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Dopamine Signaling in the Dorsal Striatum Is Essential for Motivated Behaviors: Lessons from Dopamine-deficient Mice

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Abstract

Genetically engineered mice that lack tyrosine hydroxylase in all dopaminergic neurons become hypoactive and aphagic and they starve by 4 weeks of age. However, they can be rescued by daily treatment with L-dopa, which restores activity and feeding for about 10 hours. Thus, these mice can be examined in both dopamine-depleted and dopamine replete states. A series of behavioral experiments lead to the primary conclusion that in the dopamine-depleted state these mice are not motivated to engage in goal-directed behaviors. Nevertheless, they still have a preference for sucrose, they can learn the location of food rewards, and they can form a conditioned-place preference for drugs. Dopamine signaling can be restored to the striatum by several different viral gene therapy procedures. Restoring dopamine signaling selectively to the dorsal striatum is sufficient to allow feeding, locomotion, and reward-based learning. The rescued mice appear to have normal motivation to engage in all goal-directed behaviors that have been tested. The results suggest that dopamine facilitates the output from dorsal striatum, which provides a permissive signal allowing feeding and other goal-directed behaviors.

Keywords

caudate putamen; dopamine; dopamine-deficient mice; gene knockout; motivation; nucleus accumbens; striatum; viral transduction

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> "Bilateral, complete denervation of the nigro-striatal dopamine pathway produces severe, long-lasting adipsia and aphagia, hypoactivity, difficulties to initiate activity and loss of exploratory behavior and curiosity"
>
> Urban Ungerstedt (1971)

Introduction

For this review, I describe *motivation* as the process by which animals become energized to initiate goal-directed behaviors. I discuss motivation as it relates to feeding behavior, but recognize that the concept refers to other behaviors, such as drinking, sexual activity, and nest building. Motivation, as it relates to internal drives such as those above, often reflects part of a homeostatic system. For example, when an animal has gone without food for some time there is a gradual increase in the motivation (or drive) to eat, which after feeding to satiety, abates and then gradually ascends again to the next meal. Thus, a hungry rat is motivated to obtain food. Sensory inputs from the gut, changes in circulating hormones (e.g. insulin, leptin, ghrelin), changes in circulating nutrients as well as intracellular metabolites signal 'hunger' or 'satiety' and thus modulate the motivation to eat. As internal signals of energy deficiency increase, the animal's attention becomes directed towards the goal of obtaining food. All animal trainers know that hungry animals will work harder than satiated animals, even perform extraneous tricks, to obtain food rewards. Detection of a food source,

or cues that predict one, directs the behavior of a hungry animal towards food procurement. Thus, motivation is closely associated with facilitating goal-directed behavior. It is in this sense that motivation energizes an animal to engage in certain behaviors. The literature on motivation, and the role of dopamine, is extensive and controversial; this area is subject of several thoughtful reviews, in which 'incentive salience', 'wanting' or 'Pavlovian incentive leaning' are used to describe the energizing aspect of goal-directed behavior.1–4 The end result of motivated feeding behavior is engagement of neural circuits necessary to approach a food source, followed by consummatory processes (chewing, swallowing, release of digestive enzymes, etc).4 These consummatory circuits are by and large reflexive, involving both hindbrain and autonomic nervous system in mammals and do not require dopamine or cortical circuits (but may be modulated by them).5 I describe motivation to eat as part of a homeostatic feedback system, governed primarily by internal signals, but it is also influenced by prior experience and a variety of external factors, as well.1–7 Furthermore, motivated behavior depends upon arousal, alertness and attention. A recent review by Dickinson and Balleine provides an in-depth discussion of development of ideas and experiments designed to understand motivation and learning.8

The neural circuits underlying motivated behavior are not completely understood. However, pharmacological and genetic approaches have clearly established that dopamine is essential for motivated behavior, as defined above. There are several dopaminergic systems in the mammalian brain, but viral replacement strategies that I will describe later have pinpointed the dopaminergic circuit from the substantia nigra pars compacta (SNpc) to the dorsal striatum (caudate putamen, CPu) as being a critical pathway. Thus, adult mice completely lacking dopamine fail to engage in goal-directed feeding behaviors and they will starve to death, whereas mice with dopamine signaling restored only to the CPu eat sufficiently for survival.8 These observations help to localize the circuitry involved in motivation; however, the inputs to the nigrostriatal pathway that establish motivation and the outputs from the striatum that ultimately engage the consummatory feeding circuits remain enigmatic. After describing the evidence supporting the importance of dopamine in motivated behavior, I will suggest how the SNpc>CPu dopamine circuit may be activated and how its output facilitates motivated behaviors such as feeding.

It is important to point out that the experiments and conclusions described below are derived mainly from the work of many students in my laboratory during the last 12 years who have been studying a genetic model of dopamine deficiency and selective dopamine restoration. Our conclusion that the SNpc>CPu pathway is critically involved in motivation to engage in goal-directed behaviors, including eating, is contrary to a common view that dopamine signaling in the ventral striatum (ventral tegmental area to nucleus accumbens; VTA>NAc) serves these functions. There are several explanations for this disparity. One is that the VTA>NAc projection has received the most attention by those trying to understand the role of dopamine in reinforcement, reward and addiction and the results have often led to the conclusion that dopamine action in the NAc is necessary for motivation to acquire food or addictive drugs.9–12 Most reviews suggest that dopamine action in the NAc is necessary for motivation to obtain food, but perhaps not for consumption.3–4 Another reason why the VTA>NAc receives more attention in motivational research is that the degeneration of the SNpc>CPu is generally thought to be responsible for the symptoms of Parkinson's Disease, which is usually associated with dysfunction of motor control rather than motivation.

These conflicting conclusions come from different experimental approaches. We start with a mouse that cannot produce dopamine anywhere and has severe behavioral impairments, and then we restore dopamine to different brain regions and ask what behaviors are reinstated. This contrasts with the alternative approach of starting with a normal animal and then interfering with dopamine signaling using either selective application of dopamine receptor

antagonists or selective lesions of dopamine circuits by application of neurotoxins, such as 6-hydroxydopamine (6-OHDA). Each approach has strengths and limitations. The genetic approach is extremely selective and complete, but suffers from the chronic inactivation which increases the potential for compensatory mechanisms to develop. Antagonists allow examination of transient consequences of graded disruption of receptors; but selectivity of drugs, partial receptor blockade, and limited control of distribution are important issues. Lesions can also be graded and selective, but they remove all transmitters, not just dopamine, and they usually elicit gliosis with its attendant confounds. After describing our results, I attempt to rationalize the opposing views on where in the brain dopamine signaling is critical for motivating animals to eat and perform other goal-directed behaviors.

History of dopamine's role in mediating motivation

Evidence for the role of dopamine in mediating aspects of motivation came quickly after the discovery that dopamine was a neurotransmitter in its own right, and not just a precursor of norepinephrine. By the early 1960s, dopamine was established as a neurotransmitter in the brain and it was soon apparent that dopamine levels were reduced in Parkinson's disease (PD).13 Furthermore, the akinesia symptoms of PD could be ameliorated by administration of the dopamine precursor, L-dopa.14 During the next ten years, the use of L-dopa as a treatment for PD became well established. Establishing a rodent model of PD was the next breakthrough. In his classical monograph, Urban Ungerstedt described the use of 6-OHDA to lesion the nigra-striatal dopaminergic system.15 A quote from the abstract of that paper (at the beginning of this review) describes that dramatic phenotype. Although he did not use the word "motivation" in his paper, the "difficulty to initiate activity (bradykinesia) and loss of exploratory behavior and curiosity" describes a rat with a phenotype very similar to the dopamine-deficient mice that I describe below. It is noteworthy that this seminal paper describes a behavioral deficit associated with lesion of the SNpc>CPu dopaminergic projection.

Ungerstedt's experiments also provided a neurochemical explanation for the "lateral hypothalamic syndrome" that had been described previously. Surgical lesions in the lateral hypothalamus were known to produce aphagia, which was ascribed to disruption of a "feeding center" in that area.16 The similarity of "lateral hypothalamic syndrome" to the phenotype of rats with 6-OHDA lesions along with the knowledge that the medial forebrain bundle that carries dopaminergic projections to the CPu passes through the lateral hypothalamus, led Ungerstedt to the conclusion that the surgical lesions had, in fact, severed the nigrostriatal projection.15 Nearly complete ablation of dopamine neurons that project to the ventral striatum (VTA>NAc) does not interfere with normal feeding, but it does compromise the willingness of rats to work for food rewards.17 Unilateral lesions of the nigro-striatal pathway do not affect feeding behavior; in fact, they are commonly used to create a convenient model for testing various dopaminergic therapies.18–19 With incomplete 6-OHDA ablation, adult rats may gradually recover after weeks or months of hand feeding followed by substitution of a palatable diet.19 There are reports that ablation of dopamine neurons in neonatal rats allows them to survive, presumably by facilitating a compensatory adaptation.19–20 However, subsequent experiments suggest that the lesions were incomplete in those studies because surviving rats died if residual dopamine synthesis was inhibited.21–22 We demonstrated that neonatal ablation of dopamine neurons in dopamine deficient (DD) mice does not rescue them, contrary to what would be predicted if killing the neurons allowed dopamine-independent compensatory adaptations.23 Furthermore, killing dopamine neurons of DD mice does not hasten starvation, indicating that these neurons do not make other signaling molecules that are essential for survival. Because complete bilateral 6-OHDA lesions produce severe aphagia that require hand-feeding to keep animals alive, those animals are difficult to maintain and to study. By

contrast, the DD mice described below represent a conditional model in which dopamine signaling can be restored transiently each day or permanently.24

Making dopamine-deficient mice

When I entered the catecholamine field 20 years ago, with the aim of using genetic techniques to study the genes involved in catecholamine biosynthesis, I did not expect that it would lead to studying the neurobiology of motivation. Nevertheless, the advent of gene targeting techniques and the phenotypes of the various knockout mice that we made quickly revealed the importance of dopamine for motivation.

This story began when Qun-yong Zhou and Steve Thomas decided to inactivate the *Tyrosine hydroxylase* (*Th*) and *Dopamine β-hydroxylase* (*Dbh*) genes in mice, respectively. Both individual homozygous knockout (KO) mice resulted in lethality by day 14 of embryogenesis. 25, 26 These results suggested that DBH, and hence norepinephrine or epinephrine, was critical for embryonic survival. Pharmacological rescue with L-3, 4-dihydroxyphenylserine (DOPS), a precursor that can be converted directly into norepinephrine without the action of DBH, proved that mice need norepinephrine (or epinephrine) during development.26 Epinephrine is not essential for survival because mice lacking phenylethanolamine-N-methyltransferase (PNMT), the enzyme necessary to convert norepinephrine into epinephrine, develop normally.27 The exact requirement for norepinephrine during fetal development is not established, but it is most likely important for cardiovascular function.

Restoration of norepinephrine during fetal development by DOPS therapy allows the DBH-KO mice to be born and, remarkably, they do not require further intervention after birth for survival under normal care conditions. DBH-KO mice are smaller than littermates and they display ptosis, but otherwise they appear fairly normal. However, a number of interesting phenotypes can be revealed under appropriate conditions. A striking example is their sensitivity to cold. DBH-KO mice cannot activate brown fat to generate heat and they cannot vasoconstrict their blood vessels; as a consequence, they rapidly lose body temperature at 4°C and will die within a couple of hours at that temperature.28 Males are infertile because they fail to ejaculate and females fail to nurture their pups.29 DBH-KO mice also have deficits in memory retrieval and they fail to manifest a conditioned place preference for morphine.30–31 All of these phenotypes can be reversed by treatment with DOPS. Interestingly, some of the phenotypes of mice lacking norepinephrine resemble those of people with PD, suggesting that loss of both dopamine and norepinephrine contribute to PD.32

To study the role of dopamine, it was necessary to overcome the necessity of norepinephrine during development. We devised a strategy that allows dopamine to be synthesized as a precursor of norepinephrine in noradrenergic neurons, while preventing its synthesis in dopamine neurons. In essence, we inactivated the *Th* gene everywhere and then introduced a *Th* gene under the control of the *Dbh* locus, so that only noradrenergic neurons would be rescued.24 We now refer to these $Th^{-/-}$, $Dbh^{Th/+}$ mice as dopamine-deficient (DD) mice; they have two *Th*-null alleles, one wild-type *Dbh* allele, and one *Dbh* allele driving expression of *Th*. DD mice have normal levels of norepinephrine in both central and peripheral nervous systems, but have brain dopamine levels that are <1% of normal. Dopamine levels are not zero because noradrenergic neurons produce some as a precursor to norepinephrine and they can make some from L-dopa that is produced in the skin by tyrosinase.33

Development of dopaminergic pathways

One might expect that lack of dopamine would perturb development of the nervous system. The dopamine neurons themselves might not survive if they cannot signal properly, their axon terminals might be unable to find appropriate targets, or post-synaptic, medium spiny neurons (MSNs) might not develop properly in the absence of dopamine signaling. However, morphological observations and immunohistochemical analysis of markers of both dopaminergic neurons (e.g. L-amino acid decarboxylase and the dopamine transporter) and post-synaptic cells (glutamic acid decarboxylase, dopamine D1 and D2 receptors, mu-opioid receptors, enkephalin, substance P) reveal essentially no perturbations relative to 15-day-old, littermate controls when examined prior to the first L-dopa injection.24›34 Furthermore, quantitative, electron microscopic analysis of synapses onto MSNs in the CPu reveal nothing unusual.35 Perhaps the best indication that development proceeds normally in the absence of dopamine is the rapid biological response to the first injection of L-dopa. Within a few minutes after L-dopa injection, the DD mice become active, indicating that the system is poised to respond to dopamine once it can be synthesized. Electrophysiological measurements of dopamine neurons in behaving adult DD mice indicate that their firing properties are normal in the absence of dopamine.36 Thus, the excitatory inputs that drive dopamine neuron activity are also intact. As a consequence, dopaminergic neurons are firing blanks - at least, dopamine-depleted blanks. The only major adaptation to lack of dopamine signaling that we have uncovered is the hypersensitivity of post-synaptic MSNs to dopamine, a phenomenon that has been studied extensively in rats with dopamine neuron ablation.19› 37 This hypersensitivity is not due to increased dopamine receptors, but rather to an enhanced signaling downstream of receptor activation. The behavioral hyperactivity is associated with molecular changes in MSNs. For example, the *Fos* gene is readily induced by concentrations of dopamine D1 receptor agonists that have no effect in control mice. 37 Although the mechanisms that produce hypersensitivity are poorly understood, alternative signaling cascades appear to be recruited; for example, activation of the MAP kinase pathway becomes enhanced in DD mice compared to control mice.38 The locomotor hyperactivity that occurs with each daily injection of L-dopa disappears when L-dopa is administered semi-chronically (5 times a day) and this correlates with a loss of *Fos* induction and activation of the MAP kinase pathway.38 These results indicate that the adaptations by MSNs to dopamine deficiency are reversible. There may be some compensation in our DD model because acute depletion of dopamine results in more severe bradykinesia and less responsiveness to various drugs than we observe.39 Developmental analysis revealed that MSNs start out being hypersensitive to dopamine and their responsiveness abates as dopamine release commences between 6 and 10 days after birth.34 Thus, we conclude that the dopamine system develops normally in the absence of dopamine, but there are reversible, compensatory changes in post-synaptic cells that allow them to respond better to dopamine.

Phenotype of DD mice

DD mice are born at the expected Mendelian frequency and they are essentially indistinguishable from control littermates until they are about 10 days old, at which point they become recognizable as being smaller than normal. The body weight of the DD pups peaks at about day 15, after which they lose weight and succumb by 20 to 30 days of age.24 DD pups fail to thrive even if competition with larger littermates is prevented. DD mice can be kept alive by hand feeding a liquid diet; thus, their demise is due to lack of food and water rather than failure of some other system. The observation that suckling behavior is normal for the first 10 days after birth reinforces the idea that different neural circuits are involved in early suckling and adult feeding behaviors with only the latter depending on dopamine. 40

As DD mice lose weight between 15 and 30 days after birth, they become hunched and hypoactive. Fortunately, DD mice can be rescued with L-3,4-dihydroxyphenyalanine (L-dopa), the same drug that is used to treat PD. With daily L-dopa injections starting at about 10 days of age, DD mice eat, drink and grow normally.24 After each L-dopa injection (50 mg/kg body weight, the minimum amount necessary to keep them alive) the DD mice become hyperactive (10 to 20 times more active than control mice, which show no locomotor response to this dose of L-dopa) for a few hours as the L-dopa is converted to dopamine.24,41 As dopamine is metabolized, locomotor activity gradually declines and the mice become hypoactive again after about 10 hr. The peak level of dopamine is ~10% of normal 1 hr after L-dopa treatment and it declines to less than 1% of normal over the next 10 hr. Thus, during each day there is about 10 hr during which DD mice have enough dopamine for eating and other essential activities and ~14 hr when they have insufficient dopamine for normal activities. The fact that DD mice can survive with only 10% of normal dopamine reflects adaptations in MSNs that become hypersensitive to dopamine. For all of the experiments described here, we use this daily injection regimen because it provides us with mice that are either dopamine replete or dopamine depleted.

Imagine two, hungry mice that have not eaten for 24 hr being placed in a large, unfamiliar box. If they are normal laboratory mice, they will spend the first few minutes exploring each other and the new environment; however, two DD mice will stay where they are placed, with no apparent interest in each other or the new enclosure. Then, two food pellets appear. The control mice immediately begin to gnaw on the pellets, while the DD mice appear oblivious, even though the pellets are directly in front of them. All the mice are injected with L-dopa. Fifteen minutes later the control mice are unaffected by L-dopa, but the DD mice become active and begin to eat the food.

DD mice (in the absence of dopamine) are not catatonic with muscular rigidity. When hand held, they are docile but readily move to maintain their balance. They right themselves quickly after being flipped onto their back, and they swim when placed in water. However, when gently placed in a new position, even an awkward one (for example, with front paws on a 3-cm high ledge, or hanging by front paws from the edge of a large beaker), DD mice will stay in that position much longer than normal mice. There is a phase ~30 hr after the last L-dopa injection when DD mice ambulate about the cage and crawl upside down on the cage top like normal mice; however, they do not eat much food during this period.41 When they do eat, they are indistinguishable from normal mice, but they quit before they would be satiated. When given the choice of plain or sweetened water, DD mice prefer the sweet solution (sucrose or saccharin), just like normal mice.42 Thus, DD mice can taste sweet substances and they can make a choice to consume what they prefer. If one records the number of bouts of drinking from the dispenser of sweet solution using a device that records every lick, the DD mice initiate drinking ~20 times less often than control mice during a 15-hr period. However, once initiated, DD mice lick faster and drink more than the controls.42 These observations indicate that DD mice can get to food and they have the motor capacity to consume it, but they rarely initiate feeding behavior.

DD mice lack intrinsic feeding responses

Do DD mice fail to perceive hunger (the internal changes that occur during food deprivation), or do they fail to respond to hunger signals? Normal mice respond to experimentally induced hypoglycemia in two ways; they initiate feeding and they activate counter-regulatory pathways that stimulate glucose production by the liver. For example, mild hypoglycemia induced by an injection of insulin stimulates robust feeding. DD mice have no feeding response to insulin, although their counter-regulatory response is normal.43 Likewise, DD mice fail to eat in response to 2-deoxyglucose injection, which lowers

intracellular glucose, whereas control mice manifest a typical glucoprivation feeding response.43 Cellular hypoglycemia is detected by brain-stem neurons, which then trigger feeding and autonomic counter-regulatory responses.44 The fact the DD mice engage the counter-regulatory response to hypoglycemia indicates that this hunger signal is detected by the hindbrain neurons, but the feeding response is blocked in the absence of dopamine.

Likewise, normal mice eat voraciously after neuropeptide Y (NPY) is injected into the brain; however DD mice fail to respond to this treatment. NPY induces *Fos*, a marker of neuronal activation, in the hypothalamus of DD mice, again suggesting that the peptide is detected, but feeding behavior is blocked in the absence of dopamine.43 Leptin-deficient mice are hyperphagic and become morbidly obese. Mice lacking both leptin and dopamine eat voraciously and became obese when given L-dopa daily. However, whenever L-dopa injections cease, they stop eating.45 Thus, the signals that drive feeding in the absence of leptin do not override the need for dopamine. The actions of NPY and leptin are mediated primarily by hypothalamic circuits. Thus, we suggest that dopamine provides a permissive signal that allows hypothalamic signals to engage feeding behavior, but where in the brain these two signals converge is unknown.

Learning in the absence of dopamine

Perhaps DD mice have difficultly learning relationships between nutritional or hedonistic value of food and places where it can be found. We used a T-maze task to determine whether DD mice could learn to find a food pellet in the left arm of the maze. When treated with L-dopa just before training, DD mice learn this task as well as control mice. However, untreated DD mice do not explore the T maze and hence do not learn the location of the food. We discovered that caffeine stimulates some locomotion and feeding by DD mice without inducing striatal Fos as L-dopa or D1R agonists do.46 Thus, we treated DD mice with caffeine to allow to allow them explore the T-maze and eat food pellets when they were discovered. The caffeine-treated mice failed to demonstrate that they had learned where the food pellets were even after 10 days of training with 10 trials per day; they still turned left (the side with available food pellets) only half of the time, whereas controls turned left 80% of the time after a few days of training. But, when the caffeine-treated group of DD mice was then given L-dopa, they chose the left arm 70% of the time on the first day of testing. We conclude that the DD mice had learned the location of the food without dopamine, but did not manifest that learning until dopamine was restored.47

Using a similar logic, we showed that DD mice can learn to associate the pleasurable effects of morphine or cocaine with a particular environment where the drugs were administered using a conditioned place preference paradigm.48,49 The mice were exposed to the drugs in the testing chamber while dopamine depleted and in the absence of caffeine, but were given caffeine during the testing phase so that the DD mice would ambulate and demonstrate a place preference. These experiments indicate that DD mice can learn associations between food (and drugs) and particular places in the absence of dopamine (and in the absence of caffeine). In these examples of learning in the absence of dopamine, we do not know whether the DD mice learned as rapidly as normal mice or whether they used the same strategies and neural circuits as normal mice.

Motivational deficiency

To summarize, DD mice become hypophagic, adipsic and bradykinetic beginning about 10 days after birth, at the time when the dopamine system becomes functionally engaged. They appear apathetic and show minimal interaction with other mice or their environment. Although DD mice can move sufficiently to find and consume food, they do not eat enough to survive even when palatable food and water are readily available. They do not respond to

intrinsic metabolic/hormonal changes associated with food-deprivation, despite their perception of these changes. These observations suggest that DD mice starve because they are not motivated to respond to hunger signals. They also fail to build a nest by shredding a "nestlet".8 Hence, we suggest that dopamine is necessary for mice to engage in many, if not all, goal-directed or motivated behaviors. To gain additional insight, we asked where in the brain is dopamine is required to restore goal-directed behaviors.

Viral restoration of dopamine signaling in the dorsal striatum restores goal-directed behaviors

Shortly after making DD mice, we discovered that they could be rescued by daily, intraperitoneal injections of L-dopa.24 We assume that the L-dopa is taken up by dopamine neurons, metabolized to dopamine by L-amino acid decarboxylase, transported into synaptic vesicles by the vesicular monoamine transporter (VMAT2), and released when dopamine neurons are activated. Thus, the next goal was to restrict L-dopa delivery to a small region of the brain to determine where it would be sufficient to restore feeding.

The first strategy was to infect cells in the striatum with a recombinant virus so that they would make and secrete L-dopa. We reasoned that local production and secretion of L-dopa would allow axons of dopamine neurons to take it up, metabolize it to dopamine, and release dopamine in a regulated manner. We used recombinant adeno-associated viruses (AAV) driving expression of both tyrosine hydroxylase (TH) and GTP-cyclohydrolase 1 to accomplished this goal. The later virus was necessary to allow the striatal cells to make tetrahydrobiopterin, an essential co-factor for tyrosine hydroxylase. Bilateral injection of this combination of viruses into the middle of the dorsal striatum (CPu) restores feeding.8 Most virus-treated mice ate adequately for the rest of their lives without further L-dopa injections. A control virus expressing green fluorescent protein, or injection of only the virus expressing TH tyrosine hydroxylase, does not rescue feeding. When a suitably small volume (<0.5μL) of viral mixture is injected slowly, there is a small sphere of viral infection at the site of injection without obvious spread to other brain regions.8 The zone of tyrosine hydroxylase TH expression (in an otherwise *Th*-null background) can be visualized by immunohistochemistry. Chemical determination of dopamine content in punches from various brain regions confirms the local restoration of dopamine production; after injection into CPu it is typically ~25% of normal levels.8 We take advantage of the hypersensitivity of MSNs of DD mice to dopamine D1 receptor agonists, to show that *Fos* induction persists throughout the striatum of virally treated mice, except in the area expressing tyrosine hydroxylase.8 This experiment provides functional confirmation that chronic dopamine production is limited to the area of viral transduction. In a typical rescue experiment, dopamine signaling is restored to cells in the middle of the dorsal striatum that represent about 10% of the entire CPu area. Less often, rescue is achieved by viral delivery to the dorsal-lateral striatum. Rescue of feeding was never achieved with unilateral viral injections using AAV. Significantly, feeding was never restored after viral transduction in the NAc, although other behaviors were restored after viral transduction of that brain region.8,50 Locomotor activity of these virally rescued DD mice is improved, but not equivalent to that of control mice. These results, obtained with over 100 mice that were rescued by four different students, indicate that dopamine signaling that is restricted to the CPu is sufficient to allow adult DD mice to eat enough normal chow to maintain body weight for at least a year without further intervention.

A second effective viral rescue strategy uses canine adenovirus engineered to express TH. This virus infects axon terminals and is retrogradely transported. Thus, by injecting this virus into the dorsal striatum one can transduce both dopaminergic and cortical neurons that project to that injection site. Immunohistochemistry reveals intense tyrosine hydroxylase-

positive cell bodes in the SNpc, a few in the VTA, and occasional positive cells in the cortex.51 With this strategy, GTP-cyclohydrolase I is unnecessary because the dopaminergic cells already express this critical enzyme. Although some cortical neurons were transduced by this virus, tyrosine hydroxylase was not abundant (perhaps due to lack of the essential co-factor to stabilize the enzyme). More importantly, cortical neurons lack enzymes and transporters necessary for metabolism of L-dopa and secretion of dopamine; thus, they cannot acquire a dopaminergic phenotype. Like the previous method, these virally treated DD mice eat adequately for the rest of their lives. This viral approach differs fundamentally from the previous one in that L-dopa is synthesized by dopaminergic neurons that project to the dorsal striatum rather than ectopically by cells within the striatum. The rescue of feeding by this viral strategy reinforces our conclusion that dopamine signaling in the CPu is sufficient for feeding.

A third rescue strategy relies on Cre recombinase-mediated reactivation of an inactive tyrosine hydroxylase allele (Th^{fs}). This *Th* allele is disrupted by a neomycin resistance (*NeoR*) gene flanked by loxP sites that resides in the first intron. DDfs mice ($Th^{fs/fs}$, $Dbh^{Th/+}$) with two copies of the flox-stop Th^{fs} gene and a *Th* gene inserted into the *Dbh* locus, have the same phenotype as the original DD mice.52 Injection of canine adenovirus engineered to express Cre recombinase (CAV2-Cre) into the CPu of these mice results in retrograde transport and reactivation of the endogenous *Th* genes in all dopaminergic neurons that project to the site of injection. Hence, expression of the *Th* gene is permanent and under normal physiological control. Even though recombination of the *Th* gene is presumably occurring in other neurons (e.g. cortical neurons) that project to the dorsal striatum, it would not be expressed. With this strategy, injection of CAV2-Cre into the small area within dorsal striatum results in tyrosine hydroxylase staining throughout the dorsal striatum because the dopaminergic axons branch extensively with collaterals that pervade the entire region. Perhaps as a consequence of either the widespread restoration of dopamine signaling within the dorsal striatum or the higher level of dopamine in the striatum (~50% of normal), unilateral viral transduction also rescues feeding by DDfs mice.52 This is a satisfying result because unilateral 6-OHDA ablation of dopamine neurons does not disrupt feeding. Virally rescued DDfs mice (vrDDfs) eat more than control mice and gain weight to match or exceed that of littermate controls. Analysis of their feeding pattern indicates that vrDDfs mice have similar daily rhythm, but they have slightly larger meals than controls. The increased feeding may be related to the hyperactivity of vrDDfs mice at night compared to controls. The performance of vrDDfs mice on a rotarod is greatly improved compared to untreated DDfs mice, but still inferior to that of controls.52 We conclude that restoration of dopamine signaling to the CPu via this method goes a long way towards restoring normal feeding and locomotor activity. Differences in the behavior of vrDDfs mice from controls presumably reflect lack of adequate dopamine signaling in other brain regions, but it could reflect subtle consequences of mouse development without dopamine and/or multiple L-dopa injections prior to viral rescue.

The conclusion from all of the viral rescue strategies is that dopamine signaling in the CPu is sufficient to restore feeding on normal lab chow. Rats with >95% loss of dopamine neurons that project to the NAc are viable and eat normally, a result that supports the importance of dopamine signaling in the CPu for feeding.3 We have, however, noted some deficiencies that suggest feeding-related roles for dopamine outside the dorsal striatum. Virally rescued DD or DDfs mice eat the same amount as controls after a 24-hr fast when presented with breeder chow or liquid diet, but the rescued mice eat less than controls when presented with less palatable standard chow, suggesting that dopamine action in other brain regions influences food choice.43,53 Viral rescue of DD mice restores feeding responses to neuropeptide Y, but not to glucoprivation generated by either insulin or 2-deoxy-D-glucose.43 This result suggests that dopamine signaling in the hypothalamus or ventral striatum is

necessary for feeding responses to glucoprivation. The most dramatic consequence of chronic absence of dopamine outside of the dorsal striatum is gradual proliferation of lactotropes, and to a lesser extent somatotropes, in the pituitary leading to massive elevation of prolactin levels and smaller increases in growth hormone in the blood 12 months after rescue. The proliferation of lactotropes is due to loss of dopamine inhibitory tone from the hypothalamic tuberoinfundibular dopamine system.53 These hormonal changes may influence feeding behavior as virally rescued DD mice age.

If we accept that dopamine is necessary for motivated behavior, then these viral rescue experiments indicate that dopamine signaling in the dorsal striatum is sufficient to allow feeding, drinking, and nest building - all examples of motivated behaviors. Although not examined quantitatively, mice with dopamine restored only to the CPu also breed successfully, so at least some social interactions are also restored. DD mice do not perform in the appetitive T-maze task described above, but after viral rescue in the CPu, they perform as well as control mice.54 DD mice also do not perform in an operant task where they have to press a lever for food rewards, even with caffeine. However, after viral rescue in the CPu, they learn this task as well as controls. They also learn to press an active lever that was designated by a light as well as control mice. Furthermore, the rescued mice will work just as hard (140 lever presses per reward) as control mice to obtain food rewards.55 These observations suggest that dopamine signaling in the CPu is sufficient for many aspects of motivated performance. Thus, the SNpc>CPu dopamine pathway appears to be part of the circuit necessary for motivated behavior. But, how does dopamine act in the CPu to facilitate goal-directed behaviors? To answer this broad question, we need to know more about the target neurons in the CPu, which dopamine receptors they express, and the physiological consequences of dopamine receptor activation.

Dopamine receptors and feeding behavior

There are at least five dopamine receptors the dopamine D1 and D5 receptors are coupled to $G\alpha_{s/olf}$ and their activation promotes cAMP production and neuronal excitability, whereas the dopamine D2, D3 and D4 receptors are $G\alpha_{i/o}$ coupled, and their activation inhibits adenylyl cyclase and promotes hyperpolarization of neurons. Only the D1 and D2 receptors are expressed abundantly in the CPu; the other receptors are expressed at lower levels, primarily in other brain regions. I will concentrate on D1 and D2 receptors in the following discussion of how dopamine action in the CPu facilitates goal-directed behavior. Mice with genetic inactivation of either dopamine D1 or D2 receptors are viable. D2R-KO mice appear normal, but D1R-KO mice are smaller than littermates and they need to be weaned later than normal and given palatable, easily accessible food for optimal survival after weaning.56 These observations suggest that constitutive loss of D1R signaling may compromise some aspect of feeding, but this possibility has not been rigorously explored. Mice lacking both D1 and D2 receptors die as neonates (even earlier than DD mice), apparently due to gastrointestinal dysfunction.57 No effects on feeding have been described for mice lacking the other three dopamine receptors.

There is an enormous literature describing the effects of dopamine receptor antagonists on many aspects of motivated behavior and reward, usually in the context of self-administration of drugs, electrical self-stimulation, or operant performance for food rewards. The literature relating the effects of dopamine receptor antagonists on feeding *ad libitum* are more relevant to the perspective being developed here. High doses of either D2R or D1R receptor antagonists delivered systemically inhibit feeding but they are hard to interpret because they also interfere with locomotion.3,4,58,59 Delivery of lower doses of dopamine receptor antagonists, or their delivery selectively into the NAc, such that locomotion is not impaired,

does not appear to interfere with consummatory aspects of feeding, although anticipatory (or appetitive) aspects of feeding may be compromised.3,4

Surprisingly, the converse experiment of administering dopamine receptor agonists (for example, the non-specific D1 and D2 receptor agonist, apomorphine) inhibits feeding transiently.51 Likewise, drugs such as amphetamine that release dopamine into the extracellular space inhibit feeding.60 We have shown that amphetamine-mediated hypophagia still occurs in mice that lack either D1 or D2 receptors, suggesting that signaling by both may be involved in the hypophagic response.60 Interestingly, DD mice do not manifest either amphetamine- or apomorphine-mediated hypophagia; in fact, these drugs stimulate a little feeding. However, if dopamine signaling is restored to the CPu by viral transduciton, then these drugs inhibit feeding again.60 We suggest that flooding dopamine receptors in the CPu with dopamine receptor agonists masks the normal spikes of dopamine signaling that result form burst firing of dopamine neurons and this inhibits feeding.51,60 Administration of either D1 or D2 receptor agonists to DD mice stimulates locomotion, but only D2 receptor agonists facilitate feeding, but not enough to maintain their body weight. Overall, these experiments suggest that for DD mice, D2 receptor activation is more important for feeding than D1 receptor activation, but in a normal mouse, activation of either receptor has little effect and activation of both (as with apomorphine or amphetamine) inhibits feeding.

Taken together, the results are consistent with the hypothesis that dopamine signaling via both D1R and D2R in response to phasic release of dopamine from SNpc neurons that project to the CPu is necessary for feeding, and probably many other motivated behaviors. Conditional inactivation of MSNs selectively in the CPu would provide a critical test of this hypothesis.

How does dopamine signaling in the CPu facilitate goal-directed behaviors?

Dopamine axons from the SNpc innervate GABAergic MSNs, which comprise ~90% of neurons in the CPu, as well as a small population of tonically active cholinergic neurons. The dopamine neurons synapse on the dendritic spines of MSNs near glutamatergic synapses from cortical projections and to a lesser extent from thalamic projections. Approximately half of the MSNs express D2 receptors and they form the indirect output pathway, whereas the other half express D1 receptors and form the direct pathway.61

Dopamine neurons fire in a slow, irregular pattern interspersed with bursts of activity that occur in response to salient environmental stimuli.62,63 The bursts of dopamine neuron activity produce brief spikes of extracellular dopamine in target areas such as CPu and NAc. 64 The tonic activity of dopamine neurons primarily activates D2 receptors, which have a higher affinity for dopamine, whereas the transient spikes in dopamine can activate D1 receptors. MSNs display “up” and “down” states.65 The down state is one in which the cells are hyperpolarized by intrinsic properties and synapse-driven inhibitory inputs. The MSNs are driven into the up state by strong convergent glutamatergic input which, when combined with spikes in dopamine release, enhances the generation of action potentials.65, 66 Strongly activated GABAergic MSNs can also inhibit neighboring MSNs via collaterals. Thus, dopamine spikes enhance strong cortical inputs and suppress weak ones, allowing even better discrimination. The enhanced GABAergic signaling by activated MSNs presumably relays signals that allow the animal to concentrate attention on the salient events that elicited the glutamatergic input. In the absence of dopamine, the MSNs do not fire normally in response to salient events and consequently such animals may be unable to discriminate between various environmental stimuli. As a consequence, DD mice rarely

respond to salient stimuli by initiating goal-directed activities. Even when DD mice are nutritionally deprived, and their homeostatic systems are presumably indicating 'hunger,' DD mice rarely respond to the sight or smell of food. This description predicts that the apparent apathy of DD mice is due to lack of differential activation of the MSNs that send GABAergic projections via the direct and indirect pathways to the substantia nigra reticulata (SNr), globus pallidus, and other brain regions. The dopamine-modulated, GABAergic output from the CPu presumably provides a permissive signal that allows animals to respond to the sight and smell of food and direct their attention towards food retrieval and consumption when the brain perceives energy deprivation.

The dopamine motivational circuit and initiation of feeding

The specificity of which MSNs become activated depends on the activity of particular corticostriatal, glutamatergic inputs into the CPu rather than on dopaminergic inputs because many dopaminergic neurons are activated simultaneously by salient environmental stimuli and each dopaminergic neuron makes thousands of synapses onto large numbers of MSNs. Both indirect and direct outputs from the MSNs ultimately impinge on the SNr, which then relays signals to the thalamus, motor cortex and other brain regions necessary for voluntary actions. For feeding, the animal must initiate voluntary movements to approach and then consume the food. Once food is in the mouth, the hindbrain coordinates motor control of chewing, swallowing and digestion.5 The hypothalamus relays homeostatic information related to nutrients and energy balance to many brain regions including the hindbrain to help regulate the initiation and duration of meals to achieve energy homeostasis.6–7 I suggest that that the output of the SNr represents a permissive, motivational signal because the direct and indirect pathways from the D1R- and D2R-expressing MSNs, respectively, converge in the SNr where their activities are presumably integrated. However, the fact that D1R-KO or D2R-KO mice manifest many motivated behaviors, suggests that either pathway is sufficient. The important point is that in the absence of dopamine the output from the CPu does not provide a permissive, motivational signal that allows feeding or other goal-directed behaviors to begin. In the case of feeding, the absence of that permissive signal from the SNr overrides hunger signals from the hypothalamus and other brain regions. The end result is that an animal lacking dopamine signaling in the CPu will starve.

I have emphasized the role of dopamine signaling in the CPu because restoration of dopamine signaling there restores feeding. However, VTA>NAc dopamine signaling is usually thought to mediate the motivational aspects of feeding and reward.9–12 How can these opposing views be rationalized? One possibility is that the role of dopamine in motivation is distributed in both CPu and NAc and that functions normally attributed to the VTA>NAc pathway can be also performed by the SNpc>CPu pathway. In fact, contemporary ideas on the behavioral organization of the striatum turns it about 30 degrees, such that the primary gradient is dorsalateral to ventromedial with the retrorubral dopamine neurons projecting dorsolaterally, VTA neurons projecting ventromedially, and the SNpc neurons projecting to a broad intermediate zone.67,68 Thus, our viral rescue strategies that are typically centered on the center of the dorsal striatum may restore partial functions to both dorsolateral and ventromedial striatum. This idea is supported by the similar anatomy and properties of dopaminergic and glutamatergic inputs onto MSNs from dorsal or ventral striatum.68 Furthermore, the dopamine neurons in both the SNpc and VTA usually respond to salient stimuli.68 A more systematic and restricted viral rescue strategy aimed at different subregions of the striatum of DD mice may, therefore, provide better discrimination of its various functions. Another consideration is that the critical role of SNpc>CPu pathway in mediating motivation is unappreciated because of the difficulty studying it by traditional methods. Complete lesions of the SNpc>CPu pathway result in severe motor impairment as well as aphagia, thus the animals require tube-feeding for survival and most behavior

experiments are impossible because the animals are inactive. This fact explains why complete lesions of that pathway have not been examined by those who champion the importance of the VTA>NAc pathway in facilitating motivated behavior. Moreover, incomplete lesions of dopamine signaling in the dorsal striatum may be insufficient to reveal its importance; for example, we have determined that the minimal amount of dopamine necessary in the dorsal striatum for adequate feeding is between 3–5% of wild-type levels.8 Thus, SNpc>CPu lesions that remove 95% of dopamine in the CPu may still allow almost normal goal-directed behavior. The same argument applies to dopamine receptor blockade. I propose that dopamine signaling in the CPu is essential for motivation while dopamine signaling in the NAc modulates aspects of motivation and reward processing. Using a radio as an analogy, the tuning control (VTA>NAc) modulates the output but is subservient to the volume control (SNpc>CPu) which needs to be turned up for detection of the signals. Hence, most studies on dopamine action in the NAc explore the less critical modulatory actions, but miss the essential dopamine functions in the CPu.

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