

Short communication

## Dopamine transporter and D<sub>2</sub> receptor binding densities in mice prone or resistant to chronic high fat diet-induced obesity

Xu-Feng Huang<sup>a,b</sup>, Katerina Zavitsanou<sup>b</sup>, Xin Huang<sup>a</sup>, Yinghua Yu<sup>a</sup>, HongQin Wang<sup>a</sup>,  
Feng Chen<sup>c</sup>, Andrew J. Lawrence<sup>c</sup>, Chao Deng<sup>a,b,\*</sup>

<sup>a</sup> Department of Biomedical Science, University of Wollongong, NSW 2522, Australia

<sup>b</sup> Neuroscience Institute of Schizophrenia and Allied Disorders (NISAD), NSW 2010, Australia

<sup>c</sup> Howard Florey Institute, University of Melbourne, Vic. 3010, Australia

Received 23 May 2006; received in revised form 24 August 2006; accepted 28 August 2006

Available online 26 September 2006

### Abstract

This study examined the density of dopamine transporter (DAT) and D<sub>2</sub> receptors in the brains of chronic high-fat diet-induced obese (cDIO), obese-resistant (cDR) and low-fat-fed (LF) control mice. Significantly decreased DAT densities were observed in cDR mice compared to cDIO and LF mice, primarily in the nucleus accumbens, striatal and hypothalamic regions. D<sub>2</sub> receptor density was significantly lower in the rostral part of caudate putamen in cDIO mice compared to cDR and LF mice.

© 2006 Elsevier B.V. All rights reserved.

**Keywords:** Obesity; Dopamine transporter; D<sub>2</sub> receptor; High-fat diet

Dopamine regulates energy balance primarily by modulating food intake via the mesolimbic (rewarding), meso-hypothalamic (satiety), and nigro-striatal (energy expenditure) circuits of the brain [19]. The dopamine transporter (DAT) regulates synaptic dopamine concentration by reuptake of the transmitter into presynaptic terminals, thereby terminating dopaminergic neurotransmission [1]. Although a precise regulation of synaptic dopamine availability is still not fully understood, we do know that largely relies on a balance between the clearance and release rates. For example, mice with DAT knockdown have 70% elevated levels of synaptic dopamine accompanied by higher food intake [22]. However, rats fed with chow on a restricted access regimen but given access to sucrose showed an upregulation of DAT mRNA and binding but ate more than rats in the other conditions, which was suggested due to an increased dopamine release [2]. In addition, the absence of dopamine production in the dopamine knockout mouse which does not express tyrosine

hydroxylase results in an inability to initiate feeding that can be restored by gene delivery of tyrosine hydroxylase into the striatum [26].

While much is still unclear about DAT regulation, it is known that nutritional status is important. An overnight fast decreases DAT mRNA expression in the ventral tegmental area (VTA) and substantia nigra (SN), which correlates with the changes in the levels of blood insulin in rats [20]. However, restricted feeding with scheduled sucrose access results in an upregulation of VTA DAT mRNA expression and the DAT binding sites in the VTA and nucleus accumbens in rats [2]. Central administration of insulin increases the level of DAT mRNA expression in the VTA and SN [7]. Streptozotocin-induced diabetic rats have an increased DAT function for dopamine reuptake [8]. Finally in humans, sham feeding, where subjects can see food after fasting without eating, increases extracellular dopamine up to 10-fold, and effect that can be amplified by a DAT inhibitor such as methylphenidate [27]. Furthermore, drugs that block dopamine D<sub>2</sub> and D<sub>4</sub> receptors increase appetite and result in significant weight gain in both humans and experimental animals [14,15].

The role of DAT in mice resistant to chronic high-fat diet-induced obesity has not been investigated. In this study we used chronic high-fat diet-induced obese (cDIO) and obese-resistant

\* Corresponding author at: School of Health Science, University of Wollongong, Northfield Avenue, NSW 2522, Australia. Tel.: +61 2 42214934; fax: +61 2 42214096.

E-mail address: [chao@uow.edu.au](mailto:chao@uow.edu.au) (C. Deng).

(cDR) mouse models to examine if DAT responds differently in the dopaminergic mesolimbic, nigrostriatal and mesohypothalamic system in mice susceptible, or resistant, to chronic high-fat diet-induced obesity. D<sub>2</sub> receptor density was also measured in order to further clarify the role of this receptor in cDIO and cDR mice.

Thirty-five, 12-week old, C57Bl/6 male mice were obtained from the Animal Resources Center (Perth, WA, Australia). They were housed individually in environmentally controlled conditions (temperature 22 °C, light cycle from 06:00 to 18:00 h and dark cycle from 18:00 to 06:00 h), and allowed *ad libitum* access to food and water throughout the study. Mice were fed standard laboratory chow for the first week to allow them to adapt to the new environment. They were then randomized, and 28 mice were placed on a high-fat diet containing (3.8 kcal/g), 40% fat, 44% carbohydrate and 16% protein by calories that was prepared as described previously [12,23]. The remaining mice ( $n=7$ ) were placed on a low-fat diet (2.6 kcal/g, equivalent to normal laboratory-chow diet) containing 10% fat, 74% carbohydrate and 16% protein. After 6 weeks on the high-fat diet, the eight mice with the highest body weight gain were designated as the cDIO mice and the eight mice with the lowest body weight gain were designated as the cDR mice, according to the method that we and others have used previously [11,16]. These, and the seven low-fat diet (LF) control mice, were continued on their respective diets for a further 14 weeks. Twenty-four hour energy intake was measured three times during the week before the mice were killed.

Data of body weight, energy intake, and fat deposits were analyzed by a one-way ANOVA, followed by a post hoc Tukey–Kramer–HSD test and have been reported previously [12]. In brief, body weight of cDIO mice ( $46.2 \pm 1.1$  g) was significantly larger than the cDR ( $30.9 \pm 1.1$  g) and LF mice ( $28.1 \pm 0.4$  g;  $F_{2,20} = 113.400$ ,  $p < 0.001$ ; cDIO *versus* cDR,  $p < 0.001$ ; cDIO *versus* LF,  $p < 0.001$ ) after 20 weeks of dietary intervention. Body weight gain per gram of initial body weight (the ratio of BWG/IBW) was significantly higher in the cDIO ( $0.87 \pm 0.03$ ) compared to the cDR ( $0.32 \pm 0.04$ ) and LF mice ( $0.15 \pm 0.01$ ;  $F_{2,20} = 145.738$ ,  $p < 0.001$ ; cDIO *versus* cDR,  $p < 0.001$ ; cDIO *versus* LF,  $p < 0.001$ ). The visceral fat mass (the sum of epididymal+perirenal+omental fat masses) was significantly greater in the cDIO ( $6.0 \pm 0.3$  g) group compared to the cDR ( $2.2 \pm 0.4$  g) and LF mice ( $1.0 \pm 0.1$  g;  $F_{2,20} = 102.080$ ,  $p < 0.001$ ; cDIO *versus* cDR,  $p < 0.001$ ; cDIO *versus* LF,  $p < 0.001$ ). Twenty-four hour energy intake of cDIO mice ( $19.7 \pm 1.6$  kcal/mouse/day) was also greater than in the LF ( $15.6 \pm 1.1$  kcal/mouse/day) and cDR ( $15.1 \pm 0.5$  kcal/mouse/day;  $F_{2,20} = 4.68$ ,  $p = 0.023$ ; cDIO *versus* cDR,  $p = 0.058$ ; cDIO *versus* LF,  $p = 0.031$ ) mice, respectively.

The mice were killed with an overdose of sodium pentobarbitone anesthesia (120 mg/kg, i.p.) after a total of 20 weeks of dietary treatment. All mice were killed between 07:00 and 09:00 h in order to minimize circadian variation. Brains were immediately removed after death and frozen in liquid nitrogen. Four brains from each group were cut (14  $\mu$ m) at  $-17$  °C with a cryostat for binding experiments.

Binding of [<sup>125</sup>I]RTI-55 was used to assess binding density for DAT [17]. Sections were allowed to defrost, and then preincubated for 30 min in phosphate buffer (10 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.1 M sucrose, pH 7.4) at room temperature. The binding sites of DAT were defined with 50 pM [<sup>125</sup>I]RTI-55 in the presence of 50 nM fluoxetine to prevent binding to serotonin transporters. Nonspecific binding was determined with 10  $\mu$ M GBR 12909. Following incubation for 1 h at room temperature, slides were washed (1  $\times$  1 min, then 2  $\times$  20 min) in ice-cold buffer, followed by one dip in ice-cold distilled water and dried under a gentle stream of cool air.

Binding to D<sub>2</sub> receptor was visualized using [<sup>3</sup>H]Raclopride as previously described [5]. Sections were preincubated at room temperature for 1 h in buffer solution containing 50 mM Tris–HCl, 120 mM NaCl, 2 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, pH 7.4 and then incubated for 1 h at room temperature in fresh buffer in the presence of 5 nM [<sup>3</sup>H]Raclopride (76.80 Ci/mmol). Nonspecific binding was determined in the presence of 10  $\mu$ M butaclamol. Sections were washed 2  $\times$  5 min in ice-cold buffer, briefly dipped in ice-cold distilled water and dried under a stream of cold air.

Slides were stored overnight in desiccators, and then apposed for 6 h (for [<sup>125</sup>I]RTI-55 binding) to 12 weeks (for [<sup>3</sup>H]Raclopride binding) to Kodak X-OMAT AR film in the presence of standard microscalers. Autoradiographs were developed using Kodak D-19 developer and fixed with Ilford Hypam Rapid Fixer. Autoradiographic images were captured and analyzed using a computer-assisted image analysis system, Multi-Analysis, connected to a GS-690 Imaging Densitometer (Bio-Rad, USA). The density of binding was calculated by converting the optical density of the image to dpm/mm<sup>2</sup> with the aid of a standard curve generated with calibrated microscalers, as previously described [4]. Individual brain nuclei were identified with reference to a standard mouse brain atlas [21]. The striatum was divided into rostral (Interaural: 5.34–4.42 mm), intermediate (Interaural: 4.42–3.70 mm) and caudal (Interaural: 3.70–2.98 mm) according to the method used previously [5]. Binding data for each brain area were analyzed by a Kruskal–Wallis test, followed by a Mann–Whitney *U*-test (one-tailed) using the SPSS 11.5 program (Chicago, IL).

The DAT binding sites were widely distributed in the mouse brain but predominantly found in subcortical structures including mesolimbic, striatal and hypothalamic systems. A consistent pattern was found of lower DAT binding densities in the cDR mice compared to both the cDIO (Fig. 1) and LF groups. Regions of note where cDR mice had significantly lower DAT densities than the LF mice were the nucleus accumbens (cDR  $292.4 \pm 25.5$  *versus* LF  $606.2 \pm 55.4$  dpm/mm<sup>2</sup>;  $U_{4,4} = 0$ ,  $p = 0.014$ ), bed nucleus of stria terminalis (cDR  $58.1 \pm 28.1$  *versus* LF  $140.4 \pm 32.4$ ;  $U_{4,4} = 1$ ,  $p = 0.028$ ), dorsomedial hypothalamic nucleus (cDR  $137.1 \pm 35.8$  *versus* LF  $252.7 \pm 49.3$ ;  $U_{4,4} = 1$ ,  $p = 0.028$ ), and olfactory tubercle nucleus (cDR  $241.8 \pm 20.6$  *versus* LF  $506.7 \pm 46.5$ ;  $U_{4,4} = 0$ ,  $p = 0.014$ ). Moreover, the cDR mice had significantly reduced DAT densities than the cDIO mice in the amygdaloid complex (cDR  $46.0 \pm 26.9$  *versus* cDIO  $155.7 \pm 14.6$ ;  $U_{4,4} = 1$ ,  $p = 0.028$ ), globus pallidus external (cDR

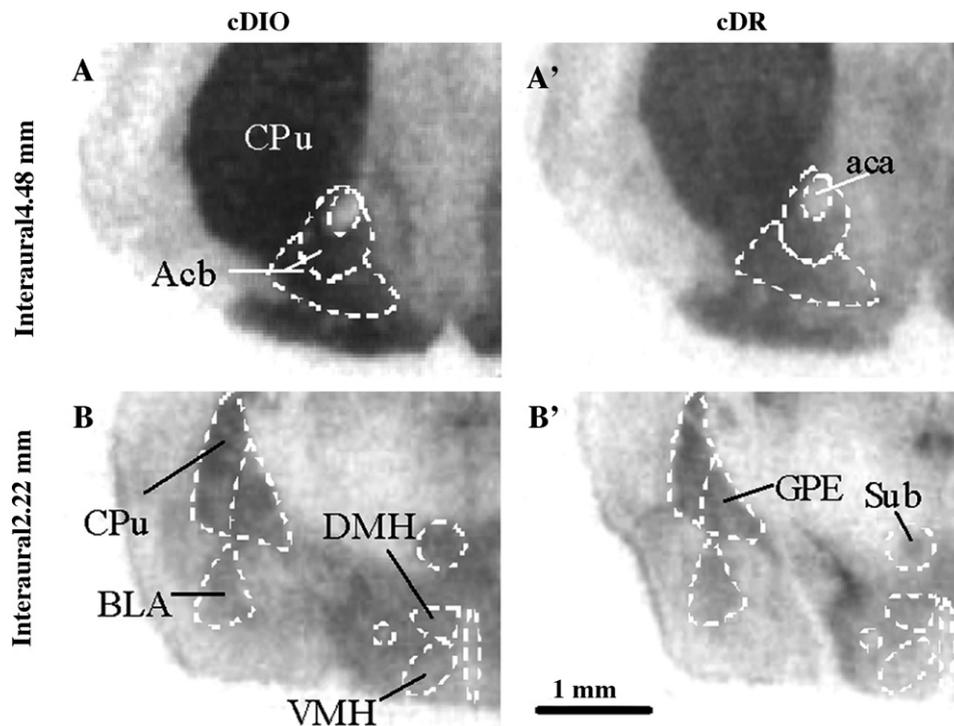


Fig. 1. Photographs depict the bindings of dopamine transporter in the brains of chronic high-fat diet-induced obese (cDIO; A and B) and obese-resistant (cDR; A' and B') mice. Note a higher binding density in the rostral caudate putamen (CPu) of the cDIO (A) comparing to cDR (A') mice. Abbreviations—aca: anterior part of anterior commissure; Acb: nucleus accumbens; BLA: anterior region of the basolateral part of amygdala; DMH: dorsomedial hypothalamic nucleus; GPE: external part of the globus pallidus; Sub: submedial thalamic nucleus; VMH: ventromedial hypothalamic nucleus.

126.3 ± 27.7 versus cDIO 233.1 ± 25.1;  $U_{4,4} = 1$ ,  $p = 0.028$ ), dorsomedial hypothalamic nucleus (cDR 137.1 ± 35.8 versus cDIO 259.7 ± 10.2;  $U_{4,4} = 1$ ,  $p = 0.028$ ), and ventromedial hypothalamic nucleus (cDR 112.8 ± 27.3 versus cDIO 208.0 ± 11.4;  $U_{4,4} = 1$ ,  $p = 0.028$ ). In the striatum, only the rostral part of the striatum (caudate putamen) had a significant reduction in DAT density in the cDR mice compared to the LF mice (cDR 505.1 ± 13.8 versus LF 667.5 ± 49.4;  $U_{4,4} = 1$ ,  $p = 0.028$ ; Fig. 1A and A'). Furthermore, DAT binding densities across brain regions were strikingly similar between the cDIO and LF groups.

[<sup>3</sup>H]Raclopride binding sites were primarily located in the striatum, nucleus accumbens and olfactory tubercle nucleus of mouse brain. The cDIO mice had significantly lower D<sub>2</sub> receptor density in the rostral part of the caudate putamen than the cDR and LF mice (cDR 88.9 ± 2.7 versus cDIO 109.1 ± 6.5,  $U_{3,3} = 0$ ,  $p = 0.05$ ; cDIO 88.9 ± 2.7 versus LF 110.1 ± 6.0,  $U_{3,3} = 0$ ,  $p = 0.05$ ). No significant difference was found in the intermediate and caudal parts of the striatum between the three groups. In the nucleus accumbens and olfactory tubercle, there were no significant differences in D<sub>2</sub> receptor density between the cDIO, cDR and LF groups.

This study compared DAT and D<sub>2</sub> receptor densities in chronic high-fat diet-induced obese (cDIO), obese-resistant (cDR) and low-fat-fed (LF) control mice. It was found that DAT binding sites were widely distributed in the mouse brain. A striking pattern emerged from the results that cDR mice had consistently lower DAT densities than cDIO and LF mice with

the major differences primarily found in the nucleus accumbens, striatal and hypothalamic regions.

The Acb is a dopamine rich region situated ventrally at the head of the striatum. It is a key structure of the mesolimbic dopamine system (the VTA projects to Acb) and relevant to food reward [29]. Eating is a highly reinforcing behavior that not only provides nutrients needed for survival, but induces feelings of gratification and pleasure [10]. This study found that cDR mice had significantly lower energy intake of a palatable food than cDIO mice. High energy intake induced obesity may reflect a disorder in rewarding system that could be due in part to abnormalities of dopamine transmission [3]. Feeding increases dopamine release in the Acb [30]. Lean rats have lower dopamine release than the obese rats [19]. Therefore, it is likely that cDR mice may have a reduced dopamine response to a palatable high-fat diet compared to cDIO mice. Our early studies found that cDR mice had a lower level of tyrosine hydroxylase mRNA expression [13], the rate-limiting enzyme in catecholamine synthesis, in the ventral tegmental area than cDIO mice. Therefore, low levels of dopaminergic VTA-Acb projection might have contributed to the cDR mice resistant to high-fat diet induced obesity compared to the cDIO mice.

This study systematically investigated the DAT and D<sub>2</sub> receptor densities in all compartments of the caudate putamen in the brains of the cDIO, cDR and LF mice. The significant differences were only found in the rostral part, but not in the intermediate and caudal parts, suggesting the existence of functional differ-

entiation between the different parts of the caudate putamen in the development of chronic high-energy diet-induced obesity. Studies have shown significant alterations of dopamine transmission in obese humans. Obese individuals (body mass index  $>40\text{ kg m}^{-2}$ ) appear to have a negative correlation between their body mass index and striatal  $\text{D}_2$  receptor availability, that is, the greater the obesity, the lower the receptor availability [28]. A similar findings have been reported in rats [9]. The present study found a significant reduction of  $\text{D}_2$  receptor density in the rostral part of the striatum of the diet-induced obese mice, which is consistent with those early reports. However, increased expression of  $\text{D}_2$  receptor mRNA has been found in the core of the nucleus accumbens and ventral part of caudal putamen of obese mice in our previous study [13]. The increased  $\text{D}_2$  receptor mRNA expression in the obese mice may be a compensatory response to the decreased activation of the  $\text{D}_2$  receptor pathway induced by a high food intake in the cDIO mice.

Finally on this issue, it has been reported that clearance of synaptic dopamine may be a combined effect of DATs and the presynaptic  $\text{D}_2$  autoreceptor. Studies have demonstrated that the mice with presynaptic  $\text{D}_2$  receptor deficiency have an increased DAT function without increased DAT binding  $B_{\text{max}}$  or  $K_{\text{d}}$  [6]. Since cDIO mice have both decreased  $\text{D}_2$  receptor binding sites and higher DAT density than cDR mice this may together promote the clearance of synaptic dopamine as has been reported previously [24], accounting at least in part of the increased food intake and fat accumulation.

Spontaneous eating rapidly but transiently increases dopamine release in the VMH of lean and obese rats. This effect is more pronounced in obese rats consuming larger meals [19]. Furthermore, a single injection of dopamine into the VMH of freely moving rats has been shown to increase meal size [19]. Enhanced release of VMH dopamine stimulates food intake [25]. On the other hand, when rats are deprived of food for 24 h, food intake brings about an immediate decrease of dopamine release to 65% of pre-eating levels in both lean and obese rats [18]. The present study showed that cDR mice had lower energy intake accompanied by low DAT densities in the VMH compared to cDIO mice, suggesting a possible low dopamine content in the VMH.

In summary, the current study has provided new information in assisting for the understanding of the neuronal basis of predisposition, or resistance, to diet-induced obesity. Dopamine transporters and  $\text{D}_2$  receptors may play an important role in contributing to the phenotypic variation. In particular, cDR mice apparently can maintain reduced levels of DAT density in the mesolimbic, nigrostriatal and hypothalamic systems in the face of palatable, high energy dense food, which may contribute to the prevention of chronic high-energy diet-induced obesity in such animals.

## Acknowledgments

This study was supported by grants from the National Health and Medical Research Council of Australia, and AstraZeneca, Mölndal, Sweden.

## References

- [1] Amara SG, Kuhar MJ. Neurotransmitter transporters: recent progress. *Annu Rev Neurosci* 1993;16:73–93.
- [2] Bello NT, Sweigart KL, Lakoski JM, Norgren R, Hajnal A. Restricted feeding with scheduled sucrose access results in an upregulation of the rat dopamine transporter. *Am J Physiol-Regul Integr Comp Physiol* 2003;284:R1260–8.
- [3] Blum K, Braverman ER, Holder JM, Lubar JF, Monastra VJ, Miller D, et al. Reward deficiency syndrome: a biogenetic model for the diagnosis and treatment of impulsive, addictive, and compulsive behaviors. *J Psychoactive Drugs* 2000;32(Suppl):i–iv.
- [4] Chen F, Lawrence AJ. 5-HT transporter sites and 5-HT1a and 5-HT3 receptors in fawn-hooded rats: a quantitative autoradiography study. *Alcoholism: Clin Exp Res* 2000;1093–102.
- [5] Delis F, Mitsacos A, Giompres P. Dopamine receptor and transporter levels are altered in the brain of Purkinje Cell Degeneration mutant mice. *Neuroscience* 2004;125:255–68.
- [6] Dickinson SD, Sabeti J, Larson GA, Giardina K, Rubinstein M, Kelly MA, et al. Dopamine  $\text{D}_2$  receptor-deficient mice exhibit decreased dopamine transporter function but no changes in dopamine release in dorsal striatum. *J Neurochem* 1999;72:148–56.
- [7] Figlewicz DP, Szot P, Chavez M, Woods SC, Veith RC. Intraventricular insulin increases dopamine transporter mRNA in rat VTA/substantia nigra. *Brain Res* 1994;644:331–4.
- [8] Galici R, Galli A, Jones DJ, Sanchez TA, Saunders C, Frazer A, et al. Selective decreases in amphetamine self-administration and regulation of dopamine transporter function in diabetic rats. *Neuroendocrinology* 2003;77:132–40.
- [9] Hamdi A, Porter J, Chandan Prasad. Decreased striatal  $\text{D}_2$  dopamine receptors in obese Zucker rats: changes during aging. *Brain Res* 1992;589:338–40.
- [10] Hoebel BG. Brain neurotransmitters in food and drug reward. *Am J Clin Nutr* 1985;42:1133–50.
- [11] Huang XF, Han M, Storlien L. The level of NPY receptor mRNA expression in diet-induced obese and resistant mice. *Mol Brain Res* 2003; 21–8.
- [12] Huang X-F, Huang X, Han M, Chen F, Storlien L, Lawrence AJ. 5-HT2A/2C receptor and 5-HT transporter densities in mice prone or resistant to chronic high-fat diet-induced obesity: a quantitative autoradiography study. *Brain Res* 2004;1018:227–35.
- [13] Huang XF, Yu Y, Zavitsanos K, Han M, Storlien L. Differential expression of dopamine  $\text{D}_2$  and  $\text{D}_4$  receptor and tyrosine hydroxylase mRNA in mice prone, or resistant, to chronic high-fat diet-induced obesity. *Brain Res Mol Brain Res* 2005;135:150–61.
- [14] Kaur G, Kulkarni SK. Studies on modulation of feeding behavior by atypical antipsychotics in female mice. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2002;26:277–85.
- [15] Lee MD, Clifton PG. Meal patterns of free feeding rats treated with clozapine, olanzapine, or haloperidol. *Pharmacol Biochem Behav* 2002;71:147–54.
- [16] Levin BE, Keeseey RE. Defense of differing body weight set points in diet-induced obese and resistant rats. *Am J Physiol* 1998;274: R412–9.
- [17] McGregor IS, Clemens KJ, Van der Plasse G, Li KM, Hunt GE, Chen F, et al. Increased anxiety 3 months after brief exposure to MDMA (“Ecstasy”) in rats: association with altered 5-HT transporter and receptor density. *Neuropsychopharmacology* 2003;28: 1472–84.
- [18] Meguid MM, Fetissov SO, Blaha V, Yang ZJ. Dopamine and serotonin VMH release is related to feeding status in obese and lean Zucker rats. *Neuroreport* 2000:2069.
- [19] Meguid MM, Fetissov SO, Varma M, Sato T, Zhang L, Laviano A, et al. Hypothalamic dopamine and serotonin in the regulation of food intake. *Nutrition* 2000;16:843–57.
- [20] Patterson TA, Brot MD, Zavosh A, Schenk JO, Szot P, Figlewicz DP. Food deprivation decreases mRNA and activity of the rat dopamine transporter. *Neuroendocrinology* 1998;68:11–20.

- [21] Paxinos G, Franklin KBJ. The mouse brain in stereotaxic coordinates. San Diego: Academic Press; 2002.
- [22] Pecina S, Cagniard B, Berridge KC, Aldridge JW, Zhuang X. Hyperdopaminergic mutant mice have higher “wanting” but not “liking” for sweet rewards. *J Neurosci* 2003;23:9395–402.
- [23] Reeves PG, Forrest H, Nielsen H, Fahey GC. AIN-93 Purified diets for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc writing committee on the reformulation of the AIN-76A Rodent diet. *J Nutr* 1993;123:1939–51.
- [24] Shimizu H, Shimomura Y, Takahashi M, Uehara Y, Fukatsu A, Sato N. Altered ambulatory activity and related brain monoamine metabolism in genetically obese Zucker rats. *Exp Clin Endocrinol* 1991;97:39–44.
- [25] Szczypka MP, Rainey MA, Palmiter RD. Dopamine is required for hyperphagia in *lep(ob/ob)* mice. *Nat Genet* 2000:102.
- [26] Szczypka MS, Mandel RJ, donahue BA. viral gene delivery seectively restores feeding and prevents lethality of dopamine-deficient mice. *Neuron* 1999:167.
- [27] Volkow ND, Wang GJ, Fowler JS, Logan J, Jayne M, Franceschi D, et al. Nonhedonic” food motivation in humans involves dopamine in the dorsal striatum and methylphenidate amplifies this effect. *Synapse* 2002;44:175–80.
- [28] Wang G-J, Volkow ND, Logan J, Pappas NR, Wong CT, Zhu W, et al. Brain dopamine and obesity. *Lancet* 2001;357:354–7.
- [29] Wise RA, Rompre PP. Brain dopamine and reward. *Annu Rev Psychol* 1989;40:191–225.
- [30] Yoshida M, Yokoo H, Mizoguchi K, Kawahara H, Tsuda A, Nishikawa T, et al. Eating and drinking cause increased dopamine release in the nucleus accumbens and ventral tegmental area in the rat: measurement by in vivo microdialysis. *Neurosci Lett* 1992;139:73–6.