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Research Report

Dysregulation of dopamine signaling in the dorsal striatum inhibits feeding

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Abstract

Dopamine signaling is an important component of many goal-directed behaviors, such as feeding. Acute disruption of dopamine signaling using pharmacological agents tends to inhibit normal feeding behaviors in rodents. Likewise, genetically engineered dopamine-deficient (DD) mice are unable to initiate sufficient feeding and will starve by ~3 weeks of age if untreated. Adequate feeding by DD mice can be achieved by daily administration of L-3,4-dihydroxyphenylalanine (L-dopa), a precursor of dopamine, which can be taken up by dopaminergic neurons, converted to dopamine, and released in a regulated manner. In contrast, adequate feeding cannot be restored with apomorphine (APO), a mixed agonist that activates D1 and D2 receptors. Viral restoration of dopamine production in neurons that project to the dorsal striatum also restores feeding in DD mice. Administration of amphetamine (AMPH) or nomifensine (NOM), drugs which increase synaptic dopamine concentration, inhibits food intake in virally rescued DD mice (vrDD) as in control animals. These results indicate that the dysregulation of dopamine signaling in the dorsal striatum is sufficient to induce hypophagia and suggest that regulated release of dopamine in that brain region is essential for normal feeding and, probably, many other goal-directed behaviors.

Theme: Neural basis of behavior
Topic: Monoamines and behavior

Keywords: Amphetamine; Apomorphine; Dopamine; Canine adenovirus; Food intake; Knockout mice; Monoamine; Nomifensine; Striatum

1. Introduction

It has been proposed that dopamine signaling in the dorsal striatum is required for feeding. As early as 1971, Ungerstedt identified the dorsal striatum as being critical for feeding using the neurotoxin 6-OHDA to lesion dopaminergic neurons [47]. Further studies verified that dopamine depletions within the striatum, including striatal regions outside of the nucleus accumbens, lead to aphagia [10,18,20,36]. In addition, genetic inactivation of *tyrosine hydroxylase* (*Th*) selectively in dopamine neurons inhibits feeding [50]. Thus, it is clear that dopamine signaling is essential for feeding; however, dysregulation of dopamine signaling can also inhibit feeding. For example, administration of dopamine receptor agonists, antagonists, or compounds that elevate synaptic dopamine such as amphetamine (AMPH) or cocaine inhibits feeding [1,6,24,29,49]. Two distinct hypotheses have been put forward to explain these results. One is that regulated (phasic) release of

Abbreviations: AAV, adeno-associated virus; AMPH, amphetamine; APO, apomorphine; CAV-2, canine adenovirus type 2; L-dopa, 3,4-L-dihydroxyphenylalanine; CPu, caudate putamen; DD mice, dopamine-deficient mice; vrDD, virally rescued dopamine-deficient mice; NAc, nucleus accumbens; NET, norepinephrine transporter; NOM, nomifensine; 6-OHDA, 6-hydroxydopamine; PBS, phosphate-buffered saline; TH, tyrosine hydroxylase; DAT, dopamine transporter

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dopamine in the dorsal striatum (caudate putamen, CPu) with transient occupancy of dopamine receptors is essential for feeding, whereas chronic occupancy of the same dopamine receptors in that brain region inhibits feeding. The other hypothesis is that dopamine signaling in the striatum (CPu and/or nucleus accumbens, NAc) is essential for feeding, whereas dopamine signaling in the hypothalamus inhibits feeding, that is, separate dopamine circuits stimulate and inhibit feeding [14,16].

The latter hypothesis evolved from experiments in which AMPH was administered to specific brain regions of rats. The greatest inhibition of feeding occurred when AMPH was injected into the lateral hypothalamus [26]. However, AMPH releases not only dopamine, but also norepinephrine and serotonin [26,43]; thus some of the inhibitory effects of AMPH might be mediated by a combination of monoamines at hypothalamic synapses. Consistent with this idea, the inhibitory effects of AMPH injected into the lateral hypothalamus could be blocked by either a dopamine D2 receptor antagonist or a beta-adrenergic antagonist (but not serotonergic antagonists) [24].

Here, we use genetically engineered DD mice to distinguish between these hypotheses. DD mice lack dopamine due to inactivation of the *Th* gene specifically in dopaminergic neurons. DD mice are born normally, but, within ~3 weeks, they become hypoactive, hypophagic, and will die of starvation without intervention [50]. Two methods have been devised that restore feeding in DD mice. The first is to restore endogenous dopamine synthesis and signaling throughout the brain by systemic injection of L-dopa, the product of tyrosine hydroxylase (TH) action and direct precursor of dopamine [31]. L-dopa is taken up by dopamine neurons, converted to dopamine, packaged into vesicles, and released in a behaviorally relevant manner throughout the dopaminergic system. DD mice become hyperactive and hyperphagic following L-dopa administration, consuming all of their daily food within ~9 h after which they return to a dopamine-depleted, severely hypoactive and hypophagic state [45,50]. Persistent feeding can also be accomplished in DD mice by restoring dopamine production in discrete brain regions using viral-mediated gene transfer strategies. Injection of recombinant adeno-associated viruses (rAAVs), expressing both human *TH* and human *GTP cyclohydrolase 1* (*GCH1*) genes, rescues feeding in DD mice when injected into the dorsal striatum [44]. When injected into this brain region, AAV infects local non-dopaminergic striatal neurons that presumably produce and secrete L-dopa, which is taken up by dopaminergic terminals and converted to dopamine for packaging and release. Here, we use another viral approach to restore feeding in DD mice by injecting a recombinant canine adenovirus type 2 (CAV-2) vector [21] expressing *Th* (*CAV-Th*) into the dorsal striatum. *CAV-Th* infects local axon terminals in the striatum and is retrogradely transported to dopamine neuron cell bodies [41] where it can drive the expression of the vector-encoded *Th* gene. Neurons transduced by *CAV-Th* then produce TH, which can be transported back to the nerve terminals where it converts L-tyrosine into L-dopa. Like gene transfer of *TH* using AAV vectors [44], *CAV-Th* injection into the dorsal striatum of DD mice restores feeding such that they no longer require daily injections of L-dopa to survive; these animals are designated as virally rescued DD (vrDD) mice.

Here, we use DD mice to investigate dopamine-dependent feeding under a variety of dopaminergic signaling states: without dopamine (no treatment), by restoring behaviorally relevant release of dopamine throughout the dopaminergic system (L-dopa treatment), or by selectively restoring relevant dopamine signaling to the dorsal striatum (viral rescue). We establish three conditions whereby regulated release of dopamine permits feeding (control, L-dopa-treated DD, and vrDD mice) and measure food intake after perturbing regulated dopamine signaling using pharmacological agents that either disrupt dopamine signaling by chronically activating dopamine receptors (APO), increase extracellular dopamine by blocking reuptake (NOM), or disrupt dopamine signaling by releasing vesicular monoaminergic stores (AMPH). We will show that dopamine release in dorsal striatum (CPu) of vrDD mice is sufficient to restore adequate feeding and that APO, AMPH, or NOM administration to these mice inhibits feeding. These results strongly support the hypothesis that dysregulation of dopamine signaling in the CPu is sufficient to block feeding.

2. Materials and methods

2.1. Animals

All mice were maintained and used in accordance with the guidelines for animal care and experimentation established by the University of Washington Animal Care and Use Committee. Mice were maintained on a mixed C57Bl/6 × 129/SvEv genetic background with standard breeder chow (Picolab, Brentwood, MO; 5LJ5 chow, 11% fat, 4.35 kcal/g) and water available ad libitum. DD mice ($Th^{-/-}$, $Dbh^{Th/+}$) which have two inactive *Th* alleles, one intact *Dopamine β-hydroxylase* (Dbh^{+}) allele and one *Dbh* allele that drives expression of *Th* (Dbh^{Th}), were created as described [50]. Control mice included animals that carry at least one intact *Th* allele and one intact *Dbh* allele; these mice produce normal levels of dopamine and norepinephrine [33,46]. Mice were housed under standard vivarium conditions on a 12 h light/dark cycle with lights on at 07:00. DD mice were maintained with daily injections of L-dopa as described [45].

2.2. Recombinant CAV-Th vector production

The expression cassette *CβA-Th-Polr2a-DsRed2*, containing the chicken β-actin promoter driving expression of rat TH, followed by the RNA polymerase 2 promoter

driving the expression of DsRed2 (Clontech) gene, was cloned into pTCAV-12a to generate pT*CAV-Th* (a pretransfer plasmid) using standard molecular biology procedures. This pretransfer plasmid was linearized and recombined with pTG5412 in *Escherichia coli* BJ5183 [8,22] to generate the transfer plasmid p*CAV-Th*. p*CAV-Th* was linearized and transfected into DKCre cells [40] to generate *CAV-Th*, a recombinant E1-deleted, replication-incompetent CAV-2 vector (for review, see [22]) expressing TH and DsRed. Vector preparation, purification, and titration were performed as described [21,42]. The *CAV-Th* stock preparation had a titer of 6.0×10^{12} physical particles/mL.

2.3. Intracerebral injection of recombinant CAV vector

Mice were anesthetized and placed into a stereotaxic frame (Cartesian Instruments, Sandy, OR), and the head was leveled in the *x*, *y*, and *z* planes using lambda and bregma as landmarks. Coordinates (in mm) for bilateral injections into the dorsal striatum were 0.8 rostral, 2.0 medial and lateral to bregma, and 3.6 beneath the skull surface according to the atlas of Franklin and Paxinos [32]. *CAV-Th* (1.0 μL) was injected through a 5-μL Hamilton syringe at a rate of 0.25 μL/min. After each injection, the needle remained stationary for 2 min and was then raised 0.1 mm for 2 min before it was removed. Mice remained on daily L-dopa treatments for 1 week after viral injection. Mice that maintained their body weight after 2 weeks without L-dopa treatment were designated as vrDD and used for the feeding studies.

2.4. Drugs

Except for L-dopa (see below), all drugs were obtained from Sigma (St. Louis, MO) and were administered intraperitoneally at a volume of 10 μL/g body weight. D-amphetamine sulfate (AMPH) was dissolved in PBS and administered at 2 mg/kg; nomifensine maleate (NOM) was dissolved in PBS and administered at 5, 10, or 25 mg/kg; *R*-(−)-apomorphine hydrochloride hemihydrate (APO) was dissolved in distilled water with 0.25% (w/v) ascorbic acid and administered at 30, 60, 120, 240, or 480 μg/kg. Serial dilutions of APO were made on the same day, and all aliquots were frozen and subsequently thawed on the day of use. L-dopa (Sigma) was dissolved in 0.25% (w/v) ascorbic acid in PBS and administered at 50 mg/kg (33 μL/g).

2.5. Behavioral measures

Locomotor activity was measured in chambers (20 × 20 × 40 cm) equipped with four infrared photobeams (San Diego Instruments, San Diego, CA) that were arrayed 8.8 cm apart along the long axis of the chamber. Photobeam interruptions were recorded by a computer running PASF software (San Diego Instruments), and only consecutive interruptions of adjacent photobeams were counted as an ambulation.

To measure food consumption, mice were acclimated to the activity chamber without food, but with *ad libitum* access to water for 16 h. At the start of the experiment, animals were injected with drug (either L-dopa, NOM, AMPH, or vehicle), and two fresh chow pellets (Picolab, 5LJ5) were weighed and added to the bottom of the activity chambers. Pellets were removed, weighed, and returned to the cage at 0.5, 1, and 2 h time points. Test sessions were separated by 2 days, during which animals were placed into their home cage, and food and water were available ad libitum. DD mice received their last L-dopa injection 16 h prior to an experiment when dopamine levels were ~1% of normal [2,45]. Doses of APO and NOM were given in ascending order of concentration with a vehicle or saline injection on both the first and last day of testing. For food intake experiments with vrDD mice, the order of treatments was: saline, NOM, AMPH, saline, APO, vehicle.

2.6. Histology

Mice were euthanized by lethal injection of 0.2 mL sodium pentobarbital, perfused with PBS followed by 4% paraformaldehyde (pH 7.2) in PBS, and postfixed overnight at 4 °C. Brains were immersed in 30% sucrose, frozen in isopentane, and cut on a cryostat. Free-floating sections (40 μm) were rinsed three times in PBS containing 0.3% Triton-X (PBS-TX) and incubated in 2.5% normal donkey serum (NDS) in PBS-TX to block non-specific binding. Sections were then incubated overnight in PBS-TX/NDS containing rabbit polyclonal antibodies to TH (Chemicon International, Temecula, CA; 1:1000 dilution) and rat monoclonal antibodies to the dopamine transporter (DAT) (Chemicon, 1:2000 dilution). Sections were washed three times in PBS-TX and incubated in PBS-TX/NDS containing donkey anti-rabbit-conjugated Cy2 (Jackson ImmunoResearch, West Grove, PA; 1:200) and donkey anti-rat-conjugated Cy3 (Jackson ImmunoResearch, 1:200 dilution) for 2 h. Sections were washed 3 times, placed on slides, coverslips were applied, and viewed using a Nikon fluorescence microscope.

3. Results

3.1. APO fails to induce normal food consumption by DD mice

To compare the efficacy of endogenous dopamine release with exogenous dopamine receptor activation on food consumption, saline, L-dopa, or APO (30, 60, 120, 240, or 480 μg/kg) was administered to DD mice, and food intake was measured at 60 min (Fig. 1A). Repeated measures ANOVA was used to analyze food intake following vehicle or APO treatment, which revealed a main effect of treatment

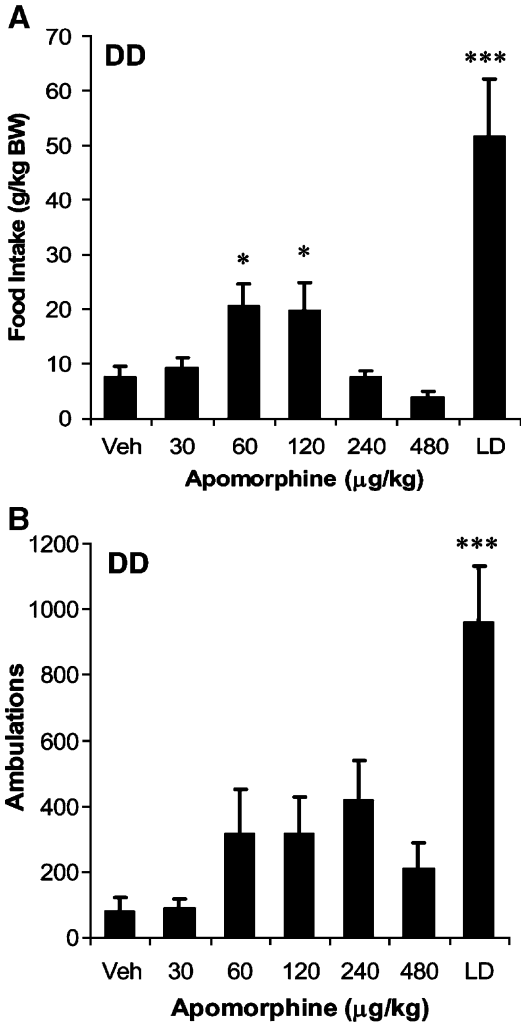

Fig. 1. Apomorphine (APO) fails to stimulate sufficient feeding by DD mice. (A) Food consumption by DD mice in response to APO and L-dopa. Food intake measured as g/kg body weight. (Body weight range; DD: 14.4–19.0 g) (B) Locomotion of DD mice in response to APO and L-dopa (n = 12). Data represent means ± SEM; *P < 0.05 compared to vehicle; ***P < 0.001 compared to vehicle and APO.

[F(5,55) = 8.081, P < 0.001]; Tukey's post hoc analysis revealed a small but significant increase in food consumption by the 60 and 120 μg/kg doses of APO compared with vehicle (P < 0.05). Repeated measures ANOVA for food intake following vehicle, APO, or L-dopa treatments revealed a main effect of treatment [F(6,66) = 12.863, P < 0.001]; Tukey's post hoc analysis revealed that L-dopa treatment significantly increased food intake compared with all doses of APO and with vehicle (P < 0.001). The effects of APO on feeding were not present at longer time points, whereas L-dopa continued to promote feeding for several hours [45]. These data show that this non-specific dopamine receptor agonist stimulates a little feeding (less than 21% of that achieved with L-dopa) but does not sustain food intake in DD mice, whereas regulated release of dopamine following L-dopa treatment allows them to eat enough to survive.

The doses of APO were chosen based on previous studies with mice [11]. However, because DD mice are hypersensitive to dopamine receptor agonists [19], we monitored DD mice and recorded locomotor behavior after APO administration. The locomotor effects of saline, L-dopa, and APO were monitored for 60 min (Fig. 1B). Repeated measure ANOVA of vehicle, APO, or L-dopa treatment revealed a main effect of treatment [F(6,66) = 10.318, P < 0.001]; Tukey's post hoc analysis revealed that L-dopa treatment significantly increased locomotion compared with all doses of APO or saline (P < 0.001). Because it is well established that L-dopa induces robust locomotor behavior in DD mice, we wanted to analyze the APO and vehicle groups independently from L-dopa. This second analysis revealed a main effect of treatment [F(5,55) = 3.25, P < 0.05]. Stereotypy was occasionally observed after administration of the 480 μg/kg dose, while higher doses reliably induced stereotypy (data not shown). Note that some doses of APO (e.g. 240 μg/kg) that stimulate locomotion without stereotypy do not stimulate feeding.

3.2. Dysregulation of dopamine signaling disrupts feeding behavior

Control, L-dopa-treated DD, or vrDD mice that were fasted for 16 h were treated with vehicle or APO (120 μg/kg), and food consumption was measured after 30 min (Fig. 2). This dose of APO was chosen because it represents the peak of feeding and locomotion and did not induce stereotypy. Repeated measures ANOVA revealed a main effect of treatment [F(1,25) = 5.90, P < 0.05]; Tukey's post hoc analysis revealed that APO inhibited food intake in all

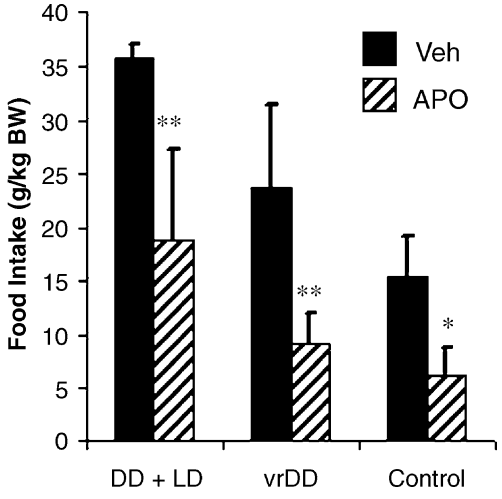

Fig. 2. Apomorphine (APO) inhibits feeding when dopamine is present. Food consumption of DD + L-dopa (LD), vrDD, and control mice 30 min following treatment of vehicle or 120 μg/kg APO. DD + L-dopa animals were pretreated with L-dopa 3 h before treatment. Data represent means ± SEM. DD + L-dopa (n = 8), vrDD (n = 8), and control (n = 7). Food intake measured as g/kg body weight. (Body weight range; DD: 13.9–16.0 g; vrDD: 14.0–23.2 g; control: 20.0–33.3 g) *P < 0.05; **P < 0.01 compared to vehicle.

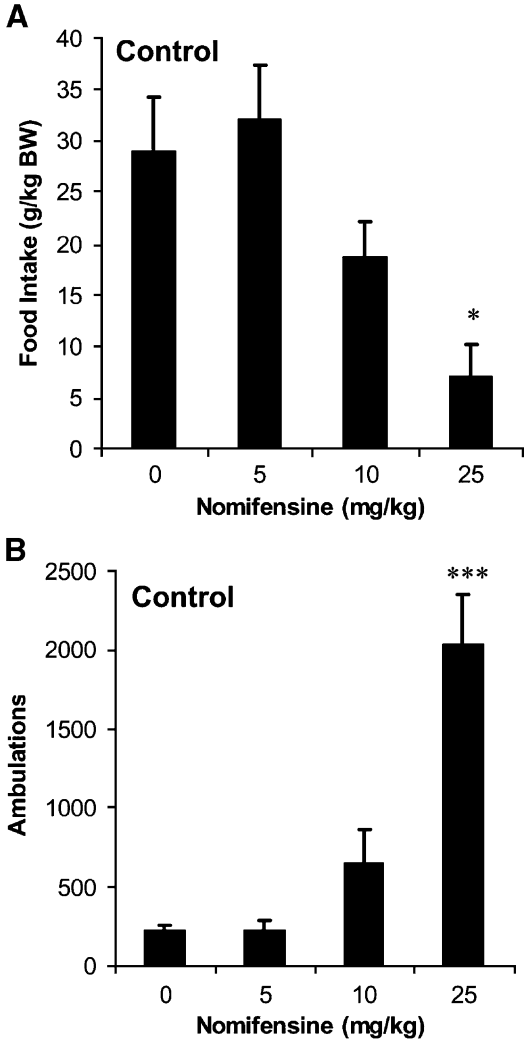

Fig. 3. Nomifensine (NOM) inhibits feeding and stimulates locomotion of control mice. (A) Food consumption of control mice ($n = 8$) was measured 1 h after administration of 0, 5, 10, or 25 mg/kg NOM. (B) Total ambulations of control mice for 1 h after administration of 0, 5, 10, or 25 mg/kg NOM. Data represent mean ± SEM; *$P < 0.05$; ***$P < 0.001$ compared to vehicle.

groups compared with vehicle ($P < 0.05$). A second analysis revealed that feeding behavior was inhibited 39% to 43% across all groups (data not shown).

As an alternative to using the general dopamine receptor agonist (APO), we also tested AMPH, which releases monoamines, including dopamine, into the extracellular space from synaptic vesicles [43]. In an additional experiment, NOM, an inhibitor of the dopamine transporter (DAT) and norepinephrine transporter (NET), was also administered to increase extracellular dopamine in the striatum [4].

A preliminary experiment was conducted to determine an appropriate dose of NOM for behavioral testing. Control mice were administered saline or NOM (5, 10, or 25 mg/kg), and then food consumption (Fig. 3A) and locomotor behavior (Fig. 3B) were measured. Repeated measures ANOVA revealed a main effect of dose on feeding behavior [$F(3,21) = 9.54$, $P < 0.001$]. Tukey's post hoc analysis revealed that the highest dose decreased food consumption significantly compared with all other doses ($P < 0.01$). Analysis of locomotor activity also revealed a main effect of NOM on locomotor activity [repeated measures ANOVA, $F(3,21) = 30.08$, $P < 0.001$]. Tukey's post hoc analysis revealed that the 25 mg/kg dose increased locomotion significantly compared with all other doses ($P < 0.001$).

NOM (25 mg/kg) or AMPH (2 mg/kg, an intermediate dose that stimulates locomotion and inhibits feeding by control mice [6]) was administered to vrDD and control mice, and food intake was measured at 1 h (Figs. 4A, B). Repeated measures ANOVA revealed a main effect of treatment in vrDD [$F(2,12) = 7.95$, $P < 0.01$, Fig. 4A] and in controls [$F(2,14) = 12.29$, $P < 0.001$, Fig. 4B]; Tukey's post hoc analysis revealed that both AMPH and NOM treatments significantly decreased food intake compared with saline ($P < 0.05$) in both genotypes; AMPH and NOM treatments did not differ from each other. These results

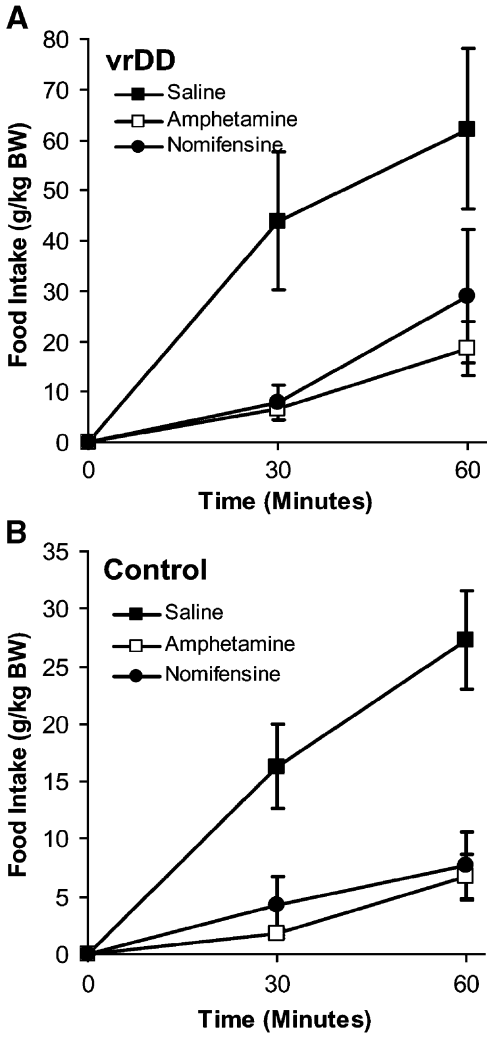

Fig. 4. Nomifensine (NOM) or amphetamine (AMPH) inhibit food consumption in vrDD and control mice. (A) Cumulative food consumption of fasted vrDD mice ($n = 7$) 1 h after NOM, AMPH, or saline injection. (B) Cumulative food consumption by fasted control mice ($n = 8$) after similar treatments. Data represent means ± SEM.

demonstrate that vrDD and control mice decrease food consumption similarly following a pharmacologically induced increase in synaptic dopamine levels.

3.3. Histological confirmation of restoration of tyrosine hydroxylase in dorsal striatum

Immunohistochemistry for TH was performed to measure the extent of viral transduction. Representative sections from control (Figs. 5A–C), vrDD (Figs. 5D–F), and DD (Figs. 5G–I) animals are shown. All brains analyzed from the vrDD mice (n = 6) were similar to that shown in Fig. 5. With the exception of noradrenergic neurons, TH is not expressed in the brains of DD mice, and therefore antibodies against TH (green) can be used to identify areas of viral transduction. Antibodies against DAT (red) identify dopaminergic neurons regardless of whether TH is present. Note that dopamine signaling was restored in the striatum (Fig. 5D) and SNc (Fig. 5E) of vrDD mice but not in the hypothalamus (Fig. 5F). In contrast, TH staining was completely absent in all these areas of DD mice (Figs. 5G–I). We did not quantify the extent of viral transduction (as either the number of TH-positive cells or as extent of DA restoration) because the important aspect of these experiments was to achieve sufficient, but spatially restricted, dopamine signaling to restore adequate feeding. Occasional non-dopaminergic cells in the cortex were TH-positive, presumably due to transduction of the corticostriatal projections (data not shown); however, it is unlikely that these cells could make or release dopamine.

4. Discussion

We have shown previously that viral transduction of a small region of the dorsal striatum with AAV expressing *TH* and *GCH1* is sufficient to rescue feeding by DD mice [44]. After a single bilateral injection, DD mice eat enough chow to maintain body weight for the duration of their lifespan. While viral treatment allows for adequate feeding, we cannot conclude that the feeding pattern and response to signals that normally elicit feeding are normal. Indeed, we have shown that these vrDD mice do not respond to 2-deoxyglucose or insulin by increasing their food intake [15]. Thus, dopamine action in other brain regions may contribute to feeding behaviors in intact animals. Nevertheless, we can conclude that dopamine action in other brain regions, including the hypothalamus, is not essential for feeding when food is available ad libitum. Importantly, dopamine signaling in the NAc, a brain region often associated with reward behavior, is not necessary (or sufficient) to rescue feeding [44].

Here, we used a new viral approach to restore feeding that takes advantage of the affinity of CAV-2 for dopaminergic terminals and its retrograde transport to cell bodies in the SNc [40,42]. This strategy also results in long-lasting dopamine synthesis. All mice that were functionally rescued

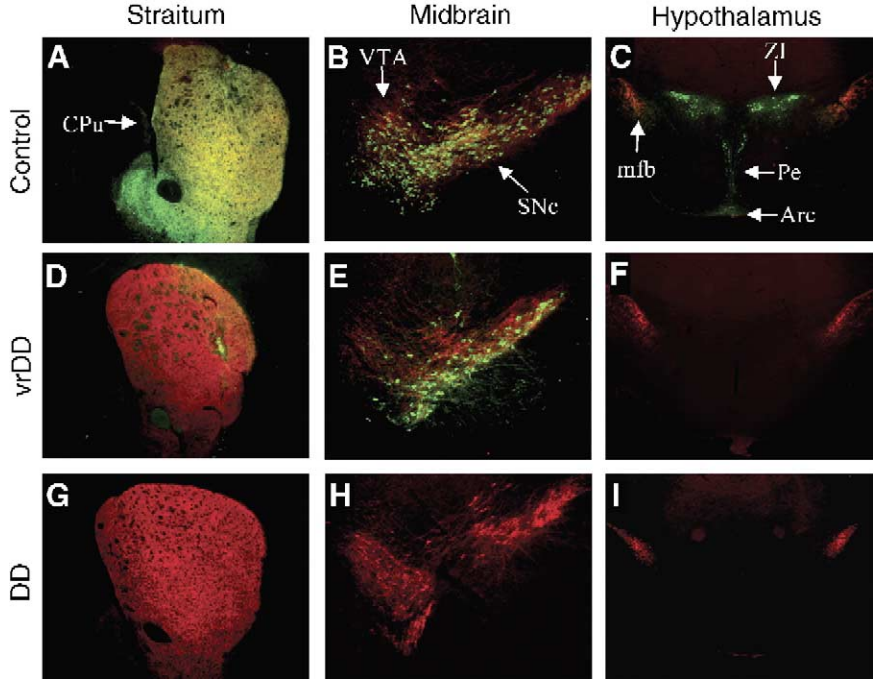

Fig. 5. Immunohistochemistry showing the striatum (A, D, G), midbrain (B, E, H), and hypothalamus (C, F, I) of control (A–C), vrDD (D–F), and DD (G–I) mice. vrDD mice were injected into the striatum with 1.0 μL *CAV-Th.* TH immunostaining is shown in green; DAT immunostaining is shown in red. CPu, caudate putamen; VTA, ventral tegmental area; SNc, substantia nigra compacta; mfb, medial forebrain bundle; ZI, zona incerta (A13 dopamine cells); Arc, arcuate hypothalamic nucleus; Pe, periventricular hypothalamic nucleus.

at 1 week after viral injection (8 of 11 mice) continued to eat adequately for the rest of their lives, surviving over 8 months. This strategy allows us to target TH expression and L-dopa production directly to the dopaminergic neurons, which rules out potential confounds caused by aberrant L-dopa production within striatal cells. We have shown previously that dopamine neurons fire normally in the absence of dopamine [34]; thus, we assume that, after viral transduction, they release dopamine in a physiologically relevant manner.

We conducted several experiments in control, DD, and vrDD mice. Our experiments show that APO fails to stimulate robust feeding in DD mice, although there is a brief stimulatory effect on feeding at low doses. We assume that APO activates the same dopamine receptors that are engaged by dopamine that is released physiologically and that the main difference between the two is that APO activates receptors chronically, whereas dopamine activates them in a phasic manner, e.g. after a burst of action potentials. However, it is also possible that APO activates receptors that are more distant from synapses and hence rarely engaged physiologically. Our results with APO complement previous results obtained with dopamine D1 and D2 receptor-selective agonists (SKF 81297 and quinpirole) that also fail to sustain adequate feeding by DD mice alone, in combination, or upon repeated administration ([19] and unpublished observations). Of the agonists tested thus far, quinpirole has the greatest effect on feeding by DD mice, but, even with optimal amounts of quinpirole, the mice lose too much weight and have to be rescued with L-dopa after a few days [19]. Our results with DD mice are equivalent to those obtained with rats that are severely hypophagic after extensive bilateral 6-OHDA lesions of dopaminergic neurons, and feeding cannot be restored with dopamine-receptor agonists [37]. While small amounts of dopamine-receptor agonist can stimulate a little feeding by dopamine-deficient animals, the same amount of agonist can be inhibitory in normal rodents as discussed below.

Prior experiments showed that dopamine-receptor agonists [30], including APO [9], inhibit feeding by rats and mice [11]. This APO-mediated effect has been postulated to be caused by activation of D2 receptors in the hypothalamus [27]. Here, we used control mice (with normal dopamine signaling) and vrDD mice (with endogenous dopamine signaling restored selectively in the dorsal striatum) to investigate this hypothesis. We found that APO inhibited feeding in control mice, as predicted. APO also inhibited feeding in the vrDD mice, whereas this dose of APO (120 μg/kg) stimulated a little feeding in DD mice. If the inhibitory effect of APO is mediated by activation of D2 receptors in the hypothalamus, it is difficult to understand how restoring dopamine signaling to the dorsal striatum in the vrDD mice can reverse the pharmacological effects of APO.

A more telling test of the hypothesis is derived from the experiments with NOM and AMPH, which respectively block dopamine uptake and purge monoaminergic vesicles. Both of these drugs flood synapses with endogenous dopamine, resulting in chronically activated dopamine receptors. Importantly, extracellular dopamine can only accumulate at sites where dopamine release normally occurs. Thus, in the case of vrDD mice, extracellular dopamine can only accumulate in the dorsal striatum after treatment with AMPH or NOM. Thus, a parsimonious interpretation of our data is that the inhibitory effect of AMPH or NOM on feeding by vrDD mice is due to effects within the dorsal striatum. We suggest that excess extracellular dopamine in the dorsal striatum masks the important signaling properties of phasic (or regulated) dopamine release that occurs in normal mice and in vrDD mice with restitution of TH to dopamine neurons in the SNc.

A substantial body of literature is consistent with the idea that systemic AMPH treatment reduces appetite by release of catecholamines within the lateral hypothalamus [3,9,23–25,28,40]. Leibowitz found that the hypophagic effects of low doses of AMPH injected directly into the lateral hypothalamus can be attenuated with local administration of dopamine receptor antagonists [24]. However, the hypophagic effect of higher doses of AMPH, which cause excessive release of dopamine, cannot be blocked by dopamine receptor antagonists [13,38], perhaps because excessive DA release in the striatum interferes with feeding behavior. We used DD and vrDD mice to test this hypothesis. DD mice do not eat much after dopamine levels fall to ~1% of normal, but they do eat a little. AMPH treatment does not block that small amount of feeding; however, it does inhibit feeding after viral restoration of dopamine signaling specifically to the dorsal striatum using AAV [6], a result that is equivalent to what we have shown here. However, because AMPH releases all monoamines, one could argue that AMPH-induced hypophagia depends on dopamine release in the striatum and norepinephrine and/or serotonin release elsewhere (e.g. the hypothalamus). The experiments with NOM, a DAT/NET inhibitor, were performed to test this possibility. The affinity of dopamine for NET is twice its affinity for DAT [5]. Hence, even though there is little noradrenergic innervation of the dorsal striatum, it may be necessary to inhibit both NET and DAT to adequately prevent dopamine reuptake. To examine whether inhibition of NET function contributes to hypophagia, we tested *Dbh*-null mice that lack the ability to make norepinephrine and epinephrine and found that inhibition of food intake by NOM was the same as in control mice (data not shown). This result is consistent with previous results showing that AMPH-mediated hypophagia also does not depend on norepinephrine [6]. We conclude that disruption of normal dopamine signaling in the dorsal striatum is sufficient to block feeding, but we cannot rule out the possibility that excess extracellular dopamine in the hypothalamus (as achieved by local injection of AMPH) may also disrupt feeding.

Dopamine neurons that project to the striatum have complex firing patterns that include bursts of action potentials. Burst firing of dopamine neurons and transient increases in striatal dopamine levels correlate with feeding and the presentation of feeding-related cues [17,35]. Bursts are elicited by salient environmental stimuli, and they result in a much greater release of dopamine in the striatum than tonic single-spike firing [12,17,39,48]. Thus, under these conditions, there can be large fluctuations of extracellular dopamine, which results in transient and probably differential activation of dopamine receptors in the striatum. Coincidence of these spikes in dopamine release with glutamatergic cortical input is thought to shape the output of striatal projection neurons and to be important for certain types of striatal plasticity [7]. In contrast, flooding the synapses with dopamine (AMPH or NOM) or by occupying receptors with synthetic dopamine agonists (APO) may mask these behaviorally relevant fluctuations in extracellular dopamine. We suggest that execution of goal-directed behaviors, such as feeding, depends on transient occupancy of dopamine receptors that are coupled to corticostriatal signaling. If regulated release of dopamine is important for some goal-directed behaviors, that becomes an important consideration when using cell transplantation for treatment of Parkinson's disease.

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