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ORIGINAL ARTICLE

Does activation of midbrain dopamine neurons promote or reduce feeding?

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BACKGROUND: Dopamine (DA) signalling in the brain is necessary for feeding behaviour, and alterations in the DA system have been linked to obesity. However, the precise role of DA in the control of food intake remains debated. On the one hand, food reward and motivation are associated with enhanced DA activity. On the other hand, psychostimulant drugs that increase DA signalling suppress food intake. This poses the questions of how endogenous DA neuronal activity regulates feeding, and whether enhancing DA neuronal activity would either promote or reduce food intake.
METHODS: Here, we used designer receptors exclusively activated by designer drugs (DREADD) technology to determine the effects of enhancing DA neuronal activity on feeding behaviour. We chemogenetically activated selective midbrain DA neuronal subpopulations and assessed the effects on feeding microstructure in rats.
RESULTS: Treatment with the psychostimulant drug amphetamine or the selective DA reuptake inhibitor GBR 12909 significantly suppressed food intake. Selective chemogenetic activation of DA neurons in the ventral tegmental area (VTA) was found to reduce meal size, but had less impact on total food intake. Targeting distinct VTA neuronal pathways revealed that specific activation of the mesolimbic pathway towards nucleus accumbens (NAc) resulted in smaller and shorter meals. In addition, the meal frequency was increased, rendering total food intake unaffected. The disrupted feeding patterns following activation of VTA DA neurons or VTA to NAc projection neurons were accompanied by locomotor hyperactivity. Activation of VTA neurons projecting towards prefrontal cortex or amygdala, or of DA neurons in the substantia nigra, did not affect feeding behaviour.
CONCLUSIONS: Chemogenetic activation of VTA DA neurons or VTA to NAc pathway disrupts feeding patterns. Increased activity of mesolimbic DA neurons appears to both promote and reduce food intake, by facilitating both the initiation and cessation of feeding behaviour.

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INTRODUCTION

Dopamine (DA) signalling in the brain is necessary for food intake. DA-deficient mice fail to eat and die of starvation without additional L-DOPA treatment.[1] However, the precise role of DA in the control of food intake remains poorly understood. Obesity and eating disorders have been associated with alterations in the DA system, including reduced expression of striatal DA D2 receptors (D2-R).[2] Furthermore, drugs that block DA receptors (DA-R), such as antipsychotics, induce weight gain and increase risk for obesity.[3,4] Food reward and reinforcement are known to be mediated via the DA system.[5] Altogether, this suggests that DA is an important modulator of feeding behaviour. However, the causal relationship between endogenous DA neuronal activity and food intake remains elusive.

One unresolved question is whether enhanced activity of DA neurons either stimulates or inhibits feeding. On the one hand, food intake induces DA release in the striatum, associated with the rewarding properties of food,[6–8] and mesolimbic DA is crucially involved in the motivation to work for food.[9–11] On the other hand, psychostimulant drugs that increase DA signalling, such as amphetamine and methylphenidate, reduce appetite and food intake.[12–15] To determine if and how DA neuronal activity controls feeding behaviour, DA neurons should be manipulated directly. Pharmacological or genetic manipulations of post-synaptic DA signalling may affect behaviour differently compared to DA neuron activation by endogenous excitatory inputs. As such, it is unclear to what extent the effects of post-synaptic stimulation of DA signalling reflect physiological regulation of feeding.[16]

A second outstanding issue is the respective role of midbrain DA neurons in the ventral tegmental area (VTA; which primarily project to the ventral striatum) compared to the substantia nigra pars compacta (SNc; primarily projecting to dorsal striatum) in the control of feeding behaviour. Thus far, the VTA has received most attention in this context.[17–21] However, receptors for feeding hormones are present in both VTA and SNc,[22] and changes in homoeostatic and motivational state have been shown to affect VTA and SNc DA neuronal activity.[23,24] Importantly, studies in DA-deficient mice have shown that selective restoration of DA signalling in the nigrostriatal pathway (from SNc towards dorsal striatum) was sufficient to rescue and even enhance normal feeding behaviour, while restoring DA in the nucleus accumbens (NAc; ventral striatum) was not.[25,26] Altogether, these findings suggest that SNc DA neurons may play an important role in the control of food intake.[18,27] However, the direct effect of enhanced

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DA neuronal activity in the VTA or SNc on feeding behaviour remains unknown.

In this study, we took a novel approach to determine whether enhancing endogenous activity of midbrain DA neurons directly affects feeding behaviour. First, we confirmed that food intake is suppressed by pharmacological DA reuptake inhibition. Then, we tested the effects of enhanced DA neuronal activity in either the VTA or SNc on food intake and feeding microstructure, using designer receptor exclusively activated by designer drugs (DREADD) technology. To identify which pathways underlay the observed changes in feeding, we selectively activated distinct midbrain neuronal pathways. Finally, since chemogenetic activation of midbrain DA neurons has been shown to induce hyperactivity,[28–30] we reasoned that this may interfere with feeding, and therefore quantified locomotor activity.

MATERIALS AND METHODS

All experiments were carried out in accordance with Dutch and international laws (Wet op de Dierproeven, 1996) and European regulations (Guideline 86/609/EEC), and were approved by the Animal Ethics Committee of Utrecht University. xperiments were performed as previously described in Boekhoudt *et al.*[28]

Subjects and surgical procedure

TH::Cre rats[31] were bred in-house, by crossing heterozygous $Cre^{+/-}$ rats with wild type Long Evans mates (these animals were also used for experiment 2 in Boekhoudt *et al*.,[28]). Male rats were injected with Cre-dependent DREADD virus AAV5-hSyn-DIO-hM3Dq-mCherry (1 µl bilaterally, 6.4–8.0*E12 virus molecules per ml; UNC Vector Core, Chapel Hill, USA) into either the VTA or SNc. For each region, 8 Cre^{+} rats were injected, as well as 7 Cre^{-} littermates serving as control group. Stereotactic coordinates were adjusted according to the animals' body weight (all coordinates in mm relative to Bregma), and were set at AP − 5.2; ML +1.1 (5° angle); DV − 7.4 for VTA (rats 7 weeks old, mean body weight 156 gram) and AP − 5.4; ML +2.2; DV − 7.7 for SNc (adult rats, mean body weight 337 gram).

For chemogenetic activation of selective pathways, DREADD was combined with canine adenovirus expressing Cre recombinase (CAV2Cre).[30] Thirty-two male Wistar rats (Crl:WU, Charles River, Sulzfeld, Germany) were injected bilaterally with AAV5-hSyn-DIO-hM3Dq-mCherry (1 µl, 1.0 × E12 virus molecules per ml) into the VTA (AP − 5.4; ML +2.2 (10° angle); DV − 8.9; adult rats, mean body weight 325 gram). In addition, CAV2Cre (1 µl, 1.25 × E12 virus molecules per ml; IGMM, Montpellier, France) was infused bilaterally into one of three VTA projection sites: NAc (AP +1.2; ML +2.8 (10º angle); DV − 7.5; $n = 11$), prefrontal cortex (PFC) (AP +2.7; ML +1.4 (10º angle); DV − 4.9; $n = 10$), or amygdala (AP − 2.2; ML +5.0 (0º angle); DV − 9.2; $n = 11$). Rats were pseudo-randomly allocated to one of the three groups. Sample sizes were calculated based on expected effect sizes and variance.

Anaesthesia and peri-operative care for both experiments were carried out as described previously.[28]

Behavioural testing

All behavioural tests were performed in adult male rats, at least four weeks after viral infusion. Rats were housed individually in 16 PhenoTyper home cages (Noldus IT, Wageningen, The Netherlands), 43 × 43 × 90cm^3, equipped with infrared cameras, and an automated weighing system.[32] The animals were kept under a 12-h light-dark cycle (lights off 16:00) with *ad libitum* access to drinking water. The rats were mildly food restricted, by removing chow during the final 8 h of the light phase, in order to ensure similar homoeostatic states across multiple testing days.[33]

All tests were performed using a counter-balanced within-subjects design. Drugs were administered at 15:30, 30 min before access to chow. Subsequent injections were separated by at least 24 h. The effects of amphetamine and GBR 12909 treatment were tested in the TH::Cre rats, with Cre^{+} and Cre^{-} animals pooled. Each dose was counterbalanced against a vehicle treatment. Dose-response testing for clozapine-N-oxide (CNO) was performed using a Latin-squared design, with 48 h in between injections. Food restriction continued during wash-out days.

Drugs

All drugs were administered intra-peritoneally at a volume of 0.1 ml per 100 g body weight. The selective DREADD ligand CNO (kindly provided by Bryan Roth and purchased at the NIMH Chemical Synthesis and Drug Supply Program; dose 0.03–1.0 mg kg^{-1}) and amphetamine (*d*-amphetamine sulphate, OPG Utrecht, the Netherlands; dose 0.3 and 1.0 mg kg^{-1}) were dissolved in sterile saline (0.9% NaCl). GBR 12909 (GBR 12909 dihydrochloride, Sigma-Aldrich, Schnelldorf, Germany; 3.0 and 10 mg kg^{-1}) was dissolved in MQ water.

Tissue preparation and immunohistochemical analysis

Tissue preparation and immunohistochemistry were performed as previously described.[28] Sucrose-saturated brains were sliced at 40 µm. Presence of hM3Dq-mCherry and tyrosine hydroxylase (TH) was visualised using primary antibodies Rabbit anti-dsRed (Clontech #632496, Leusden, The Netherlands) and Mouse anti-TH (MerckMillipore #MAB318, Amsterdam, The Netherlands), respectively, and secondary antibodies Goat anti-Rabbit Alexa 568 and Goat anti-mouse Alexa 488 (both Abcam, #ab175471 and #ab150117, Cambridge, UK). Fluorescent pictures of mounted brain slices (×5 and × 10 magnification) were taken with a Zeiss Axioscope A1 microscope and Axiovision software to analyse expression patterns. Confocal pictures (×20 magnification) were taken with a Olympus Fluoview 1000 microscope and FluoView software. Expression of DREADD (hM3Dq-mCherry) and TH was analysed using ImageJ. dsRed- and TH-immunoresponsive cells were counted within the targeted region (VTA or SNc), between − 5.2 and − 6.0 mm from Bregma.

Data analysis

No animals were excluded from analysis based on DREADD expression. Because of a defective feeder scale, one rat in the VTA > PFC group was excluded from the analysis. Experimenters were not explicitly blinded for experimental treatments or group allocation. All behavioural data were computed automatically.

Feeding data were collected every 12 s and analysed using a custom-made macro in Microsoft Excel. Within this macro, definition for a meal was set at at least 0.3 g (equivalent to 1 kCal) and at least 5 min intermeal interval. Food intake was analysed for the first two hours of chow access, as drugs were physiologically active during this period and this procedure yielded robust results within animals over multiple days. The following parameters were calculated: total food intake (g), total number of meals, average meal size (total intake/number of meals), total time spent feeding (min) and average meal duration (total time spent feeding/number of meals). For the first meal, the latency to start (min), size (g), and meal interval (time between first and second meal) were measured.

Total distance moved was analysed with EthoVision XT9 and XT11, as described previously.[28] Analysis of activity (total time and frequency) was based on continuous activity recordings in EthoVision (threshold for 'active state' set at 3%).

Statistical analyses were performed in SPSS 16.0. Non-parametric tests were used for number of meals and latency to start feeding. Other parameters were natural log transformed to allow for parametric testing. Pairwise comparisons (amphetamine or GBR 12909 compared to vehicle) were carried out with paired samples *t*-test or Wilcoxon signed rank test. Effects of CNO treatment compared to saline were tested using a repeated measures general linear model, with experimental Group as between-subjects factor and treatment as within-subjects factor. This was followed by LSD *post hoc* comparisons between treatments in the case of a significant main effect or interaction. Greenhouse–Geisser and Huyn–Feldt corrections were applied to adjust for sphericity (when Mauchley's epsilon < 0.7 or > 0.7, respectively). As non-parametric equivalents, Friedman test and Wilcoxon signed rank test were used. Threshold for statistical significance was set at $\alpha = 0.05$, all tests were two-sided.

RESULTS

Pharmacological DA reuptake inhibition suppresses food intake

To affirm the anorectic properties of pharmacological DA stimulation in our setup, we tested the effects of amphetamine (AMPH; a DA and noradrenalin reuptake inhibitor and releaser) and GBR 12909 (GBR; a selective DA reuptake inhibitor) on feeding microstructure and total food intake.

Both amphetamine (0.3 and 1.0 mg kg^{-1}) and GBR 12909 (10 mg kg^{-1}) treatment affected multiple aspects of food intake (Figure 1). Exemplifying feeding patterns following treatment are depicted in Figure 1a and b. The low dose of GBR 12909, 3.0 mg kg^{-1}, was not sufficient to affect feeding behaviour (all $P>0.1$). Both amphetamine (0.3 and 1.0 mg kg^{-1}) and GBR 12909 (10 mg kg^{-1}) decreased average meal size (Figure 1c; AMPH $P<0.0005$; GBR $P=0.012$), while only amphetamine treatment significantly reduced average meal duration (Figure 1d; AMPH: 0.3 mg kg^{-1} $P=0.001$, 1.0 mg kg^{-1} $P<0.0005$; GBR $P=0.071$) and first meal size (AMPH: 0.3 and 1.0 mg kg^{-1} $P<0.0005$; GBR $P=0.182$, data not shown). Meal frequency was significantly

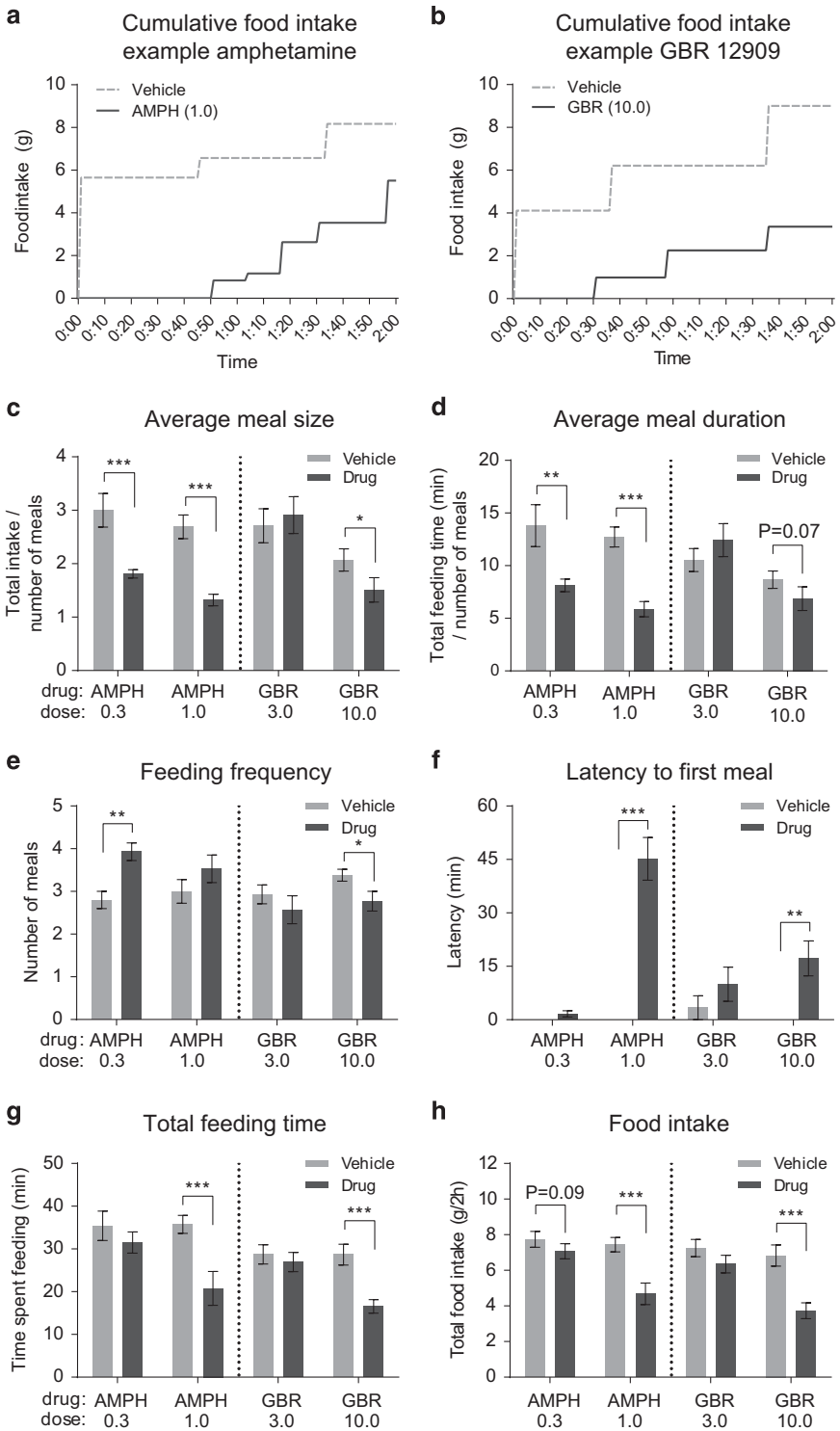

Figure 1. Effects of pharmacological DA stimulation, by amphetamine or GBR 12909, on feeding patterns. Representative examples of feeding patterns following treatment with amphetamine (1.0 mg kg^{-1}, (**a**) or GBR 12909 (10 mg kg^{-1}, (**b**). (**c**–**h**) Effect of amphetamine and GBR 12909 on average meal size (**c**), average meal duration (**d**), meal frequency (**e**), latency to start feeding (**f**), total feeding time (**g**) and total food intake (**h**). Data are presented as mean ± s.e.m., $n=13$–15 per group. *$P<0.05$, **$P<0.01$, ***$P<0.001$ drug compared to vehicle.

increased with 0.3 mg kg^{-1} amphetamine (Figure 1e; $P=0.003$; 1.0 mg kg^{-1} $P=0.242$), yet decreased following GBR 12909 treatment (Figure 1e; $P=0.035$). The interval between the first and second meal was not affected (all doses $P>0.1$, data not shown).

The high doses of amphetamine and GBR 12909 delayed the latency to start feeding (Figure 1f) and decreased total feeding time (Figure 1g; 1.0 mg kg^{-1} AMPH and GBR all $P<0.01$; 0.3 mg kg^{-1} AMPH $P=0.25$ and $P=0.095$, respectively). Altogether, these effects resulted in a significant decrease in total food intake (Figure 1h; 1.0 mg kg^{-1} AMPH and GBR both $P<0.0005$; 0.3 mg kg^{-1} AMPH $P=0.087$). Thus, pharmacological stimulation of DA signalling by amphetamine or GBR 12909 suppressed feeding behaviour.

Chemogenetic activation of DA neurons in VTA, but not SNc, disrupts feeding behaviour

To test whether chemogenetically increased excitability of DA neurons is sufficient to affect feeding behaviour, food intake was measured in rats expressing the excitatory DREADD hM3Dq in DA neurons in the VTA (VTA:Dq+) or SNc (SN:Dq+). Immunohistochemical analysis confirmed DREADD expression throughout the VTA and SNc, respectively (Figure 2a and b, see also Boekhoudt *et al*.,[28]), as well as co-localisation with TH (96% in VTA:Dq+ group, 99% in SN:Dq+ group, see Boekhoudt *et al*.,[28]). Both groups showed substantial expression efficiency: 55 and 84% of DA cells expressed hM3Dq-mCherry in VTA:Dq+ and SN:Dq+ groups, respectively.[28] No DREADD expression was observed in Cre-negative control groups (VTA:Dq- and SN:Dq-, not shown).

A representative example of cumulative food intake is depicted in Figure 2c. CNO treatment significantly decreased the average meal size in VTA:Dq+ rats (Figure 2d; Treatment effect and Treatment*Group interaction $P\leqslant 0.01$; *post hoc* CNO vs saline VTA:Dq+ group $P<0.0005$). A trend was observed towards an increased meal frequency (Figure 2e; CNO vs saline VTA:Dq+ $Z=-2.121$, $P=0.063$; other groups $P>0.1$). Combined, these effects resulted in a modest yet significant decrease in total food intake in the VTA:Dq+ group (Figure 2f; Treatment effect $F_{1,26}=7.325$, $P=0.012$; Group*Treatment interaction $F_{3,26}=6.345$, $P=0.08$; *post hoc* CNO vs saline VTA:Dq+ $P=0.001$, other groups $P>0.1$). CNO treatment had no effect on feeding behaviour in the SN:Dq+ group or control groups ($P>0.1$).

All animals typically started feeding immediately (within 1 min) upon access to chow (Figure 2c, Table 1), and CNO treatment did not affect the latency to eat (Table 1). Consistent with a smaller average meal size, CNO reduced the first meal size in VTA:Dq+ rats (Table 1; Treatment effect and Treatment×Group interaction $P<0.05$; *post hoc* CNO vs saline VTA:Dq+ $P<0.0005$, other groups $P>0.1$). The average meal duration and total time spent feeding were not affected (Table 1).

In summary, chemogenetic activation of VTA DA neurons decreased meal size and total food intake, while activation of SNc DA neurons did not affect feeding behaviour.

Dose-dependent effects of CNO on meal size and frequency in VTA:Dq+ rats

To investigate the effect of VTA DA neuron activation in more detail, we performed a dose-response test in VTA:Dq+ rats. CNO treatment significantly decreased average meal size (Figure 3a),

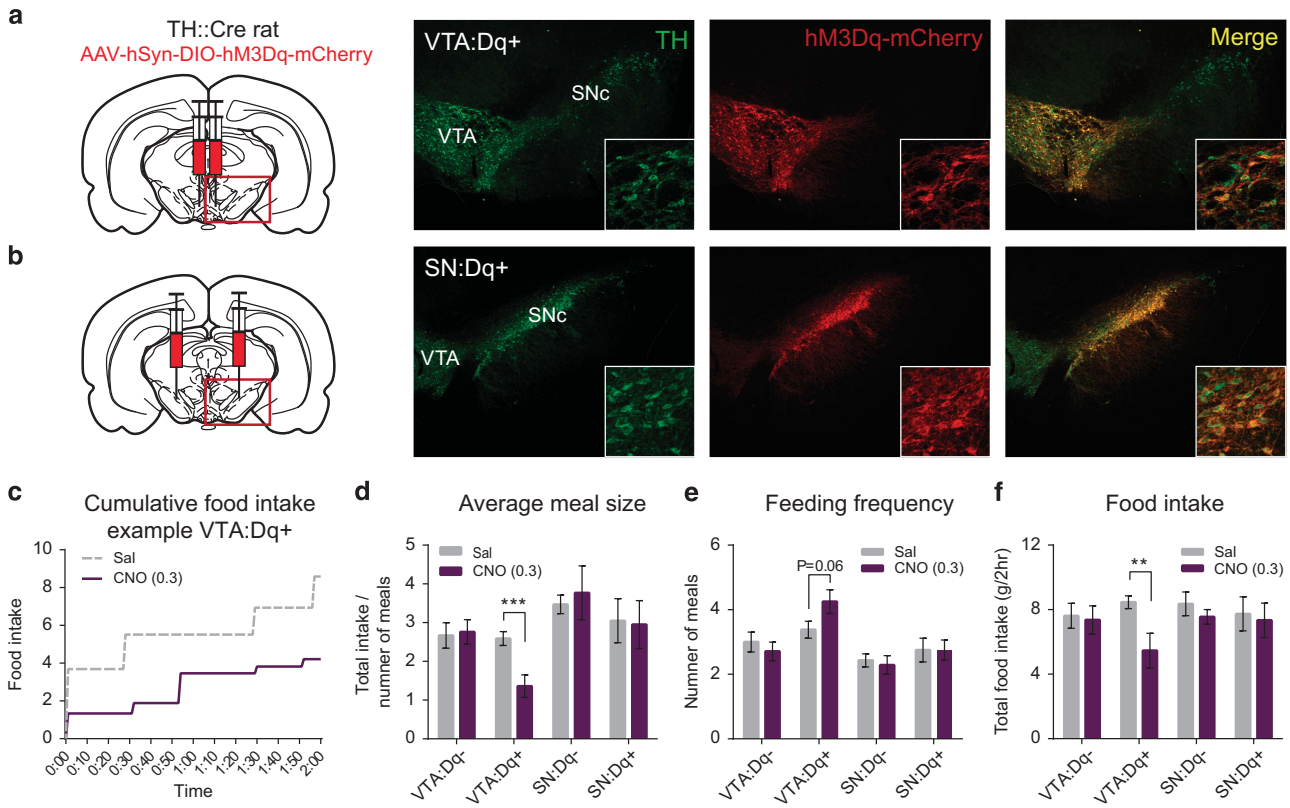

Figure 2. Chemogenetic activation of DA neurons in VTA, but not SNc, affects feeding patterns. TH::Cre rats were infused with Cre-dependent DREADD virus into either VTA (**a**) or SNc (**b**). Right panels show expression of DREADD (hM3Dq-mCherry) in DA (TH-immunoreactive) neurons in VTA (VTA:Dq+) and SNc (SN:Dq+). (**c**) Representative feeding pattern of VTA:Dq+ rat following treatment with saline (Sal) or CNO (0.3 mg kg^{-1}), showing cumulative intake over time. Each vertical step represents a meal. (**d**–**f**) Effects of CNO treatment on average meal size (**d**), meal frequency (**e**), and total food intake (**f**) in VTA:Dq+ and SN:Dq+ rats, and Cre- control groups (VTA:Dq- and SN:Dq-). Error bars represent mean ± s.e.m. $n=7$–8 per group. $^{**}P<0.01$, $^{***}P<0.001$ CNO compared to saline.

Table 1. Additional measures of feeding microstructure, following chemogenetic activation of DA neurons in VTA or SNc

	Latency to start first meal (min)				*First meal size (g)*				*First meal interval (min)*			
	SAL	*s.e.m.*	*CNO*	*s.e.m.*	*SAL*	*s.e.m.*	*CNO*	*s.e.m.*	*SAL*	*s.e.m.*	*CNO*	*s.e.m.*
VTA:Dq-	**0.00**	0.00	**3.00**	3.00	**3.39**	0.50	**3.55**	0.74	**24.57**	7.74	**34.86**	12.01
VTA:Dq+	**0.00**	0.00	**1.15**	1.15	**4.33**	0.44	**2.34** [a]	0.76	**25.53**	5.35	**22.35**	6.80
SN:Dq-	**0.00**	0.00	**0.00**	0.00	**4.65**	0.54	**4.59**	0.69	**29.14**	7.93	**40.30**	4.76
SN:Dq+	**0.00**	0.00	**0.00**	0.00	**3.73**	0.77	**3.18**	0.88	**28.73**	5.96	**25.63**	4.41

	Average meal duration (min)				*Total feeding time (min)*			
	SAL	*s.e.m.*	*CNO*	*s.e.m.*	*SAL*	*s.e.m.*	*CNO*	*s.e.m.*
VTA:Dq-	**12.38**	2.40	**12.67**	1.38	**34.49**	4.61	**33.71**	4.22
VTA:Dq+	**12.29**	1.24	**10.19**	2.44	**40.83**	5.19	**42.25**	9.45
SN:Dq-	**15.26**	0.97	**16.09**	2.79	**36.66**	2.95	**32.60**	3.38
SN:Dq+	**13.42**	1.87	**13.93**	1.73	**33.88**	3.37	**35.60**	2.85

Data represent mean and s.e.m. following treatment with either saline (SAL) or CNO. $n = 7$–8 per group. [a]$P < 0.0005$ CNO compared to saline. Other tests not significant ($P > 0.1$). The mean values are indicated in bold.

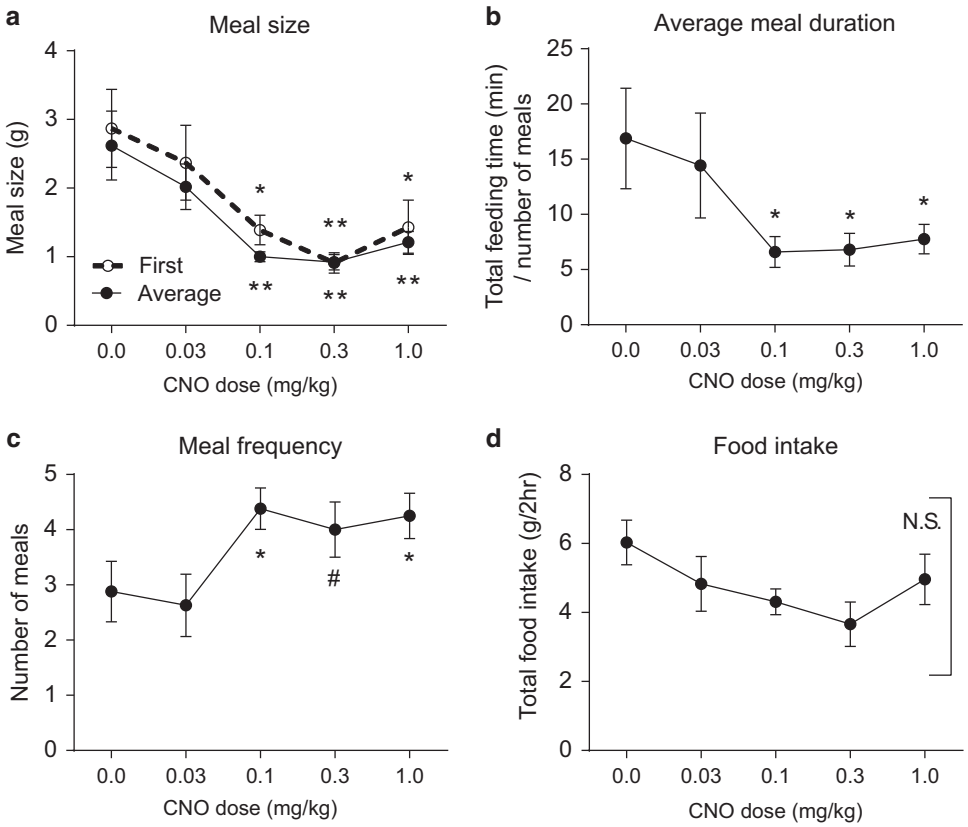

Figure 3. Dose-dependent effects of VTA DA neuron activation on feeding microstructure. Dose-response curves for effects of CNO treatment on feeding in VTA:Dq+ rats. Effects of multiple doses of CNO (0.03–1.0 mg kg^{-1}) on average meal size and first meal size (**a**), average meal duration (**b**), meal frequency (**c**), and total food intake (**d**). Data are presented as mean ± s.e.m. ($n = 8$). $^{\#}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, CNO compared to saline. N.S. not significant. The mean values are indicated in bold.

first meal size (Figure 3a) and average meal duration (Figure 3b; Treatment effects $P < 0.0005$, $P = 0.001$ and $P = 0.028$, respectively) at doses of 0.1 mg kg^{-1} CNO and higher (Figure 3a and b; *post hoc* tests CNO vs saline: 0.1, 0.3 and 1.0 mg kg^{-1} all $P < 0.05$), but not 0.03 mg kg^{-1} (Figure 3a and b; all $P > 0.1$).

In addition, CNO treatment increased the meal frequency (Figure 3c; Chi-square = 10.59, $P = 0.024$) at 0.1 and 1.0 mg kg^{-1} (CNO vs saline: $P < 0.05$; 0.03 mg kg^{-1} $P = 0.8$, 0.3 mg kg^{-1} $P = 0.078$). There was no main effect on total food intake (Figure 3d; Treatment effect $F_{4,28} = 1.909$, $P = 0.137$). Consistent with the single-dose experiment, CNO treatment did not affect the latency to start feeding or total feeding time (both $P > 0.1$, data not shown).

Taken together, CNO doses of 0.1 mg kg^{-1} and higher decreased meal size and duration in VTA:Dq+ rats and increased feeding frequency, while 0.03 mg kg^{-1} CNO was not sufficient to affect feeding behaviour.

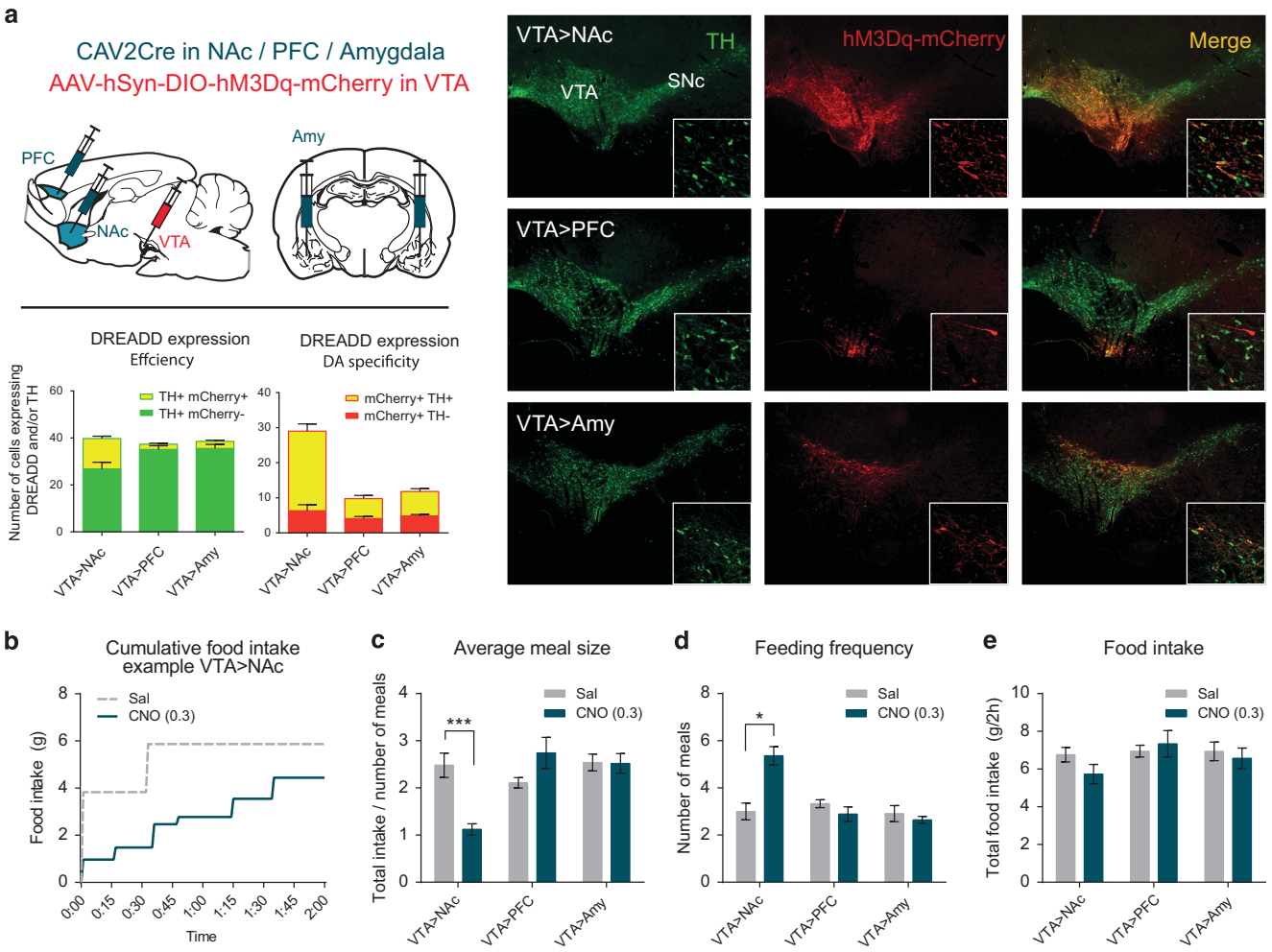

Figure 4. Effects of chemogenetic activation of selective neuronal pathways from VTA to NAc, PFC, or amygdala on feeding patterns. (**a**) Rats were infused with Cre-dependent DREADD virus in VTA, and CAV2Cre in either NAc, PFC, or amygdala, to induce DREADD expression in selective VTA projection neurons. DREADD efficiency shows fraction of DAergic (TH+) cells expressing DREADD (mCherry+), while DREADD specificity shows fraction of DREADD expressing cells that was DAergic. Figures represent average number of cells per rat in a unilateral VTA slice, based on 4.1 ± 1.6 samples per rat, $n = 6–7$ rats per group. (**b**) Representative example of feeding pattern following saline treatment vs CNO-induced activation of the VTA to NAc pathway. (**c**–**e**) Effects of CNO treatment (0.3 mg kg^{-1}) on average meal size (**c**), meal frequency (**d**), and total food intake (**e**) in rats expressing hM3Dq in VTA neurons projecting to NAc, PFC, or amygdala. Data are presented as mean ± s.e.m. $n = 9–11$ per group. $^{*}P < 0.05$, $^{***}P < 0.001$ CNO compared to Sal.

Table 2. Additional measures of feeding microstructure, following chemogenetic activation of VTA neurons projecting to NAc, PFC, or amygdala

	Latency to start first meal (min)				*First meal size (g)*				*First meal interval (min)*			
	SAL	*s.e.m.*	*CNO*	*s.e.m.*	*SAL*	*s.e.m.*	*CNO*	*s.e.m.*	*SAL*	*s.e.m.*	*CNO*	*s.e.m.*
VTA > NAc	**0.00**	0.00	**1.93**	1.93	**3.44**	0.40	**1.52****	0.28	**28.16**	10.09	**11.05***	1.26
VTA > PFC	**3.09**	3.09	**0.00**	0.00	**3.35**	0.41	**3.74**	0.69	**10.78**	1.66	**12.82**	3.53
VTA > Amy	**0.00**	0.00	**1.85**	1.85	**3.56**	0.33	**2.86**	0.38	**22.35**	4.15	**12.35**$^{\#}$	1.96

	Average meal duration (min)				*Total feeding time (min)*			
	SAL	*s.e.m.*	*CNO*	*s.e.m.*	*SAL*	*s.e.m.*	*CNO*	*s.e.m.*
VTA > NAc	**13.17**	1.81	**6.48*****	1.40	**35.20**	2.70	**31.71**	5.08
VTA > PFC	**10.37**	0.55	**15.36**	1.94	**34.76**	2.91	**41.73**	5.25
VTA > Amy	**13.17**	0.94	**13.62**	0.92	**35.38**	1.84	**35.33**	2.61

Data represent mean and s.e.m. following treatment with either saline (SAL) or CNO. $n = 9–11$ per group. $^{\#}P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$,$^{***}P < 0.001$. CNO compared to saline. Other tests not significant ($P > 0.1$). The mean values are indicated in bold.

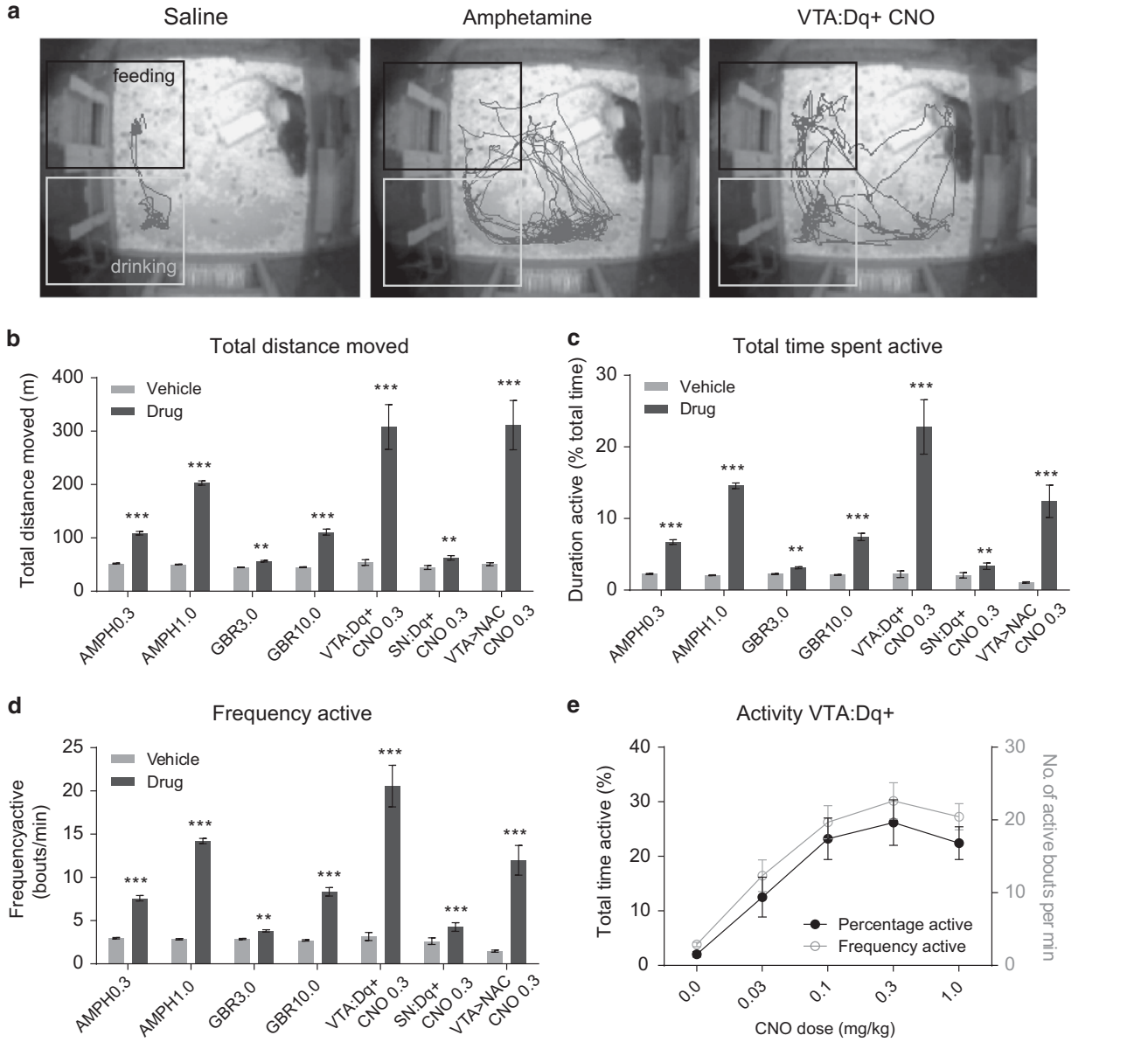

Figure 5. Effect of enhanced DA signalling on locomotor patterns. (**a**) Track visualisation during first 10 min of food access. Example of locomotor activity of the same VTA:Dq+ rat (identical background image) following treatment with saline, amphetamine (1.0 mg kg^{-1}), or CNO (0.3 mg kg^{-1}). Rectangles represent feeding and drinking areas of the home cage. (**b**) Total distance moved (m/2 h). (**c**) Time spent active (% of total time). (**d**) Frequency of active bouts. (**e**) CNO dose-response curve for time spent active (black) and frequency of active bouts (grey) in VTA:Dq+ group. Data are presented as mean ± s.e.m., $n = 7$–15 per group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ drug compared to vehicle.

Decreased meal size and increased meal frequency are mediated by VTA pathway towards NAc, but not PFC or amygdala

To determine which neuronal pathway(s) originating from the VTA underlay the DA-induced effects on feeding behaviour, we selectively targeted VTA neurons projecting to NAc (VTA > NAc), PFC (VTA > PFC), or amygdala (VTA > Amy). DREADD expression in the VTA was confirmed in all groups (Figure 4a), but was most abundant in the VTA > NAc group, consistent with a major output of VTA neurons towards NAc.[34] In the VTA > NAc group, approximately 1/3 of the DA cells expressed hM3Dq, compared to less than 10% in the VTA > PFC and VTA > Amy groups (Figure 4a). Furthermore, a relatively large proportion of DREADD expressing cells was DAergic in the VTA > NAc group (nearly 80%, compared to less than 60% in the VTA > PFC and VTA > Amy groups; Figure 4a).

Representative feeding patterns of a VTA > NAc rat are depicted in Figure 4b. In the VTA > NAc group, CNO treatment significantly decreased the average meal size (Figure 4c), first meal size (Table 2) and average meal duration (Table 2; Treatment × Group interactions all $P < 0.05$; *post hoc* CNO vs saline VTA > NAc group $P < 0.0005$). These effects were not observed in VTA > PFC or VTA > Amy groups (Figure 4c, Table 2; all *post hoc* tests $P \geqslant 0.1$). In addition, the VTA > NAc group showed an increased meal frequency (Figure 4d; CNO vs saline $P = 0.012$; VTA > PFC and VTA > Amy $P > 0.1$) and shorter intervals between the first and second meal (Table 2; Treatment effect $F_{1,28} = 5.35$, $P = 0.028$; *post hoc* CNO vs saline VTA > NAc $P = 0.027$, VTA > PFC $P = 0.907$ and VTA > Amy $P = 0.062$). CNO treatment did not affect the initial latency to start feeding (Table 2), total feeding time (Table 2) or total food intake (Figure 4e; all $P > 0.1$).

To summarise, chemogenetic activation of VTA > NAc pathway decreased the average size and duration of meals, and increased feeding frequency. Activation of VTA > PFC or VTA > Amy pathway had no significant effects on feeding patterns.

Both pharmacological and chemogenetic DA stimulation increase locomotor activity

As DA-induced increased locomotion may disturb feeding, we monitored home cage locomotor activity during the feeding episode. Figure 5a shows exemplifying feeding and locomotor patterns during the first 10 min of food access, following treatment with either saline, amphetamine, or CNO in a VTA:Dq+ rat. Under saline treatment conditions, rats typically started feeding directly upon chow access, and mainly stayed in the feeding (and drinking) zone, while overall ambulatory activity was low. Amphetamine treatment suppressed the initiation of food intake —represented by diminished time spent in the feeding area—and increased locomotor activity. Following CNO treatment, VTA:Dq+ rats rapidly initiated feeding, but showed disrupted feeding behaviour, along with locomotor hyperactivity.

Both chemogenetic and pharmacological stimulation of DA signalling significantly increased the total distance moved (Figure 5b), time spent active (Figure 5c) and frequency of active bouts (Figure 5d), although the magnitude of effects differed considerably between manipulations (Figure 5b–d). CNO treatment had no effect on locomotor activity in VTA > PFC and VTA > Amy groups, or in VTA:Dq- and SN:Dq- control groups (all $P > 0.1$, data not shown). Dose-response testing in VTA:Dq+ rats showed that 0.1, 0.3, and 1.0 mg kg^{-1} CNO induced a maximal hyperactive effect (Figure 5e). The lowest dose, 0.03 mg kg^{-1}, resulted in an intermediate increase in locomotion (Figure 5e).

Thus, both chemogenetic and pharmacological stimulation of DA signalling increased locomotor activity during the feeding episode. The doses of drugs that affected feeding were also effective in inducing hyperactivity, indicating that these outcomes may be related.

DISCUSSION

In this study, we took a novel approach to determine the effects of enhanced DA neuronal activity on feeding behaviour. We selectively activated DA neuronal subpopulations, and found that chemogenetic activation of DA neurons in the VTA, but not SNc, affected feeding behaviour in rats. Specifically, activation of the mesolimbic pathway from VTA to NAc disrupted feeding microstructure, without affecting total food intake.

Chemogenetic activation of VTA DA neurons or the VTA to NAc mesolimbic pathway resulted in smaller and shorter meals, yet had modest or no effect on total food intake. Interestingly, the reduced meal size was accompanied by an increased feeding frequency. This indicates that the rats were stimulated to engage in food intake, but ongoing feeding activities were prematurely aborted. The increase in meal frequency was most prominently observed following selective activation of the VTA to NAc mesolimbic pathway. The majority of neurons in this pathway (~80%) is DAergic.[28,30] Altogether, this suggests that the reduced meal size resulted from enhanced activity of mesolimbic DA neurons. Our findings complement earlier studies showing that blockade of DA D1- or D2-R in the NAc increased meal size,[15,35] indicating a role for mesolimbic DA in meal continuation and cessation. Thus, regarding the question whether activation of dopamine neurons promotes or reduces feeding, we propose that enhancing activity of mesolimbic DA neurons both stimulates and inhibits food intake, by promoting both the initiation and cessation of feeding behaviours, respectively.

Does mesolimbic DA neuronal activity affect food intake through appetite or behavioural activation?

The reduction in meal size and duration may suggest that animals were satiated more quickly following mesolimbic DA neuronal activation.[36] However, satiety would result in postponing the initiation of the next meal, which was not observed. Also, the latency to start feeding was not affected by chemogenetic activation of VTA DA neurons or VTA to NAc pathway (in contrast to treatment with amphetamine or GBR 12909), opposing a reduction in appetite. Instead, the increase in meal frequency suggests that animals were compensating for a reduced meal size, indicating that they were motivated to eat. Taken together, we conclude that it is unlikely that chemogenetic activation of mesolimbic DA neurons directly affects appetite or satiety.

Previously, it was shown that reducing NAc DA signalling through DA depletions or DA-R antagonists affected food intake through effects on locomotor behaviour, including approach behaviour, food handling, or behavioural switching.[35,37,38] In this study, we found that enhancing NAc DA signalling by chemogenetic activation of VTA DA neurons or VTA to NAc pathway induced locomotor hyperactivity, in agreement with findings from earlier chemogenetic and pharmacological studies.[28–30,39–41] In comparison, activation of SNc DA neurons only modestly increased locomotor activity and did not affect feeding. Dose-response testing in VTA:Dq+ rats showed that a low dose of CNO was sufficient to sub-maximally increase locomotor activity, but not to affect feeding behaviour. Higher doses of CNO induced a maximal hyperactive phenotype, and resulted in disrupted feeding patterns. This suggests that the effects of enhanced DA neuronal activity on feeding behaviour—increased cessation and initiation of feeding bouts—may be secondary to effects on behavioural activity.

Previous studies have argued that antipsychotic drugs, which block the DA D2-R, induce weight gain through a larger meal size and reduced locomotor activity, mediated by diminished DA activity.[42–44] Here, we show that chemogenetic activation of VTA DA neurons or VTA to NAc pathway has the opposite effect: smaller meals and locomotor hyperactivity. Mesolimbic DA signalling regulates behavioural activation, including action selection and switching between behavioural actions.[45–47] An increase in mesolimbic DA neuronal activity may thus promote the initiation of feeding activities as well as other activities, resulting in enhanced feeding initiation, as well as a premature cessation of ongoing food intake.

Differential effects of chemogenetic DA neuronal activation compared to pharmacological DA stimulation

Our results show that enhancing excitability of midbrain DA neurons has different effects on feeding behaviour compared to pharmacological DA stimulation by reuptake inhibitors. Consistent with earlier reports, we observed that treatment with amphetamine or GBR 12909 significantly suppressed food intake.[13,14,48,49] This hypophagic phenotype was characterised by a delayed latency to start feeding, reduced meal size and duration, and less time spent engaged in feeding. Chemogenetic activation of midbrain DA neurons reproduced some of these effects, but not all.

The discrepancy in effects on food intake between psychostimulant drugs and chemogenetic activation of DA neurons likely results from different neurobiological mechanisms and different sites of action. The anorectic effects of amphetamine may partially result from enhanced noradrenergic or serotonergic signalling, through interactions with noradrenaline and serotonin transporters, respectively. However, selective DA reuptake inhibition was sufficient to suppress feeding. As amphetamine and GBR 12909 were administered systemically, DA reuptake was blocked throughout the brain, and hypophagic effects may be mediated

by other regions than the ventral striatum, such as the lateral hypothalamus.[13] Thus, while mesolimbic DA signalling appears to be crucial for aspects of feeding behaviour involving food motivation or behavioural activation, other neurobiological substrates are likely to be involved in food consumption and appetite.[50]

Chemogenetic activation of DA neurons in the SNc (projecting to dorsal striatum) or of VTA neurons projecting to PFC or amygdala did not affect feeding behaviour, suggesting that these areas were not directly involved in suppressing food intake. It should be noted that relatively few neurons were transduced when targeting VTA projections towards PFC or amygdala, thus activation of these populations may not have been sufficient to induce behavioural effects. Future studies need to further identify the neurobiological circuits that are involved in DA-mediated regulation of feeding behaviour.

CONCLUSIONS

In this study, we showed that chemogenetic activation of VTA DA neurons or VTA to NAc pathway was sufficient to affect feeding behaviour. Enhancing mesolimbic DA activity seemed to both inhibit and stimulate feeding, reflected in smaller yet more frequent meals. Our results indicate that, while DA-enhancing psychostimulant drugs may affect food intake directly through effects on appetite, chemogenetic activation of VTA DA neurons or VTA to NAc pathway does not. We propose that enhancing mesolimbic DA neuronal activity affects feeding patterns by facilitating both the cessation and initiation of feeding behaviours, and that these effects are related to an increase in behavioural activity.

These findings provide new insights into how DA neuronal activity influences food intake, and may have implications for the role of DA in overeating. However, DA signalling may differentially affect food intake in lean and obese subjects,[48] and future studies are needed in order to translate these findings to the clinical situation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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