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.
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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

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