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Focus on Neuropeptide Y

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Chapter X.

Pilot study: Predicting striatal DRD_{2/3} availability from the inhibitory effect of dexamphetamine on feeding

Abstract

The mesolimbic dopamine system is altered in obesity, though the exact changes and factors contributing to these changes are not clear. Our lab has shown that dietary factors can affect the striatal availability of the dopamine $D_{2/3}$ receptor ($DRD_{2/3}$). Indeed, $DRD_{2/3}$ availability decreases in the ventral striatum when rats consume at least 21% of daily intake from fat when they have access to an obesogenic free-choice high-fat high-sucrose (fCHFHS) diet. Also, a pilot study in our lab indicated an association between fat intake and striatal $DRD_{2/3}$ availability, and the possibility to predict striatal $DRD_{2/3}$ availability from amphetamine (AMPH)-induced food intake suppression. This procedure is less invasive than other methods, and abolishes the use of radiopharmaceuticals to determine $DRD_{2/3}$ availability.

Therefore, our aim is to determine if AMPH-induced inhibition of food intake can predict ventral striatal $DRD_{2/3}$ availability in rats fed a fCHFHS-diet or in CHOW-fed control rats. After four weeks of diet consumption, AMPH (0.5 mg/kg in 1 ml/kg) or saline was injected intraperitoneal in a randomized crossover design and food intake was measured 5 hours later. One week later, striatal $DRD_{2/3}$ binding was measured using ^{123}I -IBZM storage phosphor imaging.

Like in our previous study, the fCHFHS group was split in fCHFHS-hf (>21% intake from fat) and fCHFHS-lf (<21% intake from fat) groups. Dorsal and ventral striatal $DRD_{2/3}$ availability was comparable between the three diet groups. Furthermore, in the CHOW-fed group, AMPH-induced caloric intake suppression could predict dorsal striatal $DRD_{2/3}$ ($R^2 = 0.44$), whereas it could not in the fCHFHS-group.

It is thus possible to predict dorsal striatal $DRD_{2/3}$ binding from AMPH-induced intake suppression in CHOW-fed control rats, but not in fCHFHS-fed rats. These data indicate that even if $DRD_{2/3}$ availability is similar after consumption of an obesogenic or control diet, the functionality of the dopamine system is altered after four weeks consumption of a fCHFHS-fed diet, such that the coupling between behavioral response and dorsal striatal $DRD_{2/3}$ availability has disappeared.

Introduction

Diet plays an important role in the etiology of obesity. Consumption of palatable, energy-dense food, enriched with fats and sugars, can dysregulate peripheral and central processes involved in energy homeostasis, promoting overconsumption and the subsequent development of obesity. The mesolimbic dopamine system of the brain, with dopamine cell bodies in the ventral tegmental area (VTA) that release dopamine in the striatum, is a key component of the reward-related brain circuitry and mediates feeding-related motivational behavior (Berridge, 2007; Hernandez & Hoebel, 1988; Leigh & Morris, 2018; Meye & Adan, 2014; Wise, 2004). Importantly, studies have repeatedly shown changes in the function of the dopamine system that could contribute to disturbed feeding such as in obesity (Kenny, 2011b; Volkow et al., 2011). In particular, changes in striatal dopamine receptor D_{2/3} (DRD_{2/3}) signaling are implicated in the etiology and maintenance of obesity (van Galen et al., 2018). However, the underlying causes leading to alterations in dopamine receptor levels have not been elucidated as of yet. Interestingly, rodent studies have indicated that striatal DRD_{2/3} availability is affected by dietary factors; we have shown that dietary factors such as choice and composition of the diet affect striatal DRD_{2/3} availability. In rats that have access to a high-fat diet, the option of choice as opposed to a pelleted high-fat diet, leads to decreased dorsal striatal DRD_{2/3} availability (van de Giessen et al., 2012). Rats that consume an obesogenic diet that also contains sucrose (i.e. the free-choice high-fat high-sugar [fchFHS] diet, which has a high clinical validity [la Fleur et al., 2007; Slomp et al., 2019]), have decreased ventral striatal DRD_{2/3} availability after four weeks diet consumption, but only when they consume at least 21% fat on a daily basis. Interestingly, a pilot experiment showed that the degree of fat intake on an obesogenic choice diet correlated with the inhibition of food intake after injection of the dopamine releaser dexamphetamine (AMPH) prior to diet exposure. Rats that decreased food intake less after AMPH injection, subsequently consumed more fat when on a fchF diet (Supplemental Figure 1). These data point to an association of fat intake with ventral striatal DRD_{2/3} availability, and the possibility to predict intake or striatal DRD_{2/3} availability from the behavioral response to AMPH, a procedure that is less invasive and time-consuming than other methods to assess DRD_{2/3} availability, and abolishes the use of radiopharmaceuticals.

In this study, we therefore investigated if AMPH-induced inhibition of caloric intake can predict ventral striatal DRD_{2/3} availability in rats fed a fchFHS-diet for four weeks or CHOW-fed control rats. Rats were put on a fchFHS diet, consisting of access to chow, a 30% sucrose solution, a dish of fat, and a bottle of water (la Fleur et al., 2007) or a CHOW-diet, consisting of access to chow and a bottle of water. After four weeks of diet consumption, rats were injected with a low dose of AMPH or saline in a crossover design and food intake measured five hours later. One week later, to abolish direct effects of the dopamine releaser

AMPH on dopamine receptor levels (Habraken et al., 2001; Jongen, de Bruin, Beekman, & Booij, 2008; Nikolaus et al., 2005; Schranter et al., 2015), DRD_{2/3} binding levels were determined using the well-validated radiotracer ¹²³I-IBZM (iodobenzamide) and storage phosphor imaging.

Experimental procedures

Animals

Twenty-eight male Wistar rats (Horst, Harlan, The Netherlands) were individually housed in a temperature- (19-23 °C) and light-controlled room (lights on-lights off: 06:00-18:00). All experimental procedures were approved by the Animal Ethics Committee of the Amsterdam UMC, location Academic Medical Center of the University of Amsterdam (The Netherlands).

Procedures

The control CHOW group (N = 14; 300-330 g at arrival) received a low caloric diet consisting of *ad libitum* chow (Special Diets Services, Witham, Essex, England) and tap water. The free choice high-fat high-sugar (fcHFHS) group (N = 14; 270-300 g at arrival) received a free choice high-fat high-sugar diet (la Fleur et al., 2007; Slomp et al., 2019) consisting of *ad libitum* access to a dish of saturated fat (beef tallow, Ossewit/Blanc de Boeuf, Vandemoortele, Belgium), a bottle of 30% w/v sugar water (300 grams commercial grade sugar in 1 L water) in addition to the low-caloric chow diet and tap water. Due to ethical considerations, the control group arrived after the experimental diet group had had three weeks access to the fcHFHS diet components. Animals were assigned to a diet group upon arrival. Body weight and daily caloric intake (chow: 3.31 kcal/g, fat: 9 kcal/g, sucrose solution: 1.2 kcal/g) were monitored five times a week. All food components were refreshed twice a week.

After 4 weeks (fcHFHS group) or 1 week (CHOW group) consumption of their respective diets, intraperitoneal (i.p.) injections of saline were administered at the beginning of the light phase to habituate the animals to the procedure. Injections were administered every other day for three days. One week later, dexamphetamine sulphate (AMPH; dosis: 0.5 mg/kg in 1 ml/kg; Spruyt Hillen BV, IJsselstein, The Netherlands) or 0.9% NaCl (1 mL/kg) was administered intraperitoneal in a balanced cross-over design with two days between injection days (see Figure 1 for an overview of the experimental setup). Prior to the injection, rats were food-restricted overnight; all food components were taken out of the cage at the end of the light period at 17.00, and rats received 10 grams of chow and water *ad libitum*. AMPH was injected at the beginning of the light phase (at 09:00). Food components were replaced immediately after injection and intake measured 5 hours later.

DRD_{2/3} binding measurement

The administration of AMPH induces a massive acute release of endogenous dopamine, which consequently may induce lower binding of the radiotracer ¹²³I-IBZM to DRD_{2/3}. To prevent such direct pharmacological effects, DRD_{2/3} binding measurements were performed one week after the injections as described before (van de Giessen et al., 2013). Rats were anesthetized at the end of the light period (16:00) with intramuscular ketamine/xylazine followed by intravenous administration of approximately 37MBq (1 mCi) of the selective DRD_{2/3} tracer ¹²³I-IBZM (0.2 mL; GE Healthcare, Eindhoven, The Netherlands). Ninety minutes later (Jongen et al., 2008), animals were sacrificed by bleeding through heart puncture under anesthesia. Mesenteric, and bilateral epididymal and perirenal fat pads were excised and weighed, and brains were removed, frozen on dry ice and sliced horizontally into 50 μm slices in a microtome cryostat at -21 °C. Storage phosphor imaging was performed as described previously (Crunelle, Miller, de Bruin, van den Brink, & Booij, 2009). One in four slices containing the dorsal and ventral striatum was exposed to a Fuji BAS-MS IP for approximately 16 hours and images were scanned using a Fuji FLA-3000 phospho imager. Regions of interest (ROIs) were drawn for the dorsal and ventral striatum, left and right. ROIs drawn for the cerebellum were used to assess non-specific binding (Crunelle et al., 2009; van de Giessen et al., 2012). Ratios of striatum-to-cerebellum binding were obtained by dividing the average uptake per pixel of combined left and right dorsal striatal or ventral striatal parts by the average uptake per pixel of the cerebellum.

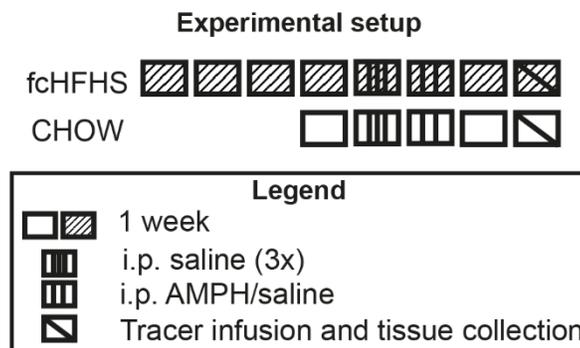


Figure 1. Experimental setup. Rats had access to a free-choice high-fat high-sugar (fcHFHS) diet for 4 weeks or a control (CHOW) diet for 1 week. Subsequently, 1 ml/kg saline was injected intraperitoneal (i.p.) to habituate animals to the procedure. Injections were administered every other day for three days. In the following week, dexamphetamine sulphate (AMPH; dose 0.5 mg/kg in 1mg/kg) or 0.9 % saline (1 mg/kg) was injected i.p. in a balanced crossover design with two days between injection days, and food intake measured at 5 hours after infusion. Animals were food-restricted overnight before each injection. After one week washout, animals were sedated, ¹²³I-IBZM radiotracer was infused and brains collected for storage phosphor imaging. See text for details.

Statistical analyses

In this study, three subgroups (consisting of a fCHFHS group with its own CHOW control group, together N = 9 or N = 10, and totalling N = 28) were started at different time points due to logistical reasons that limit the maximal number of samples that can be processed at the same time. All parameters concerning body weight and feeding behaviour were comparable between the different control groups. Therefore, the data are combined together. As the imaging analysis is more prone to variation between studies, we standardized all imaging measurements on the average of the chow controls, which was set at 100%. Subsequently, as DRD_{2/3} binding can depend on dietary preference in fCHFHS-fed rats (van de Giessen et al., 2013), the fCHFHS-fed group was split into a fCHFHS-hf (>21% fat of daily intake) and a fCHFHS-lf (<21% fat of daily intake) group based on van de Giessen et al. (2013), and all data, except for correlation analyses, are presented for the fCHFHS-hf, fCHFHS-lf, and CHOW-fed groups separately.

For the ventral striatum, several samples were lost during the slicing procedure. Response to AMPH was calculated in percentages according to $\text{Intake}_{\text{AMPH}}/\text{Intake}_{\text{veh}} * 100\%$. Results are reported for the standardized data and as mean \pm SEM. A linear regression was calculated to predict DRD_{2/3} binding based on AMPH-induced intake inhibition. All group differences were analyzed using a one-way ANOVA followed by Sidak's multiple comparisons test, or a Mixed-effects model followed by uncorrected Fisher's LSD tests. All statistical analyses were performed using Graphpad Prism version 8.0.2.

Results

Intake and adiposity measures

Total daily caloric intake differed between the groups (One-way ANOVA $F_{2,25} = 2.96$, $p < 0.0001$). *Post hoc* tests revealed that intake was higher in the fCHFHS-hf and fCHFHS-lf groups compared to the CHOW-fed group (both $p < 0.0001$). No difference was revealed between intake of the fCHFHS-hf and fCHFHS-lf groups ($p > 0.05$). fCHFHS-hf and fCHFHS-lf rats consumed comparable chow as percentage of total daily caloric intake ($t_{12} = 1.59$, $p > 0.05$). Intake of sucrose water ($t_{12} = 3.31$, $p = 0.006$) and fat ($t_{12} = 4.49$, $p = 0.0007$) as percentage of total daily caloric intake differed for the fCHFHS-hf and fCHFHS-lf rats. In accordance, body weight gain (end body weight as a percentage of body weight at the start of the experiment) was significantly higher in the fCHFHS-hf and fCHFHS-lf groups compared to the CHOW-fed group (main effect $F_{2,25} = 78.19$, $p < 0.0001$, *post hoc*s both $p < 0.0001$), but not different between the fCHFHS-hf and fCHFHS-lf groups (*post hoc*; $p > 0.05$). Fat mass as a percentage of body weight was also increased in the fCHFHS-hf and fCHFHS-lf groups compared to the CHOW-fed group: mesenteric fat (main effect $F_{2,25} = 3.979$, $p = 0.0009$; *post hoc*s both $p < 0.01$), epididymal fat (main effect $F_{2,25} = 11.21$, $p = 0.0003$; *post hoc*s both $p < 0.01$), and

perirenal fat (main effect $F_{2,25} = 6.66$, $p = 0.005$; *post hoc*s both $p < 0.05$). Fat mass as a percentage of body weight was not different between the fCHFHS-hf and fCHFHS-lf groups (all *post hoc*s $p > 0.05$). See Table 1 for an overview.

Table 1. Intake and adiposity measures per group.

	Intake/day [¶] (kcal)	Component (%) chow/sucrose/fat	Total BW gain [†] (%)	Fat mass/100 gr BW
CHOW (N = 14)	88 ± 1.4	na	121 ± 0.7	Mes. 1.32 ± 0.06 Epi. 1.29 ± 0.08 Per. 0.29 ± 0.02
fCHFHS-hf (N = 6)	110 ± 1.4*	57.6 ± 2.6 12.7 ± 2.3 [°] 31.9 ± 2.9 [°]	161 ± 6.8*	Mes. 2.37 ± 0.42* Epi. 2.19 ± 0.29* Per. 0.51 ± 0.02*
fCHFHS-lf (N = 8)	110 ± 3.5*	63.1 ± 2.2 22.0 ± 1.7 [°] 17.1 ± 1.9 [°]	161 ± 7.5*	Mes. 2.10 ± 0.19* Epi. 1.96 ± 0.13* Per. 0.44 ± 0.05*

BW = body weight, Epi. = epididymal fat pad, na = not applicable, Mes. = mesenteric fat pad, Per. = perirenal fat pad. [¶]Mean daily caloric intake over the course of the diet, [†]Total BW gain = end BW/begin BW × 100%, * $p < 0.05$ compared to CHOW group, [°] $p < 0.05$ for fCHFHS-hf vs. fCHFHS-lf. Data are presented as mean ± SEM.

AMPH decreases intake in CHOW-, fCHFHS-hf, and fCHFHS-lf groups

AMPH injections reduced total caloric intake compared to vehicle injections in all experimental groups (see Figure 2A). A two-way ANOVA analysis revealed a main effect of *Injection* ($F_{1,22} = 23.74$, $p < 0.0001$), and no significant effect of *Diet group* ($F_{2,22} = 2.53$, $p > 0.05$) or an *Interaction* ($F_{2,22} = 1.17$, $p > 0.05$) for total caloric intake after AMPH. *Post hoc* multiple comparisons showed that AMPH significantly reduced intake in all groups (CHOW; $p = 0.03$; fCHFHS-hf; $p = 0.02$; fCHFHS-lf; $p = 0.001$).

An AMPH injection reduced chow intake specifically (Figure 2B). Two-way ANOVA analysis revealed a main effect of *Injection* ($F_{1,11} = 7.93$, $p = 0.02$), and no significant effect of *Diet group* ($F_{1,11} = 1.31$, $p > 0.05$), or an *Interaction* ($F_{1,11} = 0.79$, $p > 0.05$). *Post hoc* analysis revealed a significant reduction of chow intake after AMPH in the fCHFHS-lf group ($p = 0.01$) and no reduction in the fCHFHS-hf group ($p > 0.05$). Intake of sucrose water was not affected significantly by an AMPH injection (Two-way ANOVA analysis: *Injection* $F_{1,11} = 4.00$, $p > 0.05$; *Group* $F_{1,11} = 1.86$; *Interaction* $F_{1,11} = 2.19$, $p > 0.05$; Figure 2C). Also, fat intake was not affected significantly by an AMPH injection (*Injection* $F_{1,11} = 1.48$, $p > 0.05$; *Group* $F_{1,11} = 3.54$; *Interaction* $F_{1,11} = 0.85$, $p > 0.05$; Figure 2D).

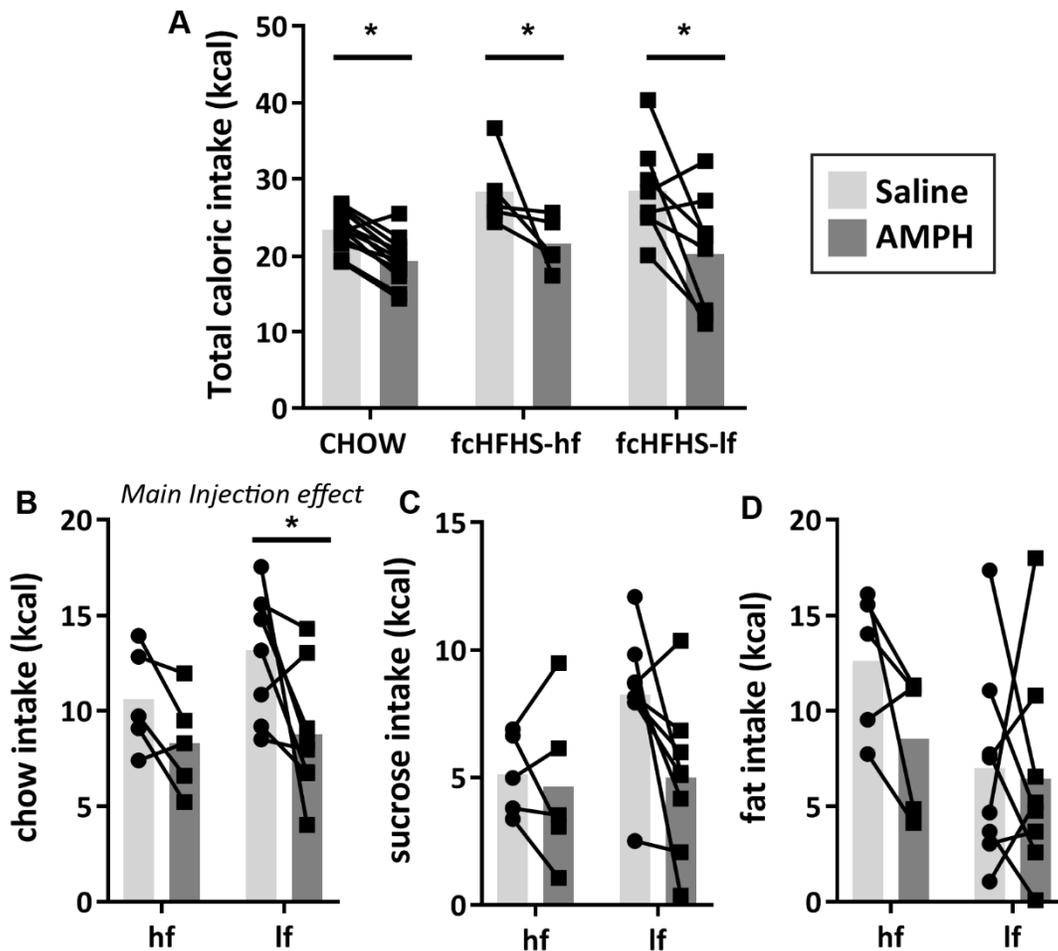


Figure 2. Intrapеритонеal AMPH decreased intake in fchFHS-hf, fchFHS-lf, and CHOW-fed rats. **A)** 0.5 mg/kg in 1 ml/kg AMPH decreases total caloric intake in CHOW- and fchFHS-fed rats (main effect of *Infusion* [$F_{1,24} = 6.34$, $p = 0.0189$], see text for details), **B)** 0.5 mg/kg in 1 ml/kg AMPH decreased caloric intake from chow in the fchFHS-lf group ($p = 0.01$) and did not decrease in the fchFHS-hf group ($p > 0.09$). AMPH did not affect intake of **C)** sucrose water, or **D)** fat. lf = fchFHS-lf, hf = fchFHS-hf. See text for details. * $p < 0.05$

fchFHS-hf, fchFHS-lf and CHOW-fed rats show comparable striatal DRD_{2/3} binding

Dorsal striatal DRD_{2/3} binding was not significantly different between the fchFHS-hf, fchFHS-lf, and CHOW-fed groups as assessed with a One-way ANOVA ($F_{2,25} = 2.441$, $p > 0.05$; see Figure 3A). Ventral striatal DRD_{2/3} binding was also not different between the fchFHS-hf, fchFHS-lf, and CHOW-fed groups as assessed with a One-way ANOVA ($F_{2,17} = 0.02$, $p > 0.05$, see Figure 3B).

When taken together, fchFHS-fed rats and CHOW-fed rats also had comparable DRD_{2/3} binding in the dorsal ($t_{26} = 1.155$, $p > 0.05$) and ventral striatum ($t_{18} = 0.7352$, $p > 0.05$, $N = 10$ /group; data not shown).

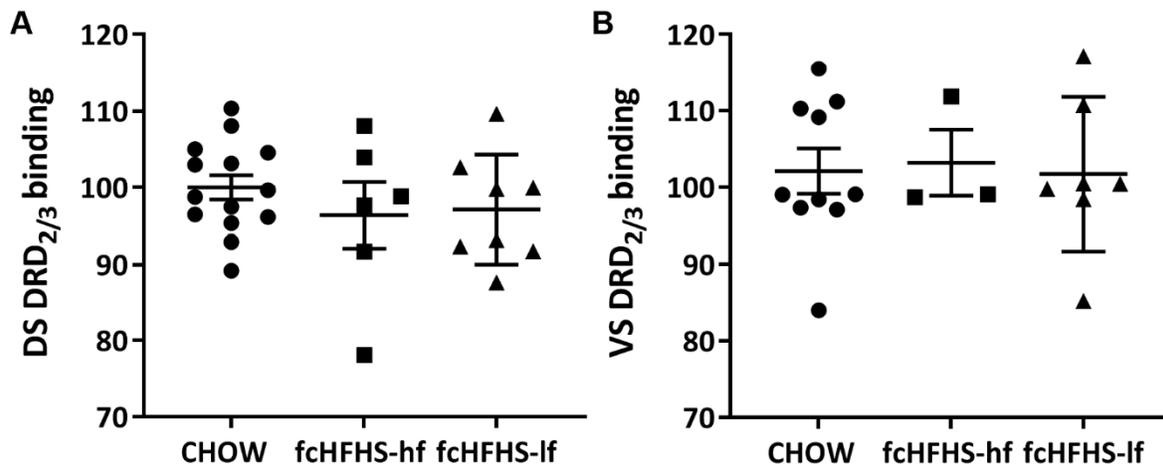


Figure 3. Striatal DRD_{2/3} binding is comparable in fCHFHS-hf, fCHFHS-lf and CHOW-fed rats. **A)** DRD_{2/3} binding in the dorsal striatum was comparable between fCHFHS-hf, fCHFHS-lf, and CHOW-fed rats ($F_{2,25} = 2.441$, $p > 0.05$), **B)** DRD_{2/3} binding in the ventral striatum was comparable between fCHFHS-hf, fCHFHS-lf, and CHOW-fed rats ($F_{2,25} = 0.02$, $p > 0.05$). Outliers were not significant as assessed with Grubb's outlier test. DS = dorsal striatum, VS = ventral striatum.

AMPH-induced inhibition of intake predicts dorsal striatal DRD_{2/3} binding in CHOW-, but not in fCHFHS-fed rats

To determine whether the response to AMPH can predict striatal DRD_{2/3} binding levels, a linear regression analysis was performed. AMPH inhibition of food intake was significantly correlated with DRD_{2/3} binding in the dorsal striatum in the CHOW-fed group ($F_{1,10} = 7.88$, $p = 0.02$, $R^2 = 0.44$; Figure 4A); i.e. more inhibition of intake by AMPH injection correlated with higher dorsal striatal DRD_{2/3} binding. In the fCHFHS-fed group, no correlation was observed between AMPH inhibition of total caloric intake with DRD_{2/3} binding in the dorsal striatum ($F_{1,11} = 0.00$, $R^2 = 0.00$, $p > 0.05$). In addition, no correlations with intake inhibition of the components of the fCHFHS diet were found (all $p > 0.05$; Figures 4B-D).

Ventral striatal DRD_{2/3} binding did not significantly correlate with AMPH-induced inhibition of intake in the CHOW-fed group ($F_{1,6} = 0.06$, $R^2 = 0.01$, $p > 0.05$; Supplemental Figure 2A), nor in the fCHFHS-fed group ($F_{1,8} = 0.04$, $R^2 = 0.01$, $p > 0.05$; data not shown). In addition, no correlation was found between DRD_{2/3} binding in the ventral striatum and AMPH-induced inhibition of intake of chow ($F_{1,8} = 0.16$, $R^2 = 0.02$, $p > 0.05$), sucrose solution ($F_{1,8} = 0.32$, $R^2 = 0.04$, $p > 0.05$), or fat intake ($F_{1,8} = 1.14$, $R^2 = 0.12$, $p > 0.05$; Supplemental Figures 2B-D).

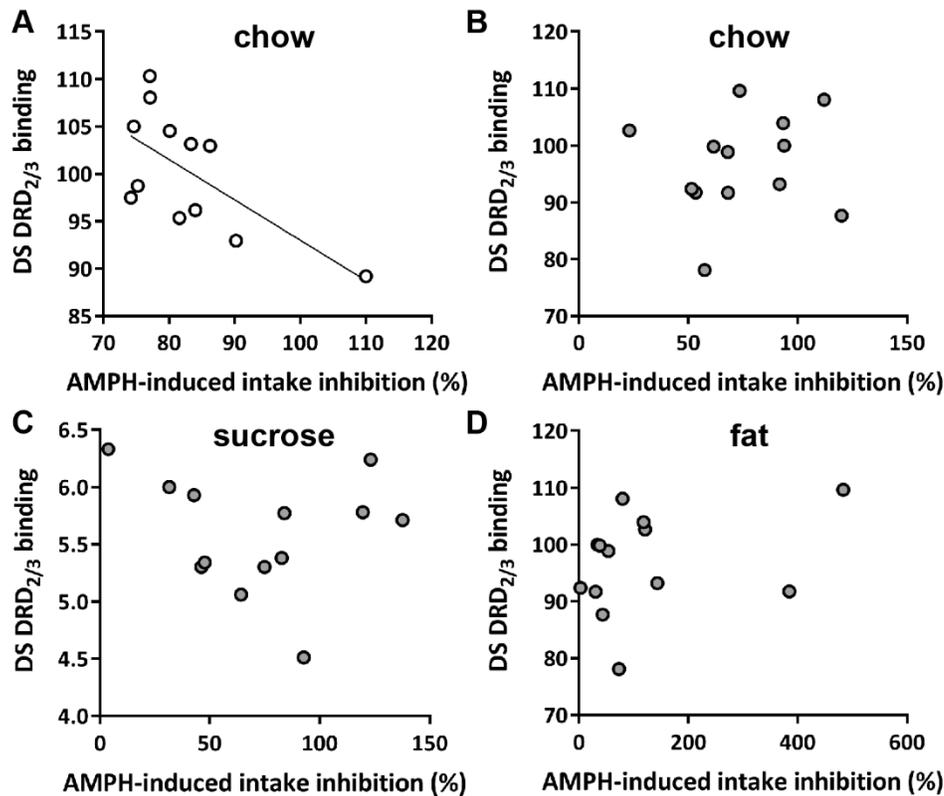


Figure 4. Dorsal striatal DRD_{2/3} binding correlates with AMPH-induced inhibition of intake in CHOW, but not in fCHFHS-fed rats. A) AMPH-induced inhibition of intake could predict dorsal striatal DRD_{2/3} binding in CHOW-fed rats ($F_{1,10} = 7.88$, $p = 0.02$, $R^2 = 0.44$, $N = 12$, 1 missing value and 1 outlier taken out according to Grubb's outlier test). **B)** In the fCHFHS-fed group, AMPH-induced inhibition of chow intake could not predict dorsal striatal DRD_{2/3} binding ($F_{1,11} = 0.16$, $R^2 = 0.01$, $p > 0.05$), nor could **C)** AMPH-induced inhibition of sucrose intake ($F_{1,11} = 0.21$, $R^2 = 0.02$, $p > 0.05$), or **D)** AMPH-induced inhibition of fat intake ($F_{1,11} = 1.15$, $R^2 = 0.09$, $p > 0.05$). AMPH = AMPH, white circles = CHOW group, grey circles = fCHFHS group, * $p < 0.05$

Discussion

Striatal DRD_{2/3} availability after obesogenic diet consumption

In this study, we investigated if AMPH-induced inhibition of food intake could predict ventral striatal DRD_{2/3} availability in rats fed a fCHFHS-diet for four weeks or CHOW-fed control rats, and if this depends on basal fat intake. First, we confirmed the intake-suppressing effects of AMPH injection irrespective of CHOW- or fCHFHS-diet consumption. Second, we determined dorsal and ventral striatal DRD_{2/3} availability, and observed that all three groups had comparable dorsal and ventral striatal DRD_{2/3} availability. Lastly, we determined whether AMPH-induced intake inhibition could predict dorsal or ventral striatal DRD_{2/3} availability. In contrast to our hypothesis, AMPH-induced intake inhibition could not predict ventral striatal

DRD_{2/3} availability, however AMPH-induced inhibition could predict DRD_{2/3} availability in the dorsal striatum in the CHOW-fed group. This correlation was absent in fCHFHS-fed animals.

Our lab has previously shown that access to a palatable diet does not lead to straightforward changes in striatal DRD_{2/3} availability, as overall no differences were found in dorsal or ventral striatal DRD_{2/3} availability between CHOW- and fCHFHS-fed rats (van de Giessen et al., 2013), which is in accordance with the data shown here. However, in rats that spontaneously consume a high fat:sugar ratio, with at least 21% of daily intake comprised of fat (i.e. fCHFHS-hf rats), ventral striatal DRD_{2/3} availability is decreased vs. CHOW-fed controls (van de Giessen et al., 2013). In our present study, we did not observe this decrease in ventral striatal DRD_{2/3} availability, which may be due to a low sample size.

Apart from our studies, several rodent studies have reported on the effects of obesogenic diet consumption on striatal DRD_{2/3} availability, with decreased, increased or no effect reported (Naef, Pitman, & Borgland, 2015). However, not all prior studies have reported the composition of the consumed diet or the striatal region that was investigated. The high variability of experimental setups has made it difficult to determine which factors play a role in affecting striatal DRD_{2/3} availability. Here, we would like to discuss a few important aspects which future studies could take into account. First, the availability of choice in the diet and the basal intake preference of the rodent should be taken into account (van de Giessen et al., 2012; van de Giessen et al., 2013). Second, it is important to note that the dorsal and ventral striatum have anatomical and functional sub-regions, and that DRD₂ receptors may respond differently to dopamine depending on the sub-region (Marcott et al., 2018), or even cell type (Gallo, 2019). Indeed, changes in DRD_{2/3} availability have been reported in a region-specific manner. For instance, Huang et al. (2006) observed decreased DRD_{2/3} availability only in the rostral part of the dorsal striatum in mice that gained weight on a high-fat diet, and rats that consume at least 21 % of daily intake from fat on a fCHFHS diet show decreased DRD_{2/3} availability specifically in the ventral striatum (van de Giessen et al., 2013). Third, a reduction in the highly glycosylated form of the DRD₂ was shown after consumption of a high-fat high-sugar cafeteria diet (P. M. Johnson & Kenny, 2010). This may indicate that post-translational changes play a role in mediating the effects of diet on DRD₂ signaling. Lastly, the radiotracer ¹²³I-IBZM, and other comparable tracers (van Galen et al., 2018), bind to both DRD₂ and DRD₃ receptor subtypes, which are both expressed in the striatum (Bouthenet et al., 1991). Changes in either dopamine receptor might thus be occluded by measuring both receptor types in our study. Future studies should take these considerations into account.

Dorsal striatal DRD_{2/3} availability: AMPH-induced feeding vs. locomotion

We found that AMPH-induced inhibition of intake could predict dorsal striatal DRD_{2/3} availability in CHOW-fed animals. The more CHOW-fed animals inhibited intake after AMPH

injection, the higher their dorsal striatal DRD_{2/3} availability. In contrast, AMPH-induced food intake could not predict dorsal striatal DRD_{2/3} availability in the fCHFHS-fed group. It seems likely that AMPH-induced dopamine release acts on a certain level of DRD_{2/3} receptors in the dorsal striatum to suppress food intake in CHOW-fed animals and not anymore in fCHFHS-fed animals. However, the predictive power of AMPH-induced intake suppression for dorsal striatal DRD_{2/3} binding was not 100%.

AMPH injection leads to a massive release of dopamine in the striatum by reversal of dopamine transporters as well as by other mechanisms (Heal, Smith, Gosden, & Nutt, 2013). It is thought that AMPH leads to intake suppression by leading to several behavioral changes that can affect feeding, such as the induction of stereotypy and increased locomotion (Salisbury & Wolgin, 1985; Wolgin, Thompson, & Oslan, 1987). It has been shown that AMPH does not affect feeding when infused directly into the dorsal striatum (G. D. Carr & White, 1986; Leibowitz, 1975a), but instead leads to stereotypy (G. D. Carr & White, 1986). Indeed, the dynamics of peripherally injected AMPH on locomotion mimic the AMPH-induced dopamine concentration in the striatum in chow-fed animals (Rowley et al., 2012). In addition, there is a negative correlation between activity and feeding in AMPH-treated rats (Cole, 1977, 1979). Apart from this, feeding-related circuits also play a role in mediating the effects of AMPH on food intake (Heal et al., 2013; Hoebel, 1977); i.e. the lateral hypothalamus (LHA) in particular mediates the direct suppressive effects of AMPH on intake as intra-LHA AMPH infusion leads to intake suppression (Leibowitz, 1975a, 1975b). Therefore, measures of locomotion may correlate more strongly with dorsal striatal DRD_{2/3} binding, and food intake suppression may correlate more strongly with measures of neurotransmission in the lateral hypothalamus.

Importantly, dopamine receptor subtype signaling interacts with each other to modulate behavior. Though several studies have looked at the involvement of DRD₁, DRD₂, and DRD₃ in mediating AMPH-induced stereotypy and AMPH-induced appetite suppression (Gilbert & Cooper, 1985; Jackson, Johansson, Lindgren, & Bengtsson, 1994; Mailman et al., 1984; Pritchard et al., 2007), these studies did not assess the role of dorsal striatal dopamine receptors specifically. The radiotracer used in our study binds to the DRD₂ and the DRD₃, which might suggest that both receptors play a role in mediating AMPH-induced intake suppression of feeding or locomotion in the dorsal striatum.

Taken together, the correlation between AMPH-induced intake suppression and dorsal striatal DRD_{2/3} availability in CHOW-fed animals, may thus be better explained as an indirect measurement of dopamine release acting on a certain level of DRD₂ and/or DRD₃ receptors to influence stereotypy, which leads to a suppression of food intake. The absence of an association between AMPH-induced food intake suppression and dorsal striatal DRD_{2/3} availability in the fCHFHS-fed group may be due to a number of factors (see *Technical*

considerations), which require further investigation. In general, it indicates a decoupling of behavior and the dopamine system.

Technical considerations

It is important to note that the variability in AMPH-induced response was relatively limited, which hinders a reliable assessment of the association between AMPH-induced intake suppression and dorsal striatal DRD_{2/3} availability. In addition, the statistically significant predictive power of AMPH-induced intake suppression on dorsal striatal DRD_{2/3} availability depends on one data point. Future studies should increase the sample size to confirm or disprove our results.

In addition, it is known that diet-induced obesity and consumption of obesogenic diets lead to alterations in the dopamine system, and several of these alterations could interact with our findings in the fCHFHS-fed group. For instance, 1) basal dopamine release can be decreased in rats fed an obesogenic diet vs. control-fed rats (Geiger et al., 2009), which may affect dopamine receptor expression and/or indirectly binding of the (radio)tracer to dopamine receptors. However, we did not observe any differences in the dorsal striatal DRD_{2/3} availability between the CHOW- and fCHFHS-fed groups. In addition, ¹²³I-IBZM binding to DRD_{2/3} is relatively unaffected by small changes in endogenous dopamine concentrations (van Wieringen et al., 2014). On the other hand, as ¹²³I-IBZM binds to both DRD₂ and DRD₃, it is still possible that changes in either receptor may have been occluded in our study. Future studies should therefore employ dopamine receptor-subtype-specific radiotracers if available.

Also, AMPH-induced dopamine release is also decreased in rats fed an obesogenic diet (Geiger et al., 2009), which may be related to changes in dopamine transporter levels (Barry et al., 2018; Jones et al., 2017; Narayanaswami, Thompson, Cassis, Bardo, & Dwoskin, 2013; J. C. Patel et al., 2019; South & Huang, 2008). If the animals in our fCHFHS-fed group have altered AMPH-induced dopamine release or dopamine transporter levels, it is possible that their behavior is not affected to the same extent when using the same AMPH dose as in the CHOW-fed group. In addition, the effects of AMPH on behavior are affected by the level of deprivation (G. D. Carr & White, 1986; Cole, 1979), which might be different for CHOW- and fCHFHS-fed rats. However, there is no apparent difference in the intake suppressing effects of AMPH between the diet groups in our study.

Lastly, in this study, DRD_{2/3} availability was measured after a washout period to prevent measurement of direct AMPH-induced pharmacological effects on the DRD_{2/3} receptors (Habraken et al., 2001; Jongen et al., 2008; Nikolaus et al., 2005). It may be that the dietary effects on the dopamine system progressed further during this period, leading to the decoupling of AMPH-induced behavior and DRD_{2/3} availability when measured at a later time point. Future studies should try to reduce the washout period to a minimum to be able to

determine the mechanisms underlying the decoupling of dopamine-mediated behavior and dorsal striatal DRD_{2/3} availability in the fCHFHS-fed group.

Taken together, several diet-dopamine system interactions prevent determining what underlies the absence of an association between dorsal striatal DRD_{2/3} availability and the behavioral response to AMPH injection in fCHFHS-fed rats. Future studies could measure multiple components of the dopamine system over time to elucidate the mechanisms underlying how diet alters the dopamine system.

Summary

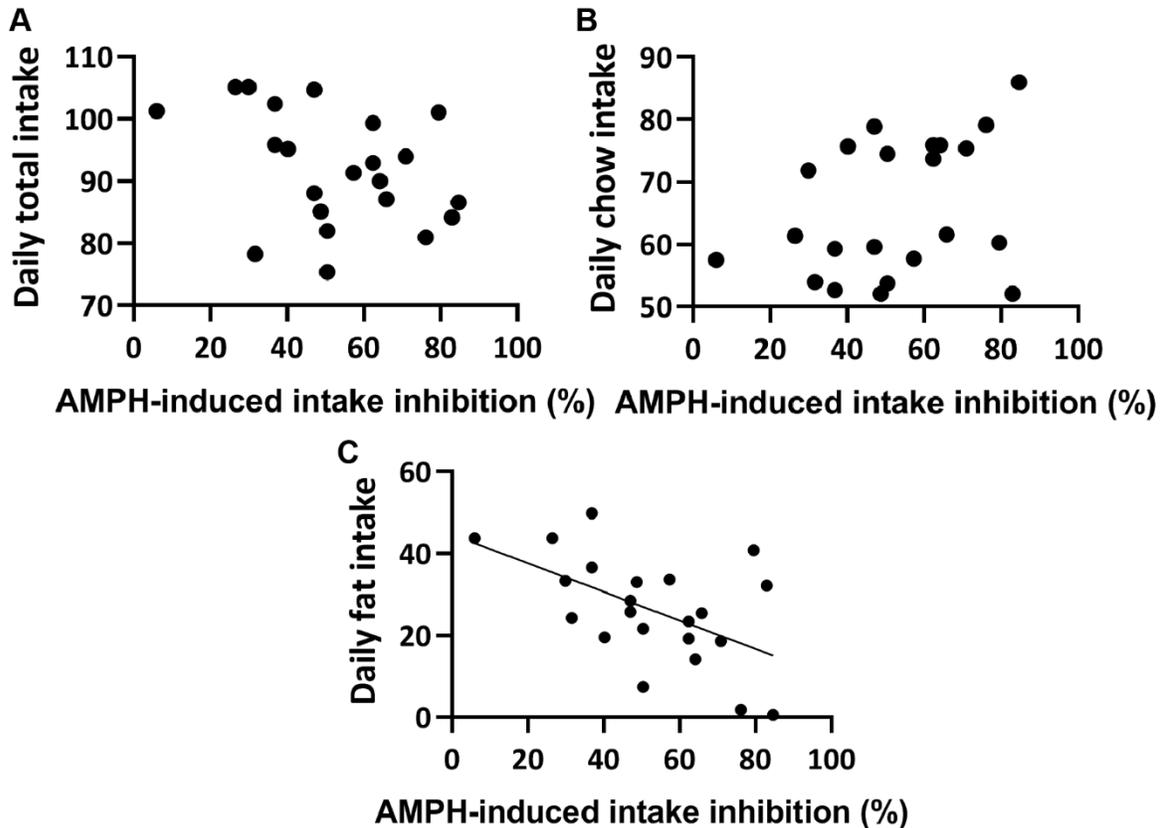
We thus conclude that four weeks consumption of a fCHFHS diet does not affect dorsal striatal DRD_{2/3} binding levels compared to consumption of a CHOW diet. In addition, AMPH-induced food intake inhibition can predict dorsal striatal DRD_{2/3} availability in CHOW-fed rats and does not predict dorsal striatal DRD_{2/3} availability in fCHFHS-fed rats. These data indicate that even if DRD_{2/3} availability levels are similar after exposure to an obesogenic or a control diet, the functionality of the dopamine system is altered after four weeks consumption of a fCHFHS-fed diet, such that the coupling between behavioral response and dorsal striatal DRD_{2/3} availability has disappeared.

Acknowledgements and author contributions

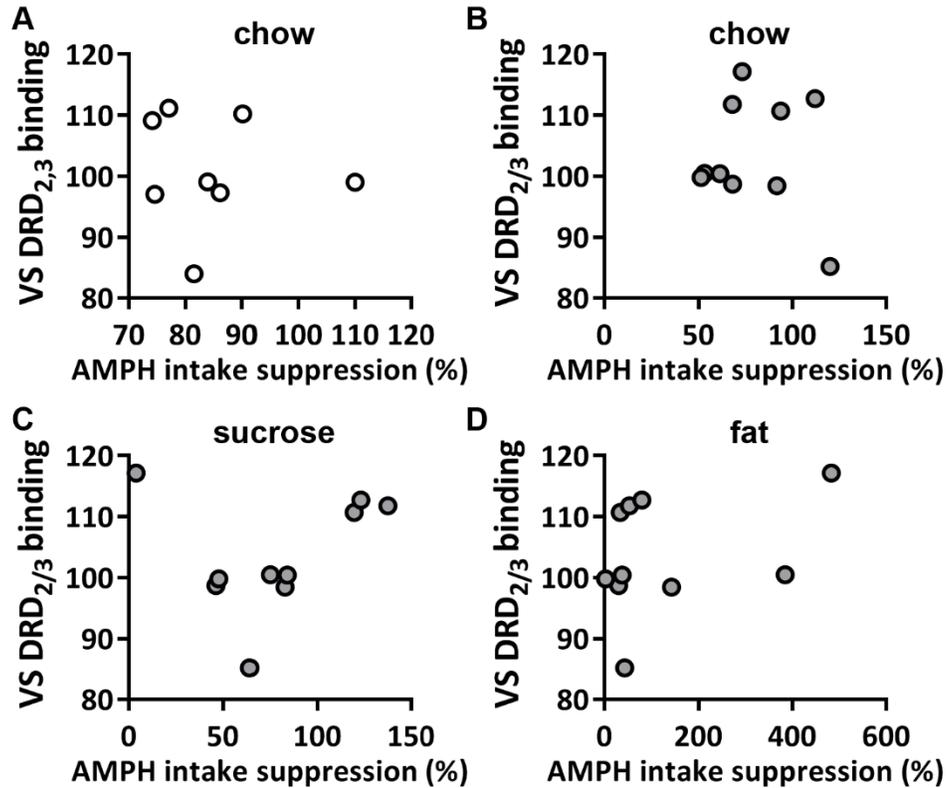
We would like to thank the IWO animal facility for their assistance, with special thanks to Sanne Hackmann, Marja van Brakel, and Ardine de Vos for administering i.p. injections.

Myrtille Gumbs performed the experiment and wrote the manuscript, Kora de Bruin performed the DRD_{2/3} availability measurements, Elsmarieke van de Giessen performed pilot experiments, Esther de Zwaal designed the experiment, and Joram Mul, Jan Booij and Susanne la Fleur critically revised the manuscript.

Supplemental figures



Supplemental Figure 1. AMPH-induced inhibition prior to exposure to fCHF diet correlates with fat intake, but not with chow intake when on a fCHF diet. 30 male Wistar rats (350-400 g) were injected intraperitoneally with amphetamine (AMPH; 1 mg/kg) or vehicle (saline) at the beginning of the dark period. Five hours after injection, food intake was measured. AMPH-induced inhibition was calculated by the percentage intake of AMPH injected rats (N = 22) normalized for vehicle intake (N = 8). Subsequently, rats were given access to a free-choice high-fat (fCHF) diet for 2 weeks. This diet consists of *ad libitum* access to chow, a bottle of tap water, and a dish of fat. Chow and fat intake were calculated over 3 days in the second week, and correlated with the prior measured AMPH-induced inhibition. **A)** Daily total, and **B)** chow caloric intake were not correlated with the AMPH-induced inhibition determined before diet exposure (respectively $F_{1,20} = 3.1$, $R^2 = 0.13$; $p = 0.09$, and $F_{1,20} = 2.7$, $R^2 = 0.12$; $p = 0.11$). **C)** Daily fat intake on a fCHF diet correlated with prior AMPH-induced inhibition ($F_{1,20} = 8.2$ $R^2 = 0.30$; $p < 0.001$). Daily intake values are presented in kcal.



Supplemental Figure 2. Ventral striatal DRD_{2/3} binding does not correlate with AMPH-induced inhibition of food intake. **A)** AMPH-induced inhibition of food intake could not predict ventral striatal DRD_{2/3} binding in CHOW-fed rats ($F_{1,6} = 7.88$, $p > 0.05$, $R^2 = 0.01$, $N = 8$). In the fCHFS-fed group ventral striatal DRD_{2/3} binding could not be predicted by AMPH-induced intake of **B)** chow ($F_{1,8} = 0.16$, $p > 0.05$, $R^2 = 0.02$, $N = 10$), **C)** sucrose solution ($F_{1,8} = 0.32$, $p > 0.05$, $R^2 = 0.04$, $N = 10$), or **D)** or fat ($F_{1,8} = 1.14$, $p > 0.05$, $R^2 = 0.12$, $N = 10$).