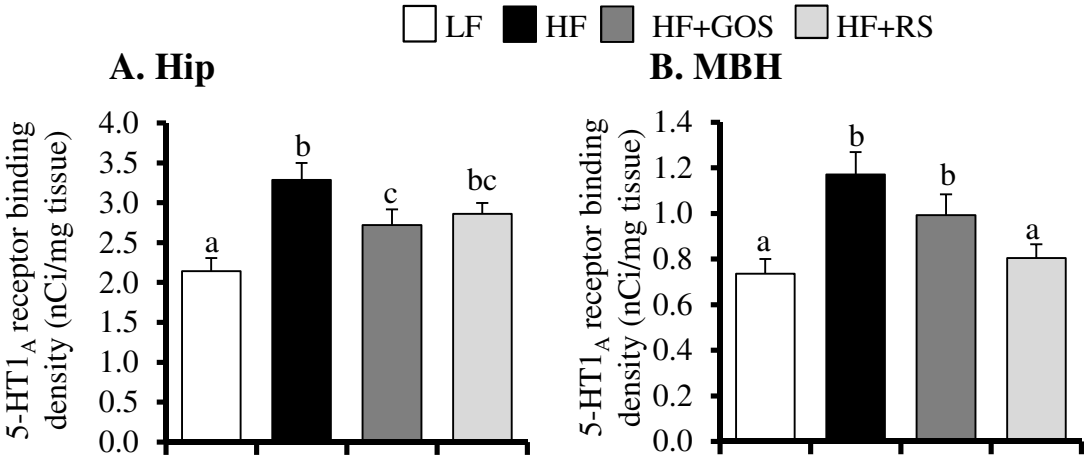

LF
HF
HF+GOS
HF+RS
A. Hip
B. MBH
5-HT1A receptor binding density (nCi/mg tissue)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
b
c
bc
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
a
b
b
a

Fig 5.

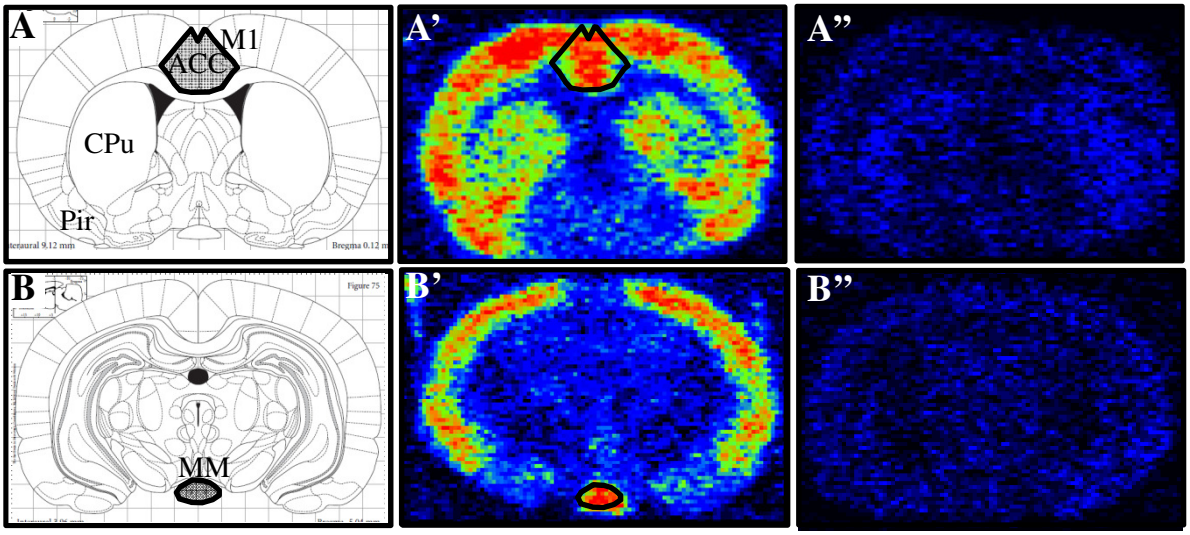
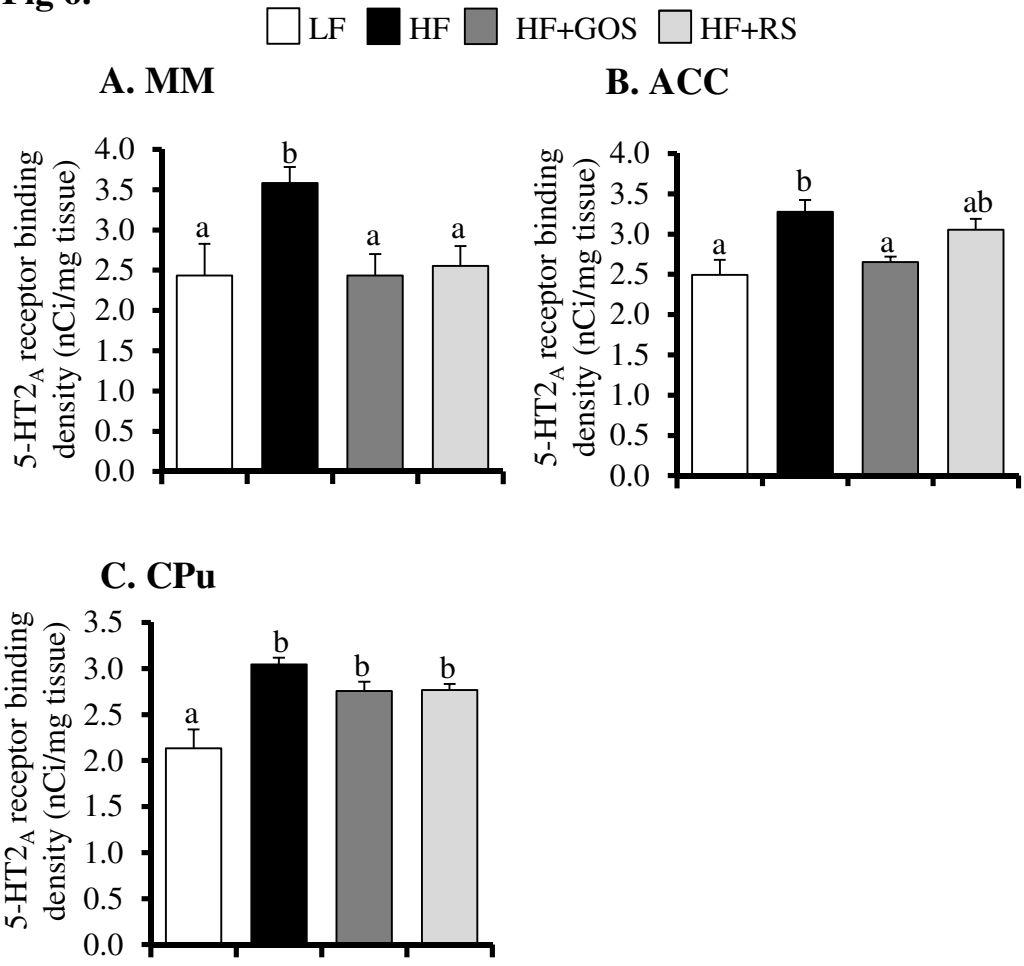


Fig 6.


LF
HF
HF+GOS
HF+RS
A. MM
B. ACC
C. CPu
5-HT2A receptor binding density (nCi/mg tissue)
a
b
a
a
a
b
a
ab
a
b
b
b

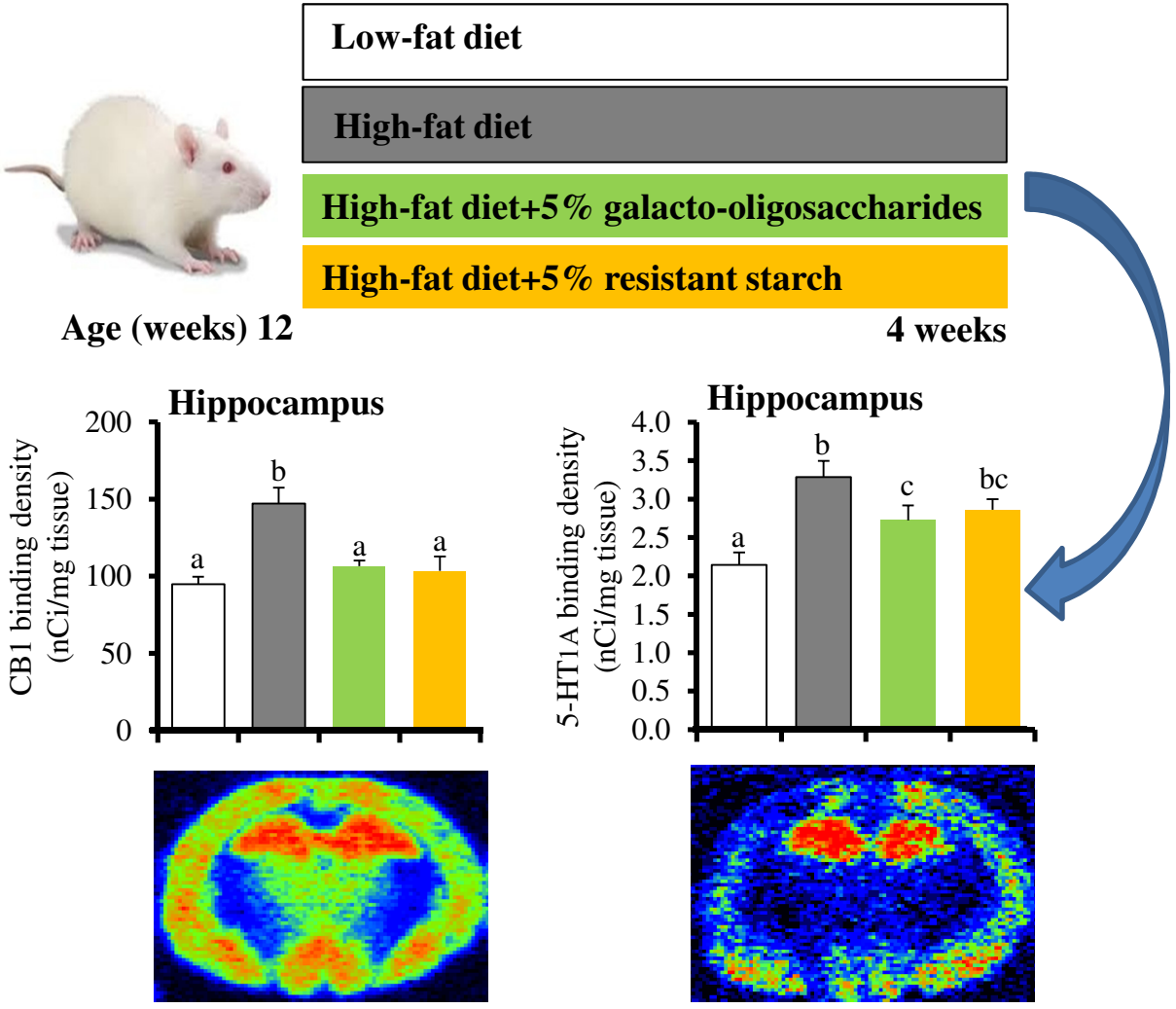

Low-fat diet
High-fat diet
High-fat diet+5% galacto-oligosaccharides
High-fat diet+5% resistant starch
Age (weeks) 12
4 weeks
Hippocampus
CB1 binding density (nCi/mg tissue)
200
150
100
50
0
a
b
a
a
Hippocampus
5-HT1A binding density (nCi/mg tissue)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
a
b
c
bc

Table 1. Specific [^{3}H]-CP55940 binding (nCi/mg tissue; mean±S.E.M.) in different brain regions of rats fed either a low or high fat (LF, HF) diet, or a HF diet supplemented with dietary fibres, galacto-oligosaccharide (GOS) (HF+GOS) or resistant starch (RS) (HF+RS), for 4 weeks.

	Mean ± SEM.				One-way ANOVA	
	LF (n=5)	HF (n=5)	HF+GOS (n=5)	HF+RS (n=5)	*F* (3, 19)	*P* value
Hip	94.70±4.99[a]	147.14±10.24[b]	106.56±3.57[a]	103.36±9.34[a]	4.763	***0.015***
MBH	37.15±1.72[a]	61.01±3.01[b]	37.30±2.38[a]	39.01±5.03[a]	11.860	***<0.001***
SN	37.68±1.81[a]	51.73±4.61[b]	36.56±2.86[a]	38.29±1.87[a]	5.644	***0.008***
VTA	40.12±1.55[a]	47.34±1.57[b]	36.20±2.66[a]	38.12±2.66[a]	4.981	***0.013***
CPu	54.10±6.20	56.78±4.10	52.72±2.74	56.82±5.35	0.270	0.846
Pir	56.13±3.20	65.76±7.12	58.72±4.12	56.74±2.09	0.949	0.440
M1	65.14±6.95	63.59±5.15	65.09±5.94	65.01±4.90	0.017	0.997
Amg	47.89±1.32	57.55±2.56	49.22±3.52	46.87±4.98	2.089	0.142
ACC	57.30±6.91	60.84±6.21	56.75±1.63	62.65±5.74	0.234	0.872

Abbreviations: Hip, hippocampus; MBH, mediobasal hypothalamus; SN, substantia nigra; VTA, ventral tegmental area; CPu, caudate putamen; Pir, piriform cortex; M1, primary motor cortex; Amg, amygdala; ACC, anterior cingulate cortex. Respective values not sharing a letter are different at $P<0.05$.

Table 2. Specific [^{3}H]-WAY-100635 binding (nCi/mg tissue; mean±S.E.M.) in different brain regions of rats fed either a low or high fat (LF, HF) diet, or a HF diet supplemented with dietary fibres, galacto-oligosaccharide (GOS) (HF+GOS) or resistant starch (RS) (HF+RS), for 4 weeks.

	Mean ± SEM.				One-way ANOVA	
	LF (n=5)	HF (n=5)	HF+GOS (n=5)	HF+RS (n=5)	*F* (3, 19)	*P* value
Hip	2.14±0.16[a]	3.29±0.21[b]	2.72±0.19[c]	2.86±0.14[bc]	6.89	***<0.001***
MBH	0.74±0.06[a]	1.17±0.10[b]	0.99±0.09[b]	0.80±0.06[a]	6.00	***0.01***
Amg	1.46±0.07	1.67±0.23	1.92±0.20	1.53±0.18	1.30	0.31
LS	2.89±0.32	2.65±0.24	2.86±0.38	2.74±0.31	0.13	0.94
ACC	1.64±0.12	1.41±0.15	1.17±0.05	1.17±0.18	2.75	0.08
M1	1.44±0.15	1.29±0.10	1.56±0.08	1.34±0.10	1.19	0.35

Abbreviations: Hip, hippocampus; MBH, mediobasal hypothalamus; Amg: amygdala; LS, lateral septal nucleus; ACC, anterior cingulate cortex; M1, primary motor cortex. Respective values not sharing a letter are different at $P<0.05$.

Table 3. Specific [^{3}H]-Ketanserin binding (nCi/mg tissue; mean±S.E.M.) in different brain regions of rats fed either a low or high fat (LF, HF) diet, or a HF diet supplemented with dietary fibres, galacto-oligosaccharide (GOS) (HF+GOS) or resistant starch (RS) (HF+RS), for 4 weeks.

	Mean ± SEM.				One-way ANOVA	
	LF (n=5)	HF n=5)	HF+GOS (n=5)	HF+RS (n=5)	*F* (3, 19)	*P* value
MM	2.44±0.39[a]	3.58±0.20[b]	2.44±0.27[a]	2.55±0.25[a]	3.803	***0.033***
ACC	2.50±0.19[a]	3.28±0.14[b]	2.65±0.07[a]	3.05±0.14[ab]	6.571	***0.004***
CPu	2.13±0.20 [a]	3.04±0.07 [b]	2.75±0.10[b]	2.76±0.07[b]	9.476	***0.001***
M1	4.45±0.50	4.81±0.43	4.96±0.23	4.77±0.41	0.281	0.839
Pir	3.20±0.22	3.48±0.30	3.19±0.33	3.83±0.34	0.995	0.420

Abbreviations: MM, medial mammillary nucleus; ACC, anterior cingulate cortex; CPu, caudate putamen; M1, primary motor cortex; Pir, piriform cortex. Respective values not sharing a letter are different at $P<0.05$.