

Research article

A combination of dietary fat intake and nicotine exposure enhances CB1 endocannabinoid receptor expression in hypothalamic nuclei in male mice



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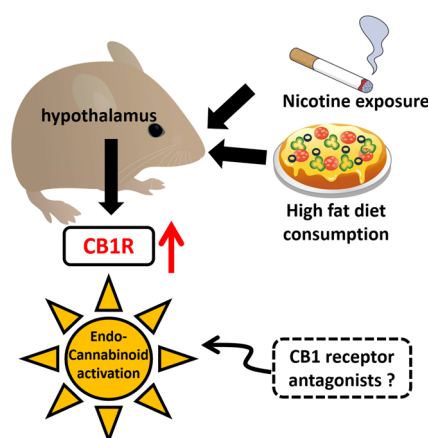
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GRAPHICAL ABSTRACT



Abbreviations: AGRP, agouti related neuropeptide; ARC, arcuate nucleus; CB1R, cannabinoid receptor 1; CRH, corticotropin releasing hormone; CRHR1, corticotropin releasing hormone receptor 1; CRHR2, corticotropin releasing hormone receptor 2; HFD, high fat diet; Hippo, hippocampus; LH, lateral hypothalamus; MCH, melanin concentrating hormone; MC3R, melanocortin 3 receptor; MC4R, melanocortin 4 receptor; NPY, neuropeptide Y; POMC, proopiomelanocortin; PVN, paraventricular nucleus; TRH, thyrotropin releasing hormone; V/DMH, ventromedial and dorsomedial hypothalamus

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ABSTRACT

Background: Cannabinoid receptor 1 (CB1R) is a GPCR expressed widely in the brain as well as in peripheral metabolic organs. Although pharmacological blockade of CB1R has been effective for the treatment of obesity and tobacco addiction, precise distribution of CB1R within the brain and potential changes by obesity or nicotine exposure have not been thoroughly addressed.

Methods: To examine CB1R distribution within the central energy center, we performed immunostaining and qPCR analysis of micro-dissected hypothalamic nuclei from male C57BL/6 mice. To address the effect of nicotine on food intake and body weight, and on potential changes of CB1R levels in the hypothalamus, mice kept on a high fat diet (HFD) for four weeks were challenged with nicotine intraperitoneally.

Results: Validity of the micro-dissected samples was confirmed by the expression of established nucleus-enriched genes. The expression levels of CB1R in the arcuate and lateral nuclei of the hypothalamus were higher than paraventricular and ventral-dorsal medial nuclei. Nicotine administration led to a significant suppression of food intake and body weight either under standard or high fat diet. Neither HFD nor nicotine alone altered CB1R levels in any nucleus tested. By contrast, treatment of HFD-fed mice with nicotine led to a significant increase in CB1R levels in the arcuate, paraventricular and lateral nuclei.

Conclusions: CB1R was widely distributed in multiple hypothalamic nuclei. The expression of CB1R was augmented only when mice were treated with HFD and nicotine in combination. These data suggest that the exposure to nicotine may provoke an enhanced endocannabinoid response in diet-induced obesity.

1. Introduction

Tobacco smoking is a popular habit with over 1.1 billion smokers worldwide [1]. It is generally accepted that current cigarette smokers weigh less than age-matched never smokers [2]. Among current smokers, however, the numbers of cigarettes smoked daily are positively correlated with the risk of developing obesity [2]. Since obesity and smoking are both high-ranked risk factors for cardiovascular morbidity and mortality, obese heavy smokers can be viewed as those at highest risk [3].

Rimonabant, an inverse agonist of endocannabinoid receptor CB1R, has been proved effective for the treatment of both obesity and tobacco addiction [4]. Although rimonabant was once approved as a drug against obesity, it was later withdrawn from the market due to psychiatric side effects [5]. Following years have witnessed continued efforts for the development of tissue-specific CB1R inverse agonists as anti-obesity drugs [6].

Endocannabinoids, e.g. anandamide or 2-arachidonoylglycerol, are endogenous substances that share psychoactive effects of plant-derived or synthetic cannabinoids. Biosynthesis, modes of action, and degradation of endocannabinoids within the brain have been intensively studied because of its importance in broad areas of human physiology [7]. Several receptors of endocannabinoids have been reported, among which CB1R, a member of GPCR, is expressed in the brain, liver and adipose tissues [7].

Within the central nervous system, CB1R is broadly expressed including hypothalamic area [7,8] Hypothalamus serves as an energy center regulating appetite and energy expenditure [9]. Pharmacological manipulation of hypothalamic functions has long been regarded as one of the basic strategies for the development of anti-obesity drugs [10]. Since adverse psychiatric outcomes of rimonabant may have resulted from its effects outside the hypothalamus such as reward centers, it is tempting to speculate that hypothalamus-specific CB1R antagonism could be a future therapeutic paradigm. However, hypothalamus comprises collections of neurons forming small nuclei with distinct physiologic functions [11]. Detailed distribution of CB1R within the hypothalamus and its potential regulation have not been fully elucidated.

Here, by our microdissection method, we have closely examined the expression of CB1R within the hypothalamus and explored potential alterations of its expression in a pathologic condition provoked by a combination of high fat diet (HFD) feeding and nicotine exposure.

2. Materials and methods

2.1. Animal experiment

Eight-week old male C57BL/6 mice were fed either standard diet (STD) (F-2, 3.73 kcal/g, 11.6% kcal fat, source: soybean; Funahashi Farm) or HFD (D12493, 5.24 kcal/g, 60% kcal fat, fat source: soybean/lard; Research Diets) *ad libitum* for four weeks on a 14-h light/10-h dark cycle at 23 °C. Mice were injected intraperitoneally with PBS, MT-II (Phoenix Pharmaceuticals) (0.5 µg/g body weight x four times/day) or nicotine bitartrate dihydrate (Sigma-Aldrich) (3 µg/g body weight x four times/day). Body weight and food intake were monitored during injections and brain areas were micro-dissected at the time of sacrifice (Suppl. Fig. 1A and B).

All animal experiments were in accordance with the law and the guideline for animal experiments of Kyoto University and Nagoya City University and were approved by the Animal Research Committee, Kyoto University and Nagoya City University.

2.2. Micro-dissection of the brain areas

Mice were sacrificed by decapitation and non-fixed brain was sagittally cut into 1mm-thick fresh slices on ice-embedded Rodent Brain Matrix (ASI Instruments, MI, USA). The slices were observed under stereoscopic microscope and microdissected according to key visible anatomical structures [12] (Suppl. Materials & Methods and Suppl. Fig. 2).

2.3. Quantitative-PCR (qPCR) analysis

Micro-dissected brain samples were soaked in pre-chilled Trizol reagent (Invitrogen) and snap frozen in liquid nitrogen. Thawed samples were homogenized and total RNA were purified using RNeasy Micro Kit (Qiagen). qPCR was undertaken using DNase (Promega), Superscript III (Invitrogen), TaqMan Master Mix (ABI) and 7300 Real Time PCR system (ABI) with primers and probes (ABI or IDT).

2.4. Immunohistochemistry

Brains were fixed and 50-µm-thick coronal sections were prepared [13], blocked in 10% donkey serum and 0.2% Triton X-100 in PBS for 30 min at room temperature and stained overnight at 4 °C with anti-

CB1R antibody (Proteintech; 17978-1-AP). Signals were amplified and visualized using the TSA Fluorescence System (PerkinElmer) [13]. Nuclei were stained with Hoechst33342 (Sigma). Confocal images were obtained using LSM880 laser-scanning microscope system (Carl Zeiss).

2.5. Statistical analyses

Figures were created using GraphPad Prism version 7.00 for Windows (GraphPad Software, www.graphpad.com). Data are shown as means $\pm$ SEM. Comparisons were undertaken by Student's *t* test using statistics function of Microsoft Excel (Vers.2016). Statistical significance was accepted at $p < 0.05$ between the groups.

3. Results

3.1. Nicotine-induced suppression of food intake and body weight in mice

Mice started on a HFD showed more rapid body weight increase than mice on a STD (Suppl. Fig. 3A). Daily energy intake measured on the fourth week was comparable between the two diet groups (Suppl. Fig. 3B). After four-week STD or HFD feeding, mice were injected intraperitoneally either with PBS, MT-II or nicotine (Suppl. Fig. 1A and B). MT-II, a melanocortin receptor agonist, was here used as a positive control, since it is widely used as a drug that reduces body weight even under HFD [14].

In STD-fed mice, injection of nicotine suppressed food intake by $29 \pm 5\%$ and the suppression was significantly greater than PBS group (Fig. 1A). MT-II suppressed food intake in HFD-fed mice by $38 \pm 5\%$ and the suppression was significantly greater than PBS (Fig. 1A). More importantly, nicotine was effective in reducing food intake by $53 \pm 13\%$ even in HFD-fed condition. The reduction was again significant compared with PBS (Fig. 1A). Body weight was decreased either by MT-II or nicotine administration both in STD and HFD-fed mice (Fig. 1B). These data suggest that nicotine is a potent weight-reducing agent even under HFD.

3.2. Expression of signature genes in micro-dissected murine hypothalamic nuclei

Since hypothalamus is the primary center of energy metabolism, we next examined hypothalamic gene expression that may account for nicotine-induced suppression of food intake and body weight. In order to perform separate nucleus-specific analyses, we microscopically dissected each hypothalamic nucleus (Suppl. Materials & Methods, Suppl. Fig. 2).

We further validated our cut-out method by qPCR analysis for region-enriched signature genes. ARC-specific neuropeptide genes; *pomc* (pro-opio-melano-cortin gene), *agrp* (agouti related neuropeptide gene) and *npv* (neuropeptide Y gene), were almost exclusively expressed in ARC (Suppl. Fig. 4A-C), while, *trh* (thyrotropin releasing hormone gene), *sf-1* (steroidogenic factor-1 gene) and *mch* (melanin

concentrating hormone gene) expression was highest in previously reported areas (Suppl. Fig. 4D-F). These data support the validity of our procedure.

3.3. Changes of the hypothalamic expression of GPCR and neuropeptides

Some hypothalamic neuropeptides relay energy signals through specific G-protein coupled receptors (GPCRs). One of the most important of such neuropeptide/receptor axes is α -MSH/melanocortin system. α -MSH peptide derived from its precursor, POMC, is produced in ARC neurons and axonally transported to PVN and released from the presynaptic terminal. α -MSH signal is then transduced through melanocortin type 4 receptor (MC4R), a GPCR enriched in postsynaptic membrane of the PVN neurons. To examine whether nicotine has any effect on the expression of GPCRs for energy-regulating neuropeptides, *mc3r* and *mc4r* expression in PVN was examined in HFD-fed mice treated with PBS, MT-II or nicotine. HFD alone did not change PVN expression of *mc3r* or *mc4r* (Fig. 2A and B). However, peripheral administration of MT-II significantly increased *mc3r* level in STD-fed mice and enhanced *mc4r* expression both in STD-fed and HFD-fed mice. In contrast, nicotine treatment did not alter the expression of these receptors in STD or HFD-fed animals (Fig. 2A-B).

Anorexigenic POMC and orexigenic NPY and AGRP are three neuropeptides essential in energy regulation and their production within the hypothalamus is limited to ARC. We therefore measured the expression of these three neuropeptides in the ARC. When mice became obese by HFD, anorexigenic *pomc* expression tended to be increased but orexigenic *agrp* and *npv* expression tended to be decreased (Fig. 2C-E). These apparently irrelevant changes have already been published and interpreted as compensatory responses to HFD-induced positive energy balance [15,16]. Upregulated *pomc* expression in HFD-fed mice was not altered by nicotine, while decreased *agrp* expression in HFD-fed mice was tended to be further suppressed (Fig. 2C and D). By contrast, *npv* expression was further and significantly decreased by nicotine in HFD-fed mice (48% decrease from HFD-fed PBS-treated mice) (Fig. 2E). Since NPY is an established orexigenic neuropeptide, NPY inhibition by nicotine may play a role in nicotine-mediated suppression of food intake and body weight.

Corticotropin releasing hormone (CRH) is another anorexigenic neuropeptide that mediates stress-induced response and plays a critical role in nicotine-induced body weight loss [17,18]. CRHR1 is a major receptor for CRH and is expressed in the hypothalamus. *crhr1* expression did not change by HFD (Fig. 2F). However, nicotine administration in HFD-fed mice led to an increase of *crhr1* expression in PVN (Fig. 2F) and may possibly be related to changes in nicotine response in HFD-fed condition.

3.4. Up-regulation of CB1R by high fat diet and nicotine in combination

cb1r expression was detected in all micro-dissected brain samples. In STD-fed mice, the expression of *cb1r* in ARC was significantly higher

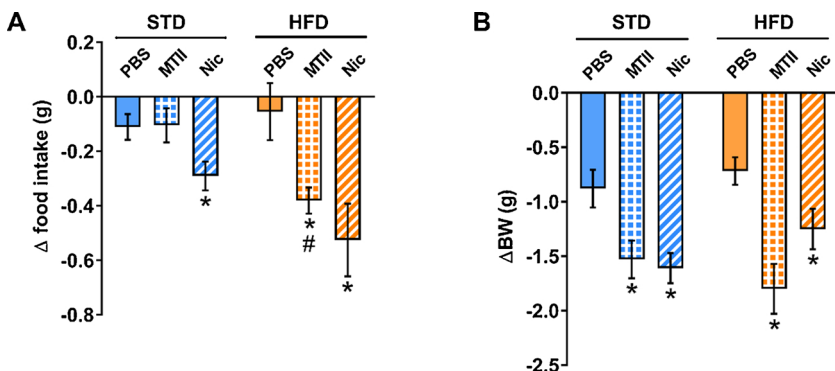


Fig. 1. Effect of MTII and nicotine on food intake and body weight. (A) Suppression of 24-h food intake in mice treated with PBS, MTII, or nicotine. Food intake during the 24-h period before and after the first injection are compared and incremental changes are shown ($n = 6$). * $p < 0.05$ vs. PBS in each diet group, # $p < 0.05$ vs. STD-MTII. (B) Changes of body weight (BW) during the 24-h period after the first injection ($n = 6$). * $p < 0.05$ vs. PBS in each diet group.

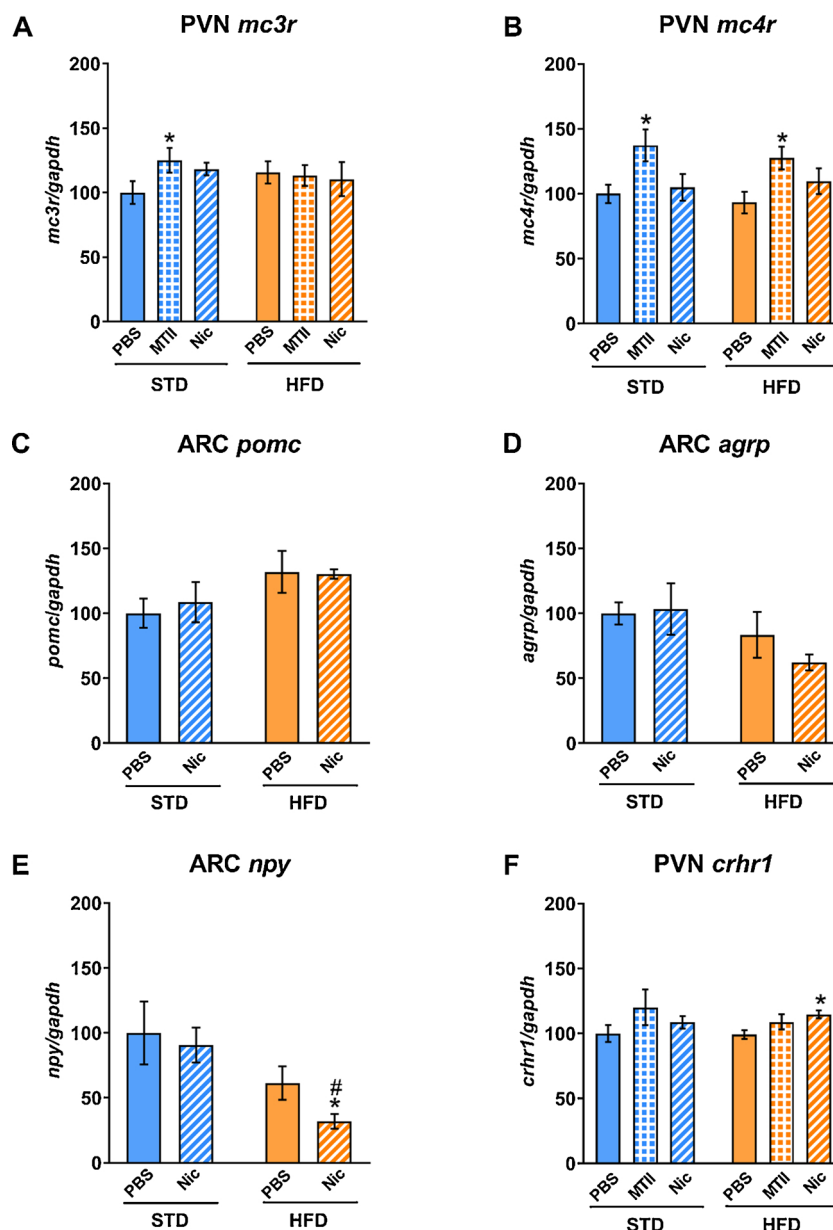


Fig. 2. Effect of HFD feeding and nicotine treatment on hypothalamic expression of GPCR and neuropeptides. Expression of *mc3r* (A) and *mc4r* (B) in PVN ($n = 4 \sim 6$). * $p < 0.05$ vs. PBS in each diet group. Expression of *pomc* (C), *agrp* (D), and *npv* (E) in ARC ($n = 4 \sim 6$). * $p < 0.05$ vs. PBS in each diet group. # $p < 0.05$ vs. STD-Nicotine. Expression of *crhr1* (F) in PVN ($n = 4 \sim 6$). * $p < 0.05$ vs. PBS in each diet group. Expression levels (%) are shown in relative to STD-PBS.

than PVN or V/DMH (Fig. 3A). In the hippocampus, *cb1r* expression was higher than ARC (Fig. 3A). To test whether the presence of *cb1r* mRNA within the hypothalamic nuclei is accompanied by CB1R immunoreactivity, we performed immunostaining using anti-CB1R antibody. CB1R protein was detected in the hippocampus and hypothalamic region (Suppl. Fig. 5). When observed in higher magnification, the signal within the hippocampus looks varicose in shape but mostly punctate in ARC, PVN and VMH (Fig. 3B-E, B'-E'). The appearance of the signals was compatible with previous report [8] and the distribution was consistent with our qPCR results. In qPCR analysis, four-week HFD did not alter *cb1r* expression except a 20% decrease observed in PVN (Fig. 3A).

In STD-fed mice, treatment with nicotine reduced *cb1r* expression only in LH and by 17% (Fig. 3F). Nicotine administration did not change *cb1r* mRNA levels in any other nucleus under STD-fed condition (Fig. 3F). Possible functional role of this significant small reduction in *cb1r* expression in LH remains to be addressed.

Interestingly, when HFD-fed mice were administered with nicotine, *cb1r* expression was significantly augmented in ARC, PVN, V/DMH, LH and hippocampus by 45%, 65%, 34%, 50% and 31%, respectively (Fig. 3G). This significant and drastic enhancement of *cb1r* expression in all hypothalamic nuclei along with hippocampus suggests that simultaneous stimuli by HFD and nicotine together, provoke events that are not caused by either one of the two. Treatment with MTII did not change *cb1r* expression in any brain area (Suppl. Fig. 6A-E).

4. Discussion

By a quantitative PCR analysis of micro-dissected mouse brain areas, we here demonstrate that expression of CB1R endocannabinoid receptor is up-regulated in mice fed on HFD and administered with nicotine. Single treatment, either with HFD or nicotine alone, does not show such an effect, suggesting a composite effect of these two insults on gene expression within the energy center of the brain.

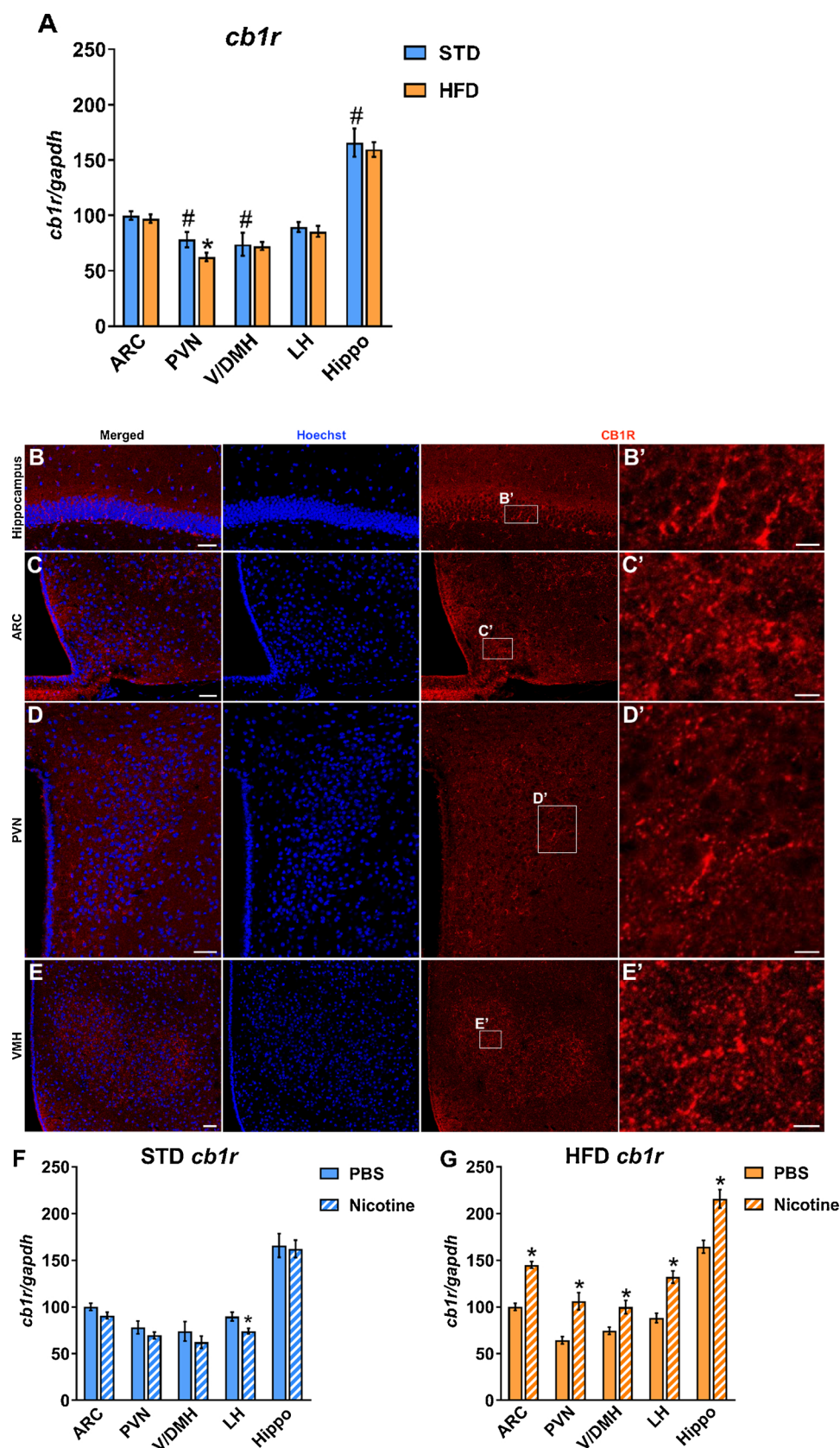


Fig. 3. Hypothalamic *cb1r* mRNA and protein expression. (A) *cb1r* expression in ARC, PVN, V/DMH, LH and Hippocampus from mice fed STD or HFD ($n = 4-6$). # $p < 0.05$ vs. STD-ARC. * $p < 0.05$ vs. STD in each nucleus. (B-E) CB1R immunoreactivity (red), nuclear staining (blue) and merged images of hippocampus (B), ARC (C), PVN (D) and VMH (E) in mice fed STD. Insets (B'-E') are magnified images of the areas indicated in the figure. Scale bars: 50 μm (B, C, D, E), 10 μm (B', C', D', E'). (F) *cb1r* expression in brain areas from STD-fed mice injected either with PBS or nicotine ($n = 4-6$). * $p < 0.05$ vs. PBS in each nucleus. (G) *cb1r* expression in brain areas from HFD-fed mice injected either with PBS or nicotine ($n = 4-6$). * $p < 0.05$ vs. PBS in each nucleus. Expression levels (%) are shown in relative to STD-PBS-ARC (A, F) or to HFD-PBS-ARC (G).

Customary intake of HFD and tobacco smoking habits are both prevalent in cultures across the globe and often co-observed in an individual. Therefore, the finding that these two factors have composite effects on the molecular dynamism of the hypothalamus may have implications in the study of both obesity and tobacco addiction. It is interesting that only in combination do these two factors influence *cb1r* expression. Combination of HFD and nicotine might trigger some specific mediators that are involved in the transcriptional regulation of *cb1r*. A future global gene network analysis is warranted to reveal the mechanism underlying this combinatorial effect.

We clearly show that nicotine potentially suppresses food intake and body weight under STD or HFD, consistent with previous studies [19]. Human epidemiological studies also show that smokers in general weigh less than non-smokers [20]. In clinical practice, weight gain associated with tobacco cessation in obese patients often poses challenges for the treatment of obesity and associated morbidities [3]. Although nicotine-induced weight loss has been established [3][20], the entire picture of how nicotine reduces body weight remains unclarified.

Body weight is principally regulated by the hypothalamic nuclei, among which ARC is the primary gateway for peripherally-derived appetite-regulating hormones such as leptin [21]. Activity of NPY/AGRP neurons in the ARC is suppressed by leptin, mediating its anorexigenic and weight-reducing effects [21]. In this study, we have found that nicotine reduces *npy* expression in the ARC under HFD, which may partly account for the observed weight-reducing effect of nicotine. Slight but significant increase of anorexigenic *crhr1* levels in the PVN by nicotine may also contribute to nicotine's weight-reducing effect.

CB1R blockade is effective for the treatment of diet-induced obesity in animal models and humans [5]. However, the target cells of CB1R inverse agonists within the appetite center have not been established. Although CB1R immunoreactivity has been reported in the hypothalamus [8], the precise and quantitative tissue distribution and its potential region-specific regulation have not been thoroughly addressed. By our own micro-dissection method, we have analyzed *cb1r* distribution and regulation within the hypothalamus. Its expression is observed throughout the hypothalamus but is higher in ARC than PVN or V/DMH. We have also analyzed the protein expression of CB1R by immunohistochemistry and demonstrated the presence of CB1R protein within these hypothalamic areas including ARC. Since ARC is a vital nucleus in regulating food intake and energy expenditure, it is tempting to speculate that CB1R in ARC play a critical role as a target of CB1R inverse agonists.

In a human study, rimonabant, a potent CB1R inverse agonist, has been proved effective in reducing body weight and improving obesity-related morbidities [22]. Although rimonabant did not seem to increase cardiovascular risk, it has unfortunately led to an increased development of neuropsychiatric adverse effects [23], leading to its withdrawal from the market. Mechanisms underlying increased rate of depression and suicidal attempts has not been clearly understood. However, if we can separate the regulation of body weight and mood control by endocannabinoids through a region-specific approach, we might be able to differently manipulate appetite and neuropsychiatric adverse events.

We demonstrate that nicotine enhances CB1R expression in hypothalamic nuclei including ARC in diet-induced obesity. Interestingly, nicotine-induced weight loss in HFD-fed mice is abolished in CB1R knockout mice [24], suggesting that CB1R is required for the hypothalamic action of nicotine.

In contrast to some other GPCRs, including β -adrenergic receptor, whose expression is down-regulated by an abundance of its ligands [25], CB1R expression is reportedly reduced by its antagonist [26]. This suggests that endocannabinoids themselves enhance CB1R expression through CB1R activation. Furthermore, increased production of anandamide within the liver in HFD-fed mice leads to an inhibition of endocannabinoid-degrading enzyme [27]. These data suggest a potential feed-forward activation of endocannabinoid system *in vivo* [27]. Our results showing that HFD and nicotine in combination induce CB1R

expression in the hypothalamus may suggest a local activation of endocannabinoid system as a whole.

Considering an essential role of CB1R in nicotine-induced body weight reduction in mice [24], potential central activation of endocannabinoid system in human obese subjects may mediate smoking-induced weight loss. Physiologic roles of enhanced CB1R expression and potential endocannabinoid activation within the hypothalamus require future studies.

In conclusion, we here show tissue distribution and regulation of CB1R endocannabinoid receptor in the hypothalamus in mice. HFD and nicotine in combination enhance CB1R expression in the hypothalamus. These data suggest a potential alteration of hypothalamic endocannabinoid activity in obese smokers.

Authors' contribution

T.G., T.T., H.M. proposed plan, T.G., T.T., M.M., K.K., T.U., Y.O. performed experiments, T.G., T.T., D.A., K.S., T.K., H.I., T.M., Y.M., H.M., N.I., K.N. gave direction to experiments, data analysis and discussion. T.G. and T.T. wrote manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.neulet.2019.134550>.

References

- [1] WHO, Prevalence of Tobacco Smoking, WHO, 2016 (Accessed 10 March 2019), <http://www.who.int/gho/tobacco/use/en/>.
- [2] S. Dare, D.F. Mackay, J.P. Pell, Relationship between smoking and obesity: a cross-sectional study of 499,504 middle-aged adults in the UK general population, *PLoS One* 10 (2015) e0123579, <https://doi.org/10.1371/journal.pone.0123579>.
- [3] M.D. Jensen, D.H. Ryan, C.M. Apovian, J.D. Ard, A.G. Comuzzie, K.A. Donato, F.B. Hu, V.S. Hubbard, J.M. Jakicic, R.F. Kushner, C.M. Loria, B.E. Millen, C.A. Nonas, F.X. Pi-Sunyer, J. Stevens, V.J. Stevens, T.A. Wadden, B.M. Wolfe, S.Z. Yanovski, H.S. Jordan, K.A. Kendall, L.J. Lux, R. Mentor-Marcel, L.C. Morgan, M.G. Trisolini, J. Wnek, J.L. Anderson, J.L. Halperin, N.M. Albert, B. Bozkurt, R.G. Brindis, L.H. Curtis, D. DeMets, J.S. Hochman, R.J. Kovacs, E.M. Ohman, S.J. Pressler, F.W. Sellke, W.K. Shen, S.C. Smith Jr., G.F. Tomaselli, American College of Cardiology/American Heart Association Task Force on Practice Guidelines, Obesity Society, 2013 AHA/ACC/TOS guideline for the management of overweight and obesity in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and The Obesity Society, *Circulation* 129 (2014) S102–S138, <https://doi.org/10.1161/01.cir.0000437739.71477.ee>.
- [4] E.V. Gelfand, C.P. Cannon, Rimobant: a selective blocker of the cannabinoid CB1 receptors for the management of obesity, smoking cessation and cardiometabolic risk factors, *Expert Opin. Investig. Drugs* 15 (2006) 307–315, <https://doi.org/10.1517/13543784.15.3.307>.
- [5] D.R. Janero, A. Makriyannis, Cannabinoid receptor antagonists: pharmacological opportunities, clinical experience, and translational prognosis, *Expert Opin. Emerg. Drugs* 14 (2009) 43–65, <https://doi.org/10.1517/14728210902736568>.
- [6] C. Wilson, Obesity: CB1R inverse agonists—antiobesity effects without the neuropsychiatric adverse effects? *Nat. Rev. Endocrinol.* 8 (2012) 564, <https://doi.org/10.1038/nrendo.2012.145>.
- [7] S. Zou, U. Kumar, Cannabinoid receptors and the endocannabinoid system: signaling and function in the central nervous system, *Int. J. Mol. Sci.* 19 (2018) 833, <https://doi.org/10.3390/ijms19030833>.
- [8] G. Wittmann, L. Deli, I. Kalló, E. Hrabovszky, M. Watanabe, Z. Liposits, C. Fekete, Distribution of type 1 cannabinoid receptor (CB1)-immunoreactive axons in the mouse hypothalamus, *J. Comp. Neurol.* 503 (2007) 270–279, <https://doi.org/10.1002/cne.21383>.
- [9] K. Timper, J.C. Brüning, Hypothalamic circuits regulating appetite and energy

- homeostasis: pathways to obesity, *Dis. Model. Mech.* 10 (2017) 679–689, <https://doi.org/10.1242/dmm.026609>.
- [10] R.A.H. Adan, Mechanisms underlying current and future anti-obesity drugs, *Trends Neurosci.* 36 (2013) 133–140, <https://doi.org/10.1016/j.tins.2012.12.001>.
 - [11] Shlomo Melmed, Kenneth S. Polonsky, P. Reed Larsen, Henry Kronenberg, *Williams Textbook of Endocrinology*, 13th edition, Elsevier, 2016.
 - [12] Y. Minokoshi, T. Alquier, N. Furukawa, Y.-B. Kim, A. Lee, B. Xue, J. Mu, F. Foufelle, P. Ferré, M.J. Birnbaum, B.J. Stuck, B.B. Kahn, AMP-kinase regulates food intake by responding to hormonal and nutrient signals in the hypothalamus, *Nature* 428 (2004) 569–574, <https://doi.org/10.1038/nature02440>.
 - [13] H. Ota, T. Hikita, M. Sawada, T. Nishioka, M. Matsumoto, M. Komura, A. Ohno, Y. Kamiya, T. Miyamoto, N. Asai, A. Enomoto, M. Takahashi, K. Kaibuchi, K. Sobue, K. Sawamoto, Speed control for neuronal migration in the postnatal brain by Gmip-mediated local inactivation of RhoA, *Nat. Commun.* 5 (2014) 4532, <https://doi.org/10.1038/ncomms5532>.
 - [14] T. Tanaka, H. Masuzaki, S. Yasue, K. Ebihara, T. Shiuchi, T. Ishii, N. Arai, M. Hirata, H. Yamamoto, T. Hayashi, K. Hosoda, Y. Minokoshi, K. Nakao, Central melanocortin signaling restores skeletal muscle AMP-Activated protein kinase phosphorylation in mice fed a high-fat diet, *Cell Metab.* 5 (2007) 395–402, <https://doi.org/10.1016/j.cmet.2007.04.004>.
 - [15] M. Ziotopoulou, C.S. Mantzoros, S.M. Hileman, J.S. Flier, Differential expression of hypothalamic neuropeptides in the early phase of diet-induced obesity in mice, *Am. J. Physiol. Metab.* 279 (2000) E838–E845, <https://doi.org/10.1152/ajpendo.2000.279.4.E838>.
 - [16] T.L. Moretto, I.D. Benfato, F.P. de Carvalho, M. Barthichoto, L. Le Sueur-Maluf, C.A.M. de Oliveira, The effects of calorie-matched high-fat diet consumption on spontaneous physical activity and development of obesity, *Life Sci.* 179 (2017) 30–36, <https://doi.org/10.1016/j.lfs.2017.04.017>.
 - [17] S.C. Heinrichs, J. Lapsansky, D.P. Behan, R.K. Chan, P.E. Sawchenko, M. Lorang, N. Ling, W.W. Vale, E.B. De Souza, Corticotropin-releasing factor-binding protein ligand inhibitor blunts excessive weight gain in genetically obese Zucker rats and rats during nicotine withdrawal, *Proc. Natl. Acad. Sci. U. S. A.* 93 (1996) 15475–15480, <https://doi.org/10.1073/PNAS.93.26.15475>.
 - [18] S.P. Kamdi, K.T. Nakhate, M.P. Dandekar, D.M. Kokare, N.K. Subhedar, Participation of corticotropin-releasing factor type 2 receptors in the acute, chronic and withdrawal actions of nicotine associated with feeding behavior in rats, *Appetite* 53 (2009) 354–362, <https://doi.org/10.1016/J.APPET.2009.07.017>.
 - [19] A. Stojakovic, E.P. Espinosa, O.T. Farhad, K. Lutfy, Effects of nicotine on homeostatic and hedonic components of food intake, *J. Endocrinol.* 235 (2017) R13–R31, <https://doi.org/10.1530/JOE-17-0166>.
 - [20] C. Bamia, A. Trichopoulou, D. Lenas, D. Trichopoulos, Tobacco smoking in relation to body fat mass and distribution in a general population sample, *Int. J. Obes.* 28 (2004) 1091–1096, <https://doi.org/10.1038/sj.ijo.0802697>.
 - [21] L. Gautron, J.K. Elmquist, K.W. Williams, Neural control of energy balance: translating circuits to therapies, *Cell* 161 (2015) 133–145, <https://doi.org/10.1016/j.cell.2015.02.023>.
 - [22] L.F. Van Gaal, A.M. Rissanen, A.J. Scheen, O. Ziegler, S. Rössner, Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study, *Lancet* 365 (2005) 1389–1397, [https://doi.org/10.1016/S0140-6736\(05\)66374-X](https://doi.org/10.1016/S0140-6736(05)66374-X).
 - [23] E.J. Topol, M.G. Bousser, K.A. Fox, M.A. Creager, J.P. Despres, J.D. Easton, C.W. Hamm, G. Montalescot, P.G. Steg, T.A. Pearson, E. Cohen, C. Gaudin, B. Job, J.H. Murphy, D.L. Bhatt, CRESCENDO Investigators, Rimonabant for prevention of cardiovascular events (CRESCENDO): a randomised, multicentre, placebo-controlled trial, *Lancet* 376 (2010) 517–523, [https://doi.org/10.1016/S0140-6736\(10\)60935-X](https://doi.org/10.1016/S0140-6736(10)60935-X).
 - [24] S.A. Bura, A. Burokas, E. Martín-García, R. Maldonado, Effects of chronic nicotine on food intake and anxiety-like behaviour in CB1 knockout mice, *Eur. Neuropsychopharmacol.* 20 (2010) 369–378, <https://doi.org/10.1016/j.euroneuro.2010.02.003>.
 - [25] J.R. Hadcock, C.C. Malbon, Down-regulation of beta-adrenergic receptors: agonist-induced reduction in receptor mRNA levels, *Proc. Natl. Acad. Sci. U. S. A.* 85 (1988) 5021–5025, <https://doi.org/10.1073/PNAS.85.14.5021>.
 - [26] J. Tam, V.K. Vemuri, J. Liu, S. Bátkai, B. Mukhopadhyay, G. Godlewski, D. Osei-Hyiaman, S. Ohnuma, S.V. Ambudkar, J. Pickel, A. Makriyannis, G. Kunos, Peripheral CB1 cannabinoid receptor blockade improves cardiometabolic risk in mouse models of obesity, *J. Clin. Invest.* 120 (2010) 2953–2966, <https://doi.org/10.1172/JCI42551>.
 - [27] J. Liu, R. Cinar, K. Xiong, G. Godlewski, T. Jourdan, Y. Lin, J.M. Ntambi, G. Kunos, Monounsaturated fatty acids generated via stearoyl CoA desaturase-1 are endogenous inhibitors of fatty acid amide hydrolase, *Proc. Natl. Acad. Sci. U. S. A.* 110 (2013) 18832–18837, <https://doi.org/10.1073/pnas.1309469110>.

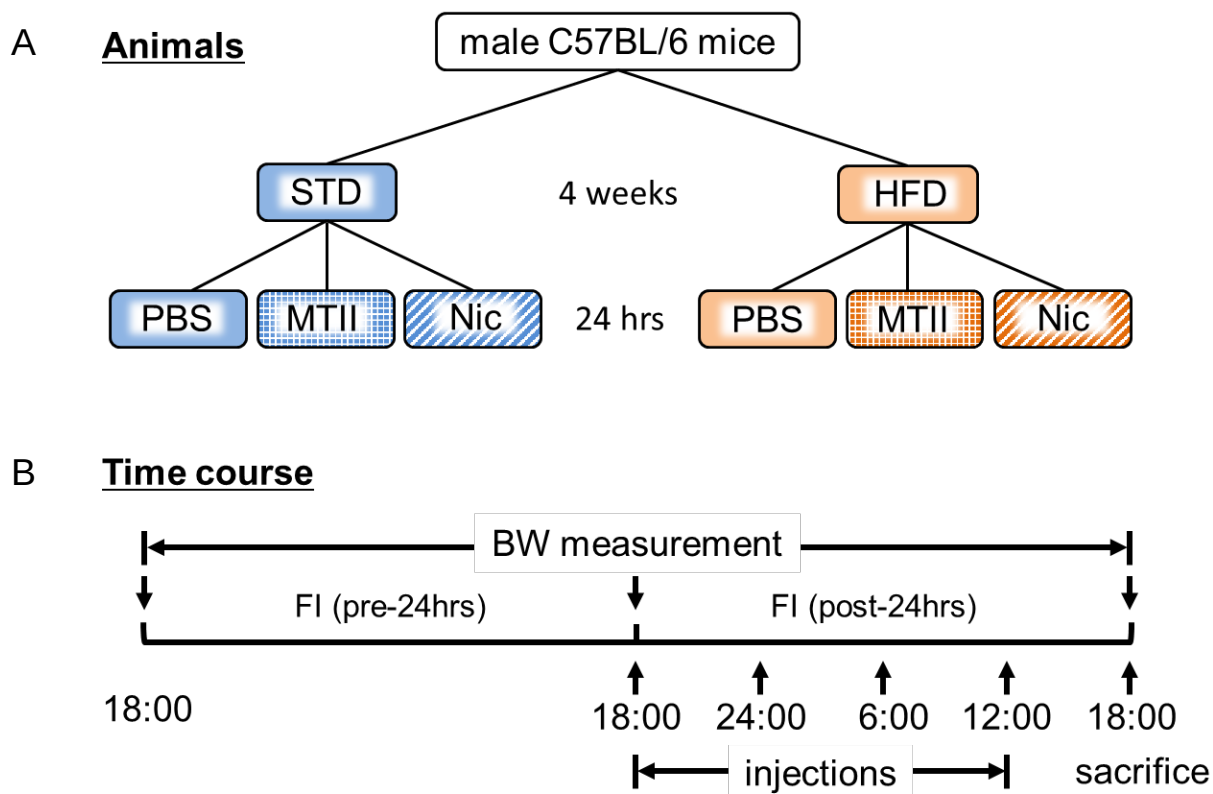
Supplementary Materials & Methods

Procedure for the microdissection

1mm slice from the median plane was used for obtaining the arcuate (ARC), ventromedial and dorsomedial (V/DMH), and paraventricular (PVN) nuclei. Arcuate nucleus was excised by first cutting from just posterior to the incisura of the optic chiasm back to the mammillary body. The mammillary body was excluded from the sample. Triangular area of dorsomedial plus ventromedial hypothalamic nucleus was excised by cutting up from the optic incisura toward the central point of the thalamic area and cutting from the center down to just anterior to the border of the mammillary body. Paraventricular nucleus was excised by cutting from the antero-ventral border of the anterior commissure and the dorsal border of the suprachiasmatic nucleus down to just dorsal to the optic incisura and from dorsal border of the anterior commissure back to the center of the thalamic area.

Lateral hypothalamic nucleus (LH) and hippocampus (Hippo) were sampled from the slice just lateral to the median-most slice. Square tissue of the lateral hypothalamic nucleus was excised with vertical cut up from the optic incisura, ventral border of the thalamus and anterior border of the mammillary body. Finally, bulb-shaped hippocampus was excised (Suppl. Fig. 2).

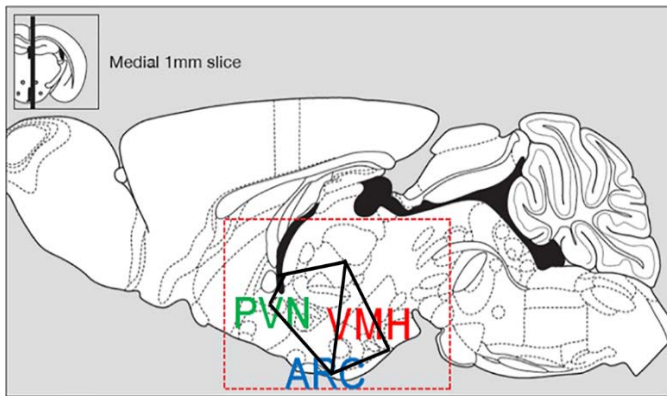
Supplementary Figure 1



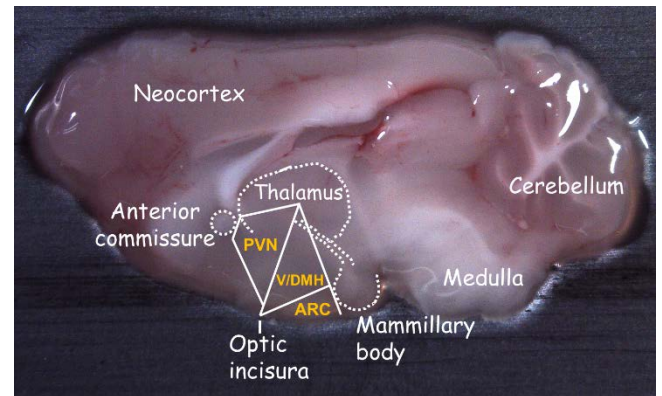
Suppl. Fig. 1. Experimental protocol. (A) Eight-week old male C57BL/6 mice were divided into two groups, fed either on a standard diet (STD) or on a high fat diet (HFD) for four weeks. Before the sampling, STD or HFD-fed animals were intraperitoneally (ip.) injected with PBS, MT-II ($0.5 \mu\text{g/g}$ body weight x four times/24hrs) or nicotine ($3 \mu\text{g/g}$ body weight x four times/24hrs). (B) Injections were performed four times consecutively with the first injection at 18:00 and with intervals of six hours. Six hours after the final injection, brain areas were immediately micro-dissected on ice. Body weight (BW) and food intake (FI) were monitored before and during injections and at the time of sacrifice.

Supplementary Figure 2

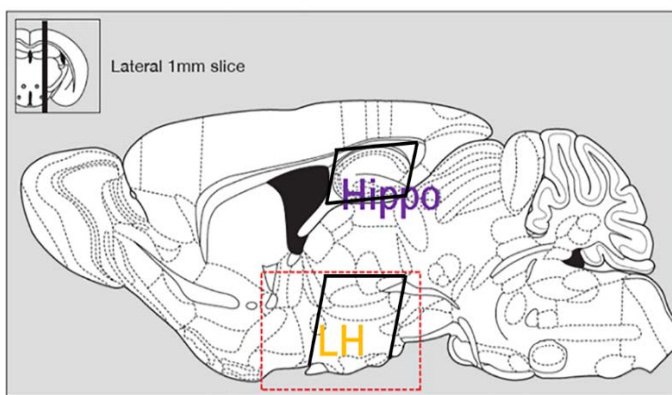
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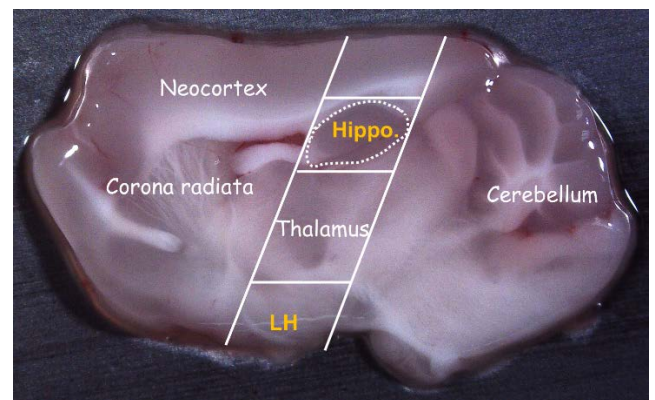
B



C

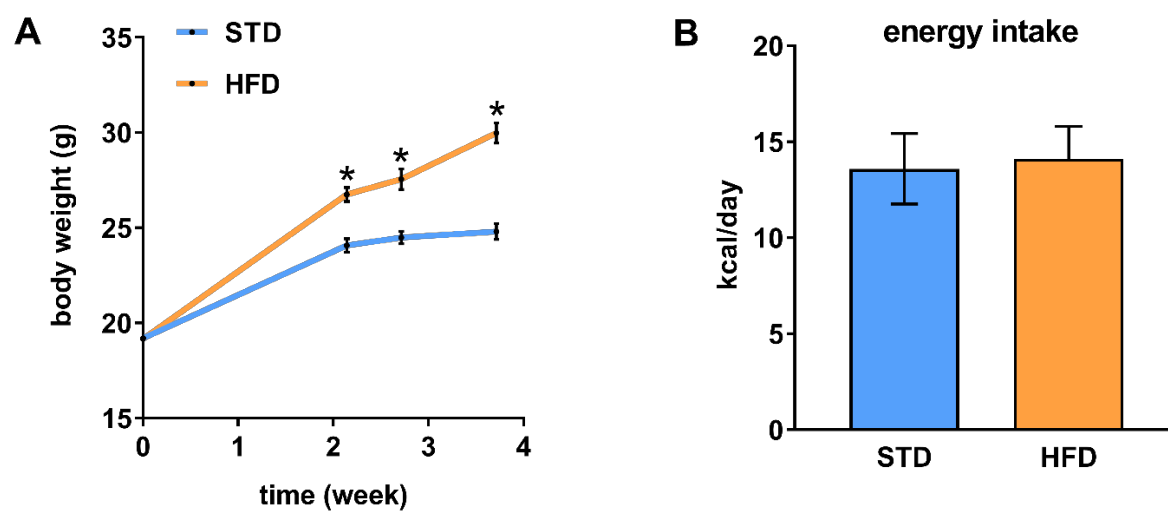


D



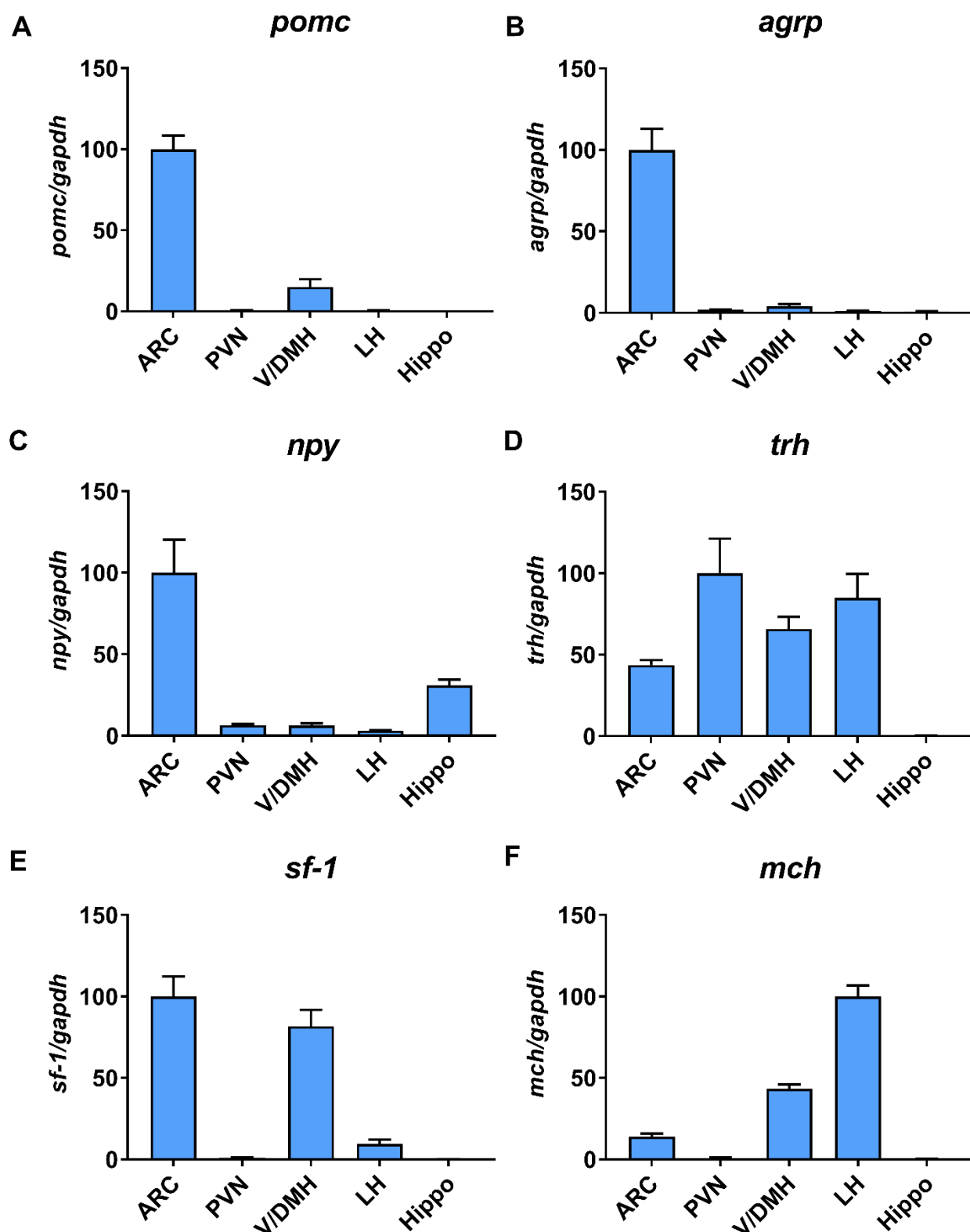
Suppl. Fig. 2. Atlas for micro-dissection. Sagittally cut 1mm-thick fresh slice from the median-most plane and 1mm lateral were prepared. Illustrations (A, C) and un-fixed brain slices (B, D) of medial (A, B) and lateral (C, D) sides are shown. Dissection under the microscope has been performed as indicated by solid lines. ARC: arcuate nucleus, PVN: paraventricular nucleus, VMH: ventromedial and dorsomedial hypothalamus (V/DMH), LH: lateral hypothalamus, Hippo: hippocampus.

Supplementary Figure 3



Suppl. Fig. 3. Effect of HFD on body weight and food intake. (A) Body weight of mice fed with STD or HFD (n=17~18). * $p < 0.05$, STD vs. HFD. (B) Baseline 24-hour energy intake of mice fed with STD or HFD (n=18).

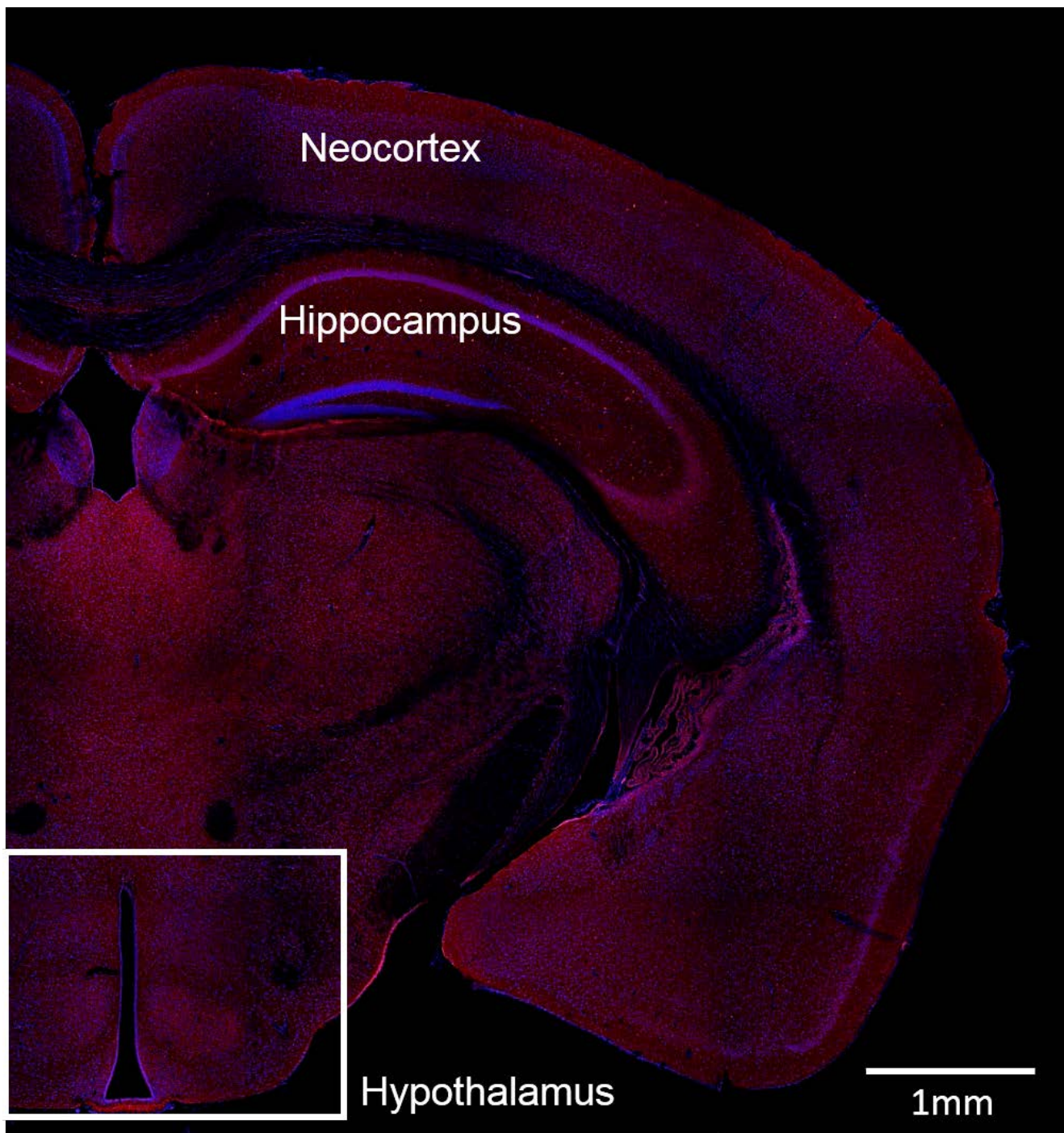
Supplementary Figure 4



Suppl. Fig. 4. Expression of signature genes in micro-dissected murine hypothalamic nuclei. Relative expression of *pomc* (A), *agrp* (B), *npv* (C), *trh* (D), *sf-1*(E) and *mch* (F) to *gapdh* in ARC, PVN, VDMH, LH and hippocampus from C57BL/6 mice (n=6). Values are shown in percentage to the nucleus with highest expression for each gene. ARC-specific neuropeptide genes, *pomc*, *agrp* and *npv* were almost exclusively expressed in ARC samples (A-C). *trh* expression was observed at highest levels in PVN and LH (4D). *sf-1* expression was observed in ARC and VDMH (E) and *mch* expression was highest in LH (F). *pomc*: pro-opio-melano-cortin gene, *agrp*: agouti related

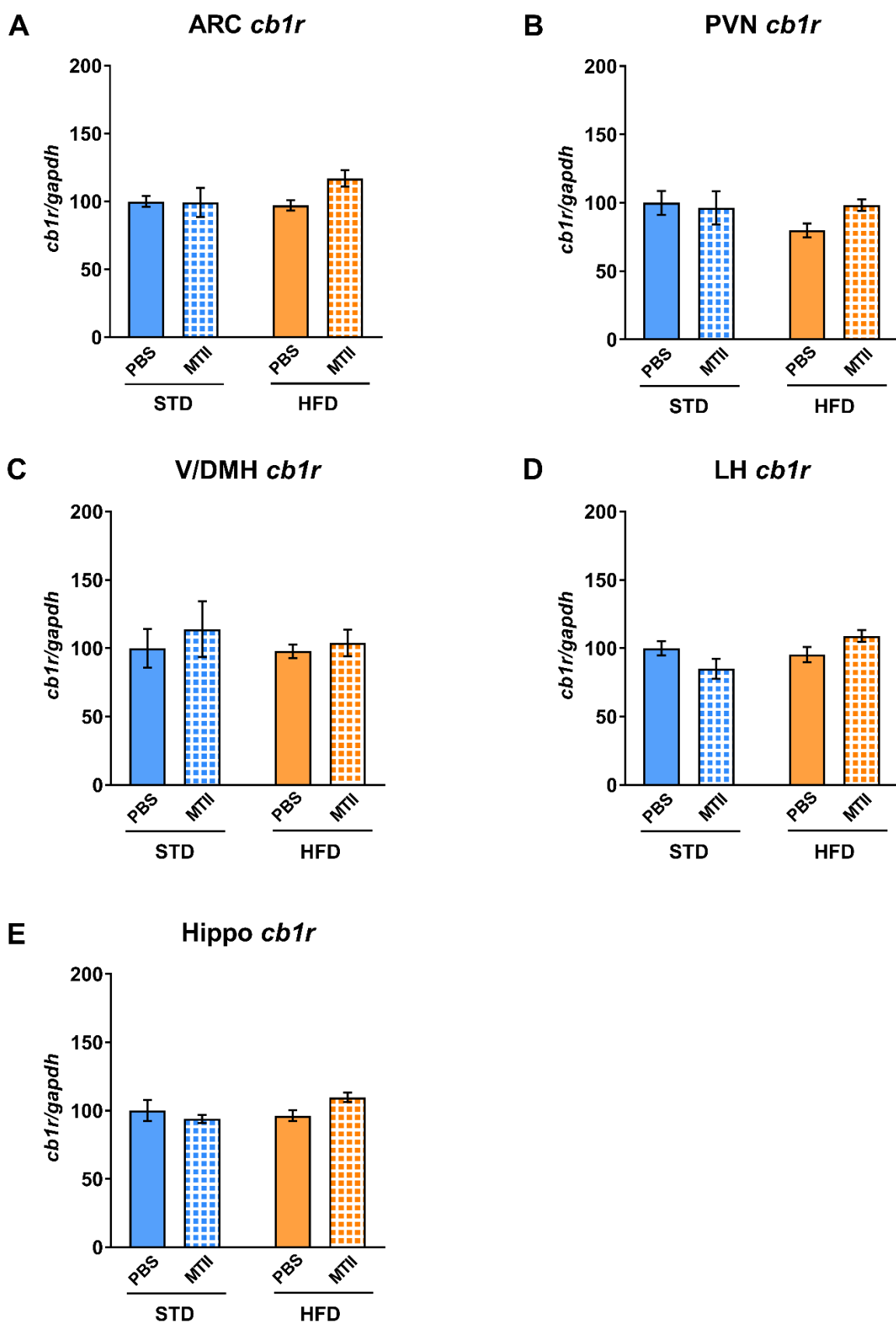
neuropeptide gene, *npv*: neuropeptide Y gene, *trh*: thyrotropin releasing hormone gene, *sf-1*: steroidogenic factor-1 gene, *mch*: melanin concentrating hormone gene.

Supplementary Figure 5



Suppl. Fig. 5. Low magnification image of the mouse brain immunostained with anti-CB1R antibody (red) and merged with nuclear staining (blue). Scale bar: 1mm.

Supplementary Figure 6



Suppl. Fig. 6. *cb1r* mRNA expression in brain areas from STD or HFD-fed mice injected either with PBS or MTII. (A) ARC, (B) PVN, (C) V/DMH, (D) LH, and (E) Hippocampus. Significant difference was not observed between PBS and MTII in either diet group or in any brain area (n=4~6). Expression levels (%) are shown in relative to STD-PBS.