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Research report

Viral restoration of dopamine to the nucleus accumbens is sufficient to induce a locomotor response to amphetamine

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Abstract

Administration of amphetamine to mice evokes hyperlocomotion. Dopamine deficient (DD) mice, in which tyrosine hydroxylase (TH) has been specifically inactivated in dopaminergic neurons, have a blunted response to amphetamine, indicating that the hyperlocomotive response requires dopamine. Dopamine production can be restored to specific brain regions by using adeno-associated viruses expressing TH and GTP cyclohydrolase 1 (GTPCH1). Restoration of dopamine specifically to the nucleus accumbens (NAc) of DD mice completely restores the ability of these mice to respond to amphetamine. This response is specific to the dopamine production in the NAc, as restoration of dopamine production to the caudate putamen (CPu) does not fully restore the hyperlocomotive response to amphetamine. These data support previous studies in which accumbal dopamine is required for producing a normal locomotor response to amphetamine and further show that release of dopamine restricted to the NAc is sufficient for this response

Theme: Neural basis of behavior

Topic: Drugs of abuse: amphetamine and other stimulants

Keywords: Nucleus accumbens; Dopamine-deficient mouse; Adeno-associated virus; Tyrosine hydroxylase

1. Introduction

The psychostimulant amphetamine has been shown to cause specific behaviors in rodents. Low dosages induce locomotion, whereas higher dosages induce stereotypic behaviors in both rats and mice [8]. Amphetamine has been shown to be rewarding in conditioned place preference and self-administration paradigms [5,21].

The neurotransmitter dopamine (DA) has been shown to be necessary for amphetamine-induced behaviors. Administration of α-methyl-*p*-tyrosine, an inhibitor of tyrosine hydroxylase (TH), depletes the brain of both DA and norepinephrine (NE) and inhibits the response to amphetamine [18,22]. DA is believed to be the more critical transmitter in amphetamine-induced responses, because specific depletion of NE does not abolish amphetamine-induced behaviors [19,28], and in fact makes mice more sensitive to amphetamine [30].

Amphetamine is believed to act on the DA transporter by inhibiting reuptake of DA [20] and by causing release of DA from presynaptic neurons through reverse transport of vesicular DA [23]. The dopaminergic pathway believed to be most important for amphetamine-induced behaviors is the mesolimbic system, which consists in part of dopaminergic projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). Infusion of amphetamine directly into the NAc induces locomotion [14,16], and specific lesions of the dopaminergic projection

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to the NAc with 6-hydroxydopamine (6-OHDA), resulting in the depletion of DA from the NAc, lead to a reduction of the locomotor response to amphetamine [7,8,12]. Similar treatments in the caudate putamen (CPu), a critical region of the nigrostriatal pathway, do not produce the same effects, indicating DA release in the NAc is necessary to produce the locomotor response to amphetamine [3].

We have used gene-targeting techniques to generate mice that lack TH specifically in dopaminergic neurons [31]. As a result, these mice are dopamine deficient (DD) but have normal levels of norepinephrine in noradrenergic neurons. These mice have a severe hypoactive phenotype, both basally and in response to a novel environment, when compared to litter-mate controls. DD mice resemble rats with bilateral 6-OHDA lesions, which are also extremely hypoactive [29]; however, unlike lesioned rats, DD mice have intact dopaminergic neurons. Treatment of DD mice with moderate doses of amphetamine evokes locomotor responses that are reduced, in terms of both duration and magnitude, relative to that of control animals [27]. Because these mice receive daily injections of the DA precursor, L-dopa, to maintain viability, we believe that the small locomotor response to amphetamine is due to release of residual vesicular dopamine from the previous day's L-dopa injection. Striatal DA receptors in DD mice are also hypersensitive to DA and DA agonists so even a small release of DA can produce behavioral activation [9]. Importantly, DD mice manifest no response to a second treatment with amphetamine, whereas wild type mice respond normally [27].

As DD mice are lacking DA in all brain regions, it is difficult to determine where in the brain DA is required for a response to amphetamine. However, DA production can be restored to specific brain regions using biosynthetic enzyme somatic cell gene transfer that establishes L-dopa production in a tightly localized brain region. By transducing cells with recombinant adeno-associated viruses (rAAVs) that express TH and GTPCH1, ectopic expression of TH can be restored to the local region where the rAAVs were injected for the lifespan of the animal [25,26]. Although this combination of TH and GTPCH1 results in DA levels that are approximately 20% of that observed in normal animals, the amount of DA produced is capable of fully rescuing multiple behavioral phenotypes in these mice [25,26]. By injecting small volumes (<1 μl) of rAAVs into regions containing the projection fields of dopaminergic neurons, DA production is restored to very specific regions [25,26]. DD mice with DA production restored to specific brain regions provide a model that is complementary to regional 6-OHDA lesions, with the benefits that DA neurons are intact. Also, unlike 6-OHDA lesions, which are often incomplete and variable between animals, DD mice do not have these problems because the lack of TH is a genetic modification. Therefore, through viral injections, we have complete control as to where DA is being produced. In this study, we show that restoration of DA to only the NAc is sufficient to restore the ability of the animals to respond to amphetamine to a level equivalent to control animals.

2. Materials and methods

2.1. Generation and maintenance of DD and virally restored mice

DD mice were created as described [31]. The $Th^{-/-}$; $Dbh^{Th/+}$ pups that represent the DD mice were identified by their runted appearance and a hyperactive response to L-dopa at 2–3 weeks of age that is absent in normal mice receiving this treatment. The mice were maintained in a modified specific pathogen-free colony, except for those described in Fig. 4B, which were housed in a conventional mouse colony. DD mice were kept alive by daily injection of L-dopa (50 mg/kg body weight, intraperitoneal) using a solution containing 1.5 mg/ml L-dopa dissolved in 2.5 mg/ml ascorbic acid. Control mice were littermates of DD mice that had at least one intact *Th* gene and one intact *Dbh* gene; these mice produce nearly normal levels of DA and norepinephrine [27]. Purina chow (5LJ5) and water were available ad libitum except during experimental procedures.

Two different viral preparations were used. The viral preparations used for the experiment represented in Fig. 4B were described previously [25,26]. For the remainder of the experiments described here, recombinant AAV vectors for TH and GTPCH1 were constructed as shown in Fig. 1. The viruses were pseudotyped AAV-1, packaged as previously described [17], and this viral type was previously characterized [11]. The vector preparations had titres of 3.6×10^{12} (TH) and 5.0×10^{12} (GTPCH1) genomic particles/ml. For intracerebral injection, mice were anesthetized with ketamine/xylazine (6.5 and 0.44 mg/ml, i.p. at a dose of 20 μl/g body weight) and placed into a stereotaxic frame (Cartesian Instruments, Sandy, OR). The head was leveled in the *x*, *y*, and *z* planes using the sagittal suture and lambda and bregma as landmarks. Coordinates used for the NAc were 1.50 anterior–posterior, 0.85 and −0.85 medial–lateral, and 4.60 dorsal–ventral, using bregma as the reference point. Coordinates for the CPu placement were 0.80 anterior–posterior, 2.00 and −2.00 medial–lateral and 3.60 dorsal–ventral. Recombinant AAV vectors for TH and GTPCH1 were mixed at a 1:1 ratio. Virus (0.65–0.80 μl) was injected through a 5-μl Hamilton syringe fitted with a 24-gauge needle at a rate of 0.25 μl/min. After each injection, the needle remained stationary for an additional 2 min and was then raised 0.1 mm and maintained in this position for an additional 2 min before being removed. Following surgery, mice were allowed to recover for at least 2 weeks before experiments commenced.

All procedures were conducted in accordance with guidelines established by the National Institutes of Health and the University of Washington Animal Care Committee.

2.2. Behavioral analysis

Ambulatory activity was measured in transparent plexiglass cages (40×20×20 cm) placed into activity chambers equipped with infrared beams (San Diego Instruments). The number of consecutive beam breaks that occurred in every 15-min interval was measured and converted to meters using the distance between beams (8.8 cm) as a conversion factor. All animals were allowed to acclimate to the chambers for 1.5 h prior to beginning a test. All locomotor tests on DD and NAc-restored mice were performed on these animals 18 h after the last treatment with L-dopa when brain DA levels are less than 1% of control animals.

Catalepsy was measured by placing the mouse's forepaws on a platform 3 cm high and the latency to removal of the forepaws was measured. Catalepsy is defined as an inability to move from this position for a period of 1 min, thus a maximum of 1 min was allowed. All mice tested for this experiment moved in less than 10 s, and thus were not considered cataleptic. Catalepsy tests were performed 15 and 30 min after administration of DA antagonists.

2.3. Drugs

All drugs were dissolved in a phosphate-buffered saline solution (PBS, 10 mM phosphate, 150 mM NaCl, pH 7.0). All vehicle controls were PBS alone All treatments were by intraperitoneal injection. Concentrations for drugs used were as follows: amphetamine (Sigma) 0.3 mg/ml, SCH23390 (Sigma) 0.01 mg/ml, haloperidol (Sigma) 0.05 mg/ml.

2.4. Histology and immunohistochemistry

Mice were killed by lethal injection of 0.2 ml sodium pentobarbital, perfused with PBS followed by 4% paraformaldehyde in PBS and postfixed overnight at 4 °C. Brains were submerged in 30% sucrose and frozen on Dry Ice. Sections (30 μm) were cut on a freezing microtome.

Immunohistochemistry for TH or c-Fos was performed as follows. Free-floating sections were rinsed three times in PBS containing 0.3% Triton-X (PBS-TX) and incubated in PBS-TX plus 2.5% normal goat serum (NGS) for 1 h to block nonspecific binding. Sections were then incubated overnight in PBS-TX containing rabbit polyclonal c-Fos antibody (Santa Cruz Biotechnology, 1:5000 dilution) or monoclonal TH antibody (Chemicon International, 1:800 dilution.) After washing three times with PBS-TX, sections were incubated in PBS-TX and either donkey anti-rabbit conjugated to Cy3 (Jackson ImmunoResearch, 1:400 dilution) or donkey anti-mouse Cy2 (Jackson Immuno-Reasearch, 1:400 dilution) for 2 h. Sections were then washed three times with PBS and viewed under a fluorescent microscope. To induce c-Fos expression, mice were treated with amphetamine at 3 mg/kg body weight and sacrificed 2 h later.

2.5. Statistical analysis

All data was analyzed for normality, and found to follow a Gaussian distribution. Data were analyzed by ANOVA, followed by Dunnett post hoc test if significant differences among groups were found when comparing more than two groups. When comparing two groups, data were compared using a paired *t*-test.

3. Results

3.1. Constructs for rAAVs

Fig. 1 shows the constructs used to produce the rAAVs

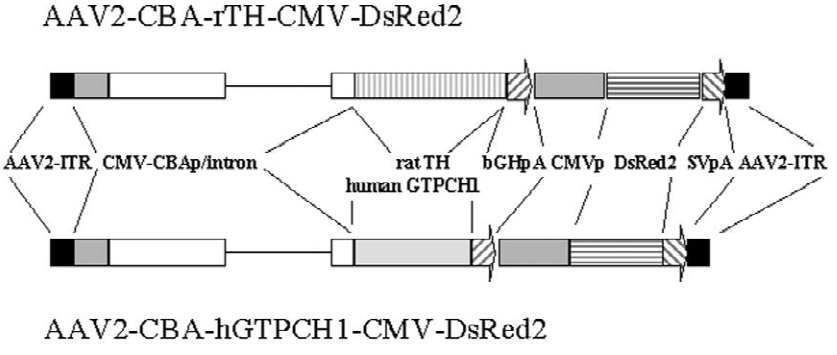

Fig. 1. Constructs used to create rAAVs for TH and GTPCH1. Each construct contains the coding region for the gene of interest, as well as the coding region for DsRed 2.

for TH and GTPCH1 for most experiments. Both constructs contain the coding region for the appropriate gene under the control of a chicken β-actin promoter, as well as a DsRed2 reporter gene, under the control of a CMV promoter. For one experiment (see below), viruses (CMV-TH and CMV-GTPCH1) described previously were used [25,26].

3.2. Injection sites and TH expression

The approximate injection sites are shown in Fig. 2. For the NAc, injection sites were targeted to the core of the NAc, slightly dorsal and lateral to the anterior commissure (A). The CPu injection sites were into the central region of the CPu (B). To confirm expression of the rAAVs, brains were examined for expression of both TH and the fluorescent reporter gene DsRed2, which is included in both the TH and GTPCH1 vectors. Fig. 2C shows expression of DsRed2 in the brain of a NAc-injected mouse. DsRed2-positive cells were observed in the region surrounding the anterior commissure, as expected. Expression of DsRed2 overlapped with expression of TH, which is seen in Fig. 2D. Fig. 2E shows a magnification of the area delineated by the white box in Fig. 2D to reveal cell bodies as well as processes that express TH. DsRed2 expression was also seen in the central CPu of CPu-injected animals (Fig. 2F). Expression of DsRed2 overlapped the area where TH expression was detected (Fig. 2G). Fig. 2H shows a magnification of the area delineated by the white box in Fig. 2G.

3.3. DA replacement in the NAc but not the CPu restores the locomotor response to amphetamine

Both DD and NAc-injected mice require daily injections of the DA precursor L-dopa in order to eat enough to survive. All experiments described below were performed 18 h after the previous day's L-dopa injection. At this point, brain DA levels are less than 1% that of control animals [27]. As previously described, CPu-injected mice have restored feeding behavior, and eat enough to survive without daily L-dopa injections [25,26].

Wild-type (WT) mice responded to amphetamine (3 mg/kg body weight) by increasing their activity for a

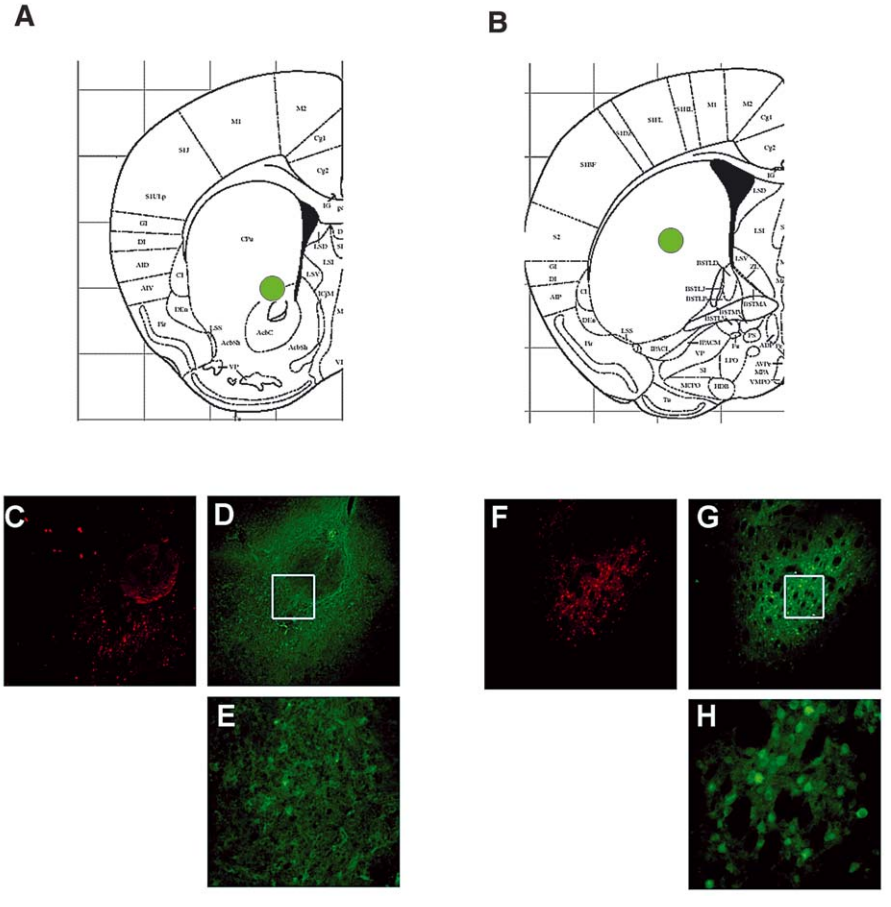

Fig. 2. Approximate injection sites and DsRed2 and TH expression in injected animals. Green circles represent injection sites for NAc-injected (A) and CPu-injected (B) mice, based on post-mortem examination of needle tracks in a subset of animals (NAc-injected=6, CPu-injected=2). (C) DsRed2 and (D) TH expression in NAc-injected mice. (E) Magnified view of area outlined by the white box in (D), showing TH expression in NAc-injected animal. (F) DsRed2 and (G) TH expression in CPu-injected mice. (H) Magnified view of area outlined by the white box in (G).

period of approximately 2 h. When a second amphetamine treatment was given 2.5 h after the first treatment, WT mice increased their activity similarly to their first response (Fig. 3A). When DD mice were treated with the same dose of amphetamine, their initial response was smaller in magnitude when compared to WT mice. A second treatment with amphetamine after 2.5 h elicited no response in DD mice (Fig. 3A).

DA production was restored in either the NAc or CPu by injection of rAAVs. Treatment of NAc-injected mice with amphetamine resulted in a locomotor response similar to that of WT animals. A second dose of amphetamine to NAc-injected mice evoked a response equal to the first (Fig. 3B). Treatment of CPu-injected mice with the same dose of amphetamine evoked a locomotor response that was less than the response of either WT- or NAc-injected mice in both magnitude and duration and a second dose of amphetamine produced a response similar to the first response.

To better understand the statistical significance of the differences between the groups of animals, we chose to sum the distances traveled for the 2.5 h following each injection. The cumulative distance traveled by the four groups of mice in response to first and second treatments with amphetamine are presented in Fig. 4A. Over the first 2.5-h period, WT mice traveled more than DD mice.

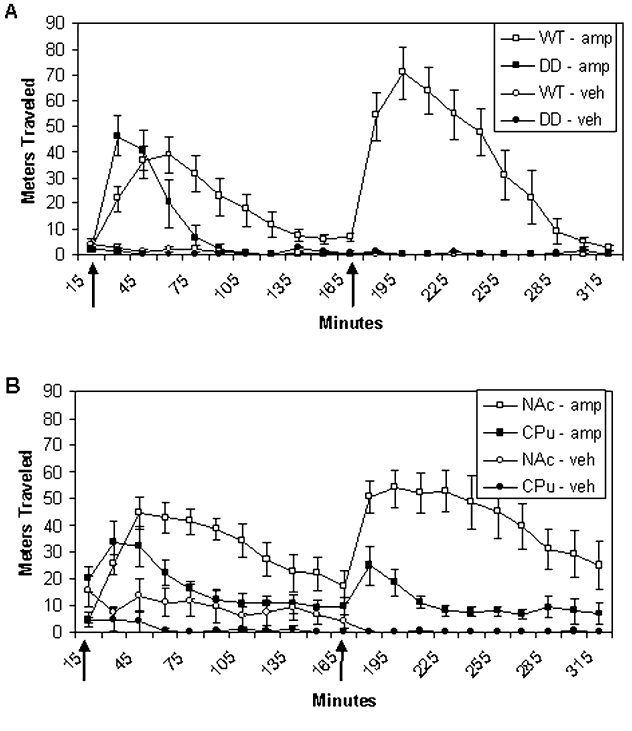

Fig. 3. Locomotor response to amphetamine. Amphetamine (3 mg/kg body weight) or vehicle was injected two times, the second dose occurring 2.5 h after the first dose. Arrows indicate time of injection. (A) WT (n=13) and DD (n=7); (B) NAc-injected (n=7) and CPu-injected (n=7). NAc-injected and DD mice were treated 18 h after their last L-dopa treatment. Error bars represent standard error of the mean.

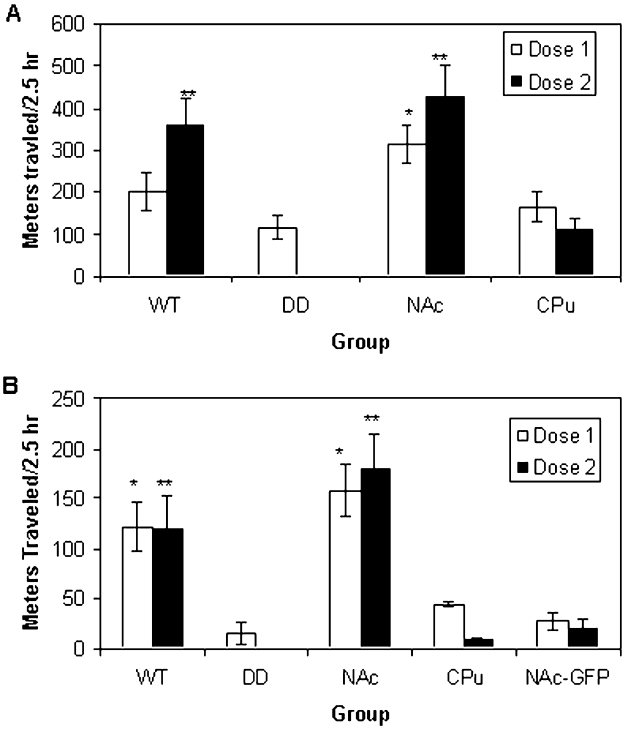

Fig. 4. Cumulative distance traveled in response to two doses of amphetamine. (A) Mice were injected with 3 mg/kg body weight amphetamine and activity was measured for 2.5 h. After 2.5 h, mice were injected a second time with 3 mg/kg body weight amphetamine and activity was measured for an additional 2.5 h. Error bars represent standard error of the mean. WT (n=13), NAc (n=7), DD (n=7), CPu (n=7). *P<0.01 compared to DD Dose 1; **P<0.01 compared to DD Dose 2. (B) Same as A, except using different groups of WT (n=8), DD (n=8), NAc-injected (n=6), CPu-injected mice (n=2) as well as NAc-injected GFP (n=5) as a control virus. Error bars represent standard error of the mean. *P<0.01 compared to DD Dose 1; **P<0.01 compared to DD Dose 2. All statistical analysis was performed using ANOVA followed by a Dunnett post hoc test.

NAc-injected mice responded similarly to WT mice following amphetamine, while CPu-injected mice responded similarly to DD mice. During the 2.5-h period following a second injection, WT mice moved slightly more than they did during the first 2.5-h period, while DD mice did not move. NAc-injected mice responded to a second injection of amphetamine with a locomotor response equivalent to that seen after the first injection of amphetamine. CPu-injected mice also responded to a second injection of amphetamine in the same manner as they responded to the first injection of amphetamine.

Experiments were also performed with DD mice that were injected with the rAAVs used previously [25,26]. The results from those studies are presented in Fig. 4B. Mice that received injections of the TH and GTPCH1 rAAVs into the NAc responded to amphetamine similarly to WT mice. In mice that received injections of a control virus expressing GFP into the NAc, amphetamine did not evoke a locomotor response, demonstrating that functional DA in this region is required for restoration of amphetamine-

induced locomotion. Injection of the TH and GTPCH1 rAAVs into the CPu did not restore amphetamine-induced locomotion to a level comparable to that seen in WT mice. Although the viruses used in this experiment were different than those described above it was clear that the mice injected into the NAc with TH and GTPCH1 rAAVs resembled WT mice, whereas the small group of CPu-injected mice ($n=2$) had a severely blunted response to both the first and second injections of amphetamine.

3.4. The amphetamine-induced response has a dopaminergic component

In WT mice, co-treatment of amphetamine with the D1 antagonist, SCH23390, and the D2 antagonist, haloperidol, significantly blocked the amphetamine-induced locomotor response. This treatment also blocked the locomotor response to of NAc-injected mice to amphetamine (Fig. 5). Because DA receptor antagonists can induce bradykinesia and even catalepsy [2], a basic catalepsy test was performed. The antagonist doses used here did not inhibit the animals' ability to move (data not shown). These data confirm that the viral restoration of dopamine is the major component in the restored amphetamine response of the NAc-injected mice.

3.5. Induction of the immediate early gene product c-Fos occurs primarily in the brain regions in which expression of viral components occur

DA receptor agonists and certain psychostimulants, when given systemically, are able to induce expression of the immediate early gene product c-Fos in specific brain regions, including the NAc and CPu [1]. To determine whether restoration of DA production also restored the induction of c-Fos, we examined c-Fos expression in WT, DD, NAc-injected, and CPu-injected mice.

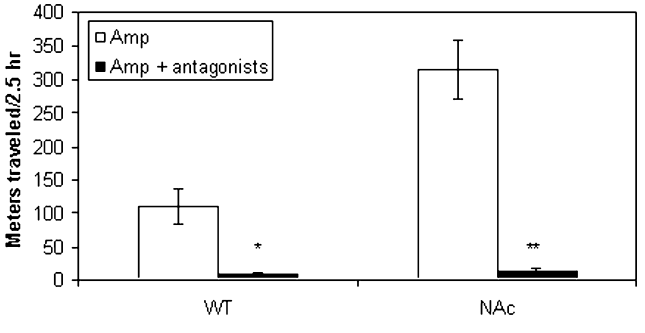

Fig. 5. Treatment with amphetamine and DA antagonists. Mice were treated with a cocktail of amphetamine (3 mg/kg body weight), the D1 antagonist SCH23390 (0.1 mg/kg body weight) and the D2 antagonist haloperidol (0.5 mg/kg body weight). NAc-injected mice were treated 18 h after their last L-dopa treatment. WT ($n=5$), NAc ($n=7$). Error bars represent standard error of the mean. *$P<0.01$ compared to WT amphetamine; **$P<0.01$ compared to NAc amphetamine. All statistical analysis was performed using a paired *t*-test.

Fig. 6A,B shows c-Fos expression in a WT mouse in both the NAc and CPu after treatment with amphetamine. Expression of c-Fos was absent in both the NAc and CPu of a DD mouse that received the same treatment (Fig. 6C,D).

In a NAc-injected mouse treated with amphetamine, c-Fos expression was present in the NAc (Fig. 6E), but absent in the CPu (Fig. 6F). This c-Fos expression pattern overlapped with the region transduced with rAAvs, as seen by the expression of DsRed2 in the NAc (Fig. 6G) but not in the CPu (Fig. 6H). In a CPu-injected mouse, expression of c-Fos was not present in the NAc (Fig. 6I), but was present in the CPu (Fig. 6J). Again, c-Fos expression overlapped with expression of DsRed2, with no DsRed2 expression in the NAc (Fig. 6K) but expression in the CPu (Fig. 6L).

4. Discussion

Amphetamine is thought to act through the mesolimbic dopaminergic pathway to induce locomotion in rodents [14,16]. Injections of amphetamine peripherally or directly into the NAc evoke a locomotor response. Loss of DA production in the NAc, a critical brain region in the mesolimbic pathway, by lesioning dopaminergic projection neurons to the NAc with 6-OHDA abolishes amphetamine-induced behaviors [7,8,12]. While this evidence indicates a role for the NAc in amphetamine-induced behaviors, it does not reveal whether DA action only in the NAc is sufficient to evoke such behaviors.

We have used viral gene therapy to selectively restore DA production to either the NAc or the CPu of mice that have a genetic disruption of DA biosynthesis. Since the injection sites are the terminal fields of dopaminergic pathways, as opposed to regions that contain dopaminergic cell bodies, it is likely that non-dopaminergic cells are being transduced. Because of this, it was necessary to inject two viruses—one of which expressed TH and a second that expressed GTPCH 1, which allows production of an essential cofactor, tetrahydrobiopterin [10,13]. Transfection of cells by both TH and GTPCH 1 allow for production and release of L-dopa by non-dopaminergic cells. Unlike the 6-OHDA model in which neurons are destroyed, DD mice have intact neurons, thus making it possible for the released L-dopa to be taken up by local terminals, converted to DA, packaged, and released. Using this technique, we have found that restoration of DA to the NAc is sufficient to evoke amphetamine-induced locomotion that is quantitatively equivalent to amphetamine-induced locomotion in WT mice following both an initial injection of amphetamine and a second injection of amphetamine.

In contrast to restoration of DA in the NAc, restoration of DA to the CPu results in a response to an initial injection of amphetamine that is not different from the

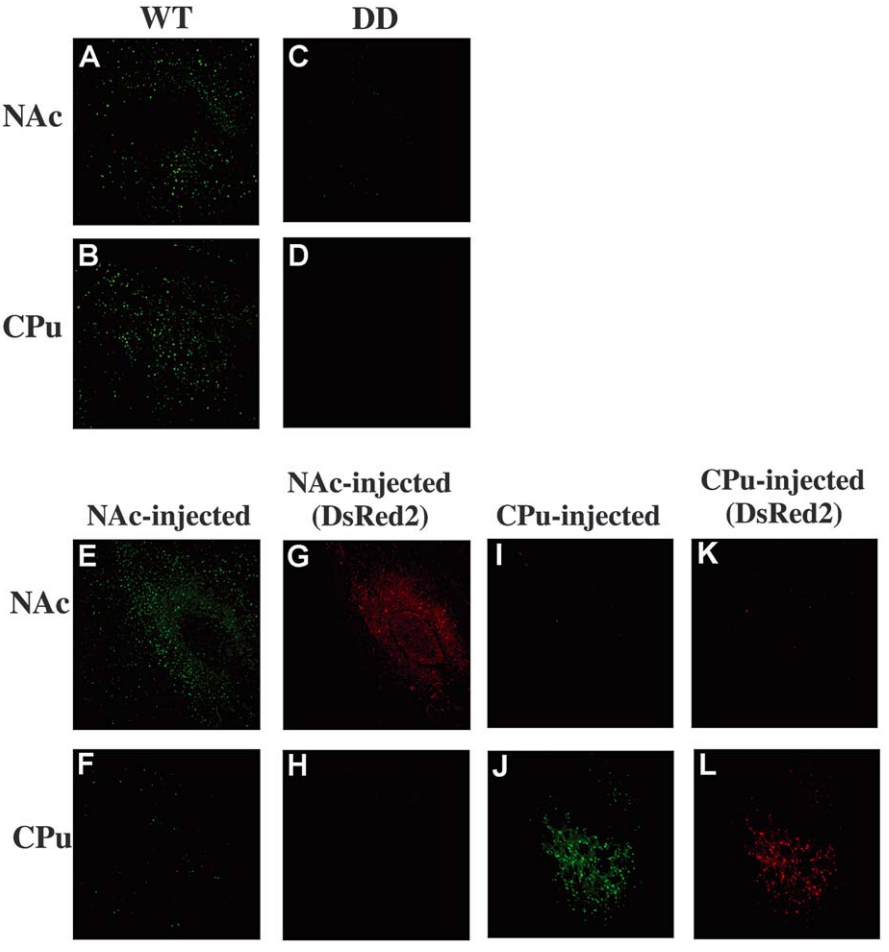

Fig. 6. Immunohistochemistry for c-Fos following amphetamine. Expression of c-Fos in the NAc (A) and CPu (B) of a WT mouse following amphetamine treatment (3 mg/kg body weight). (C) NAc of a DD mouse, (D) CPu of DD mouse. (E) c-Fos expression in the NAc of a NAc-injected mouse, as compared to CPu of the same mouse (F). (G,H) DsRed2 expression in the same sections as E and F. (I) c-Fos in the NAc of a CPu-injected mouse, compared to CPu of the same mouse (J). (K,L) DsRed2 expression in the same sections as I and J.

response of a DD mouse. However, CPu-injected mice do have a response to a second injection of amphetamine, unlike DD mice, but the response is small compared to WT or NAc-injected mice. Unlike the DD and NAc-injected animals that require daily L-dopa injections to maintain feeding, CPu-injected animals are able to eat without daily L-dopa injections [25,26]. Thus, there is no residual DA from L-dopa injections in these mice. It is also unlikely that this response is due to small amount of viral transduction in the NAc of the CPu-injected mice, as immunhistochemistry for both TH and c-Fos shows no expression of either gene in the NAc of these animals. As it appears that DA production is limited to the CPu in CPu-injected mice, we can conclude that local production of DA in this region is capable of producing a locomotor effect in response to amphetamine, although not as robust of an effect as is seen in WT- or NAc-injected mice. Previous studies have shown that the percent restoration of DA by viral transduction is similar in both the NAc and CPu (approximately 20 and 30%, respectively) [25]. However, as the CPu is a much larger brain structure than the NAc, it is possible that restoration of DA to a larger area of the CPu would lead to a greater locomotor response to amphetamine.

While we have targeted two distinct brain regions, the NAc and the CPu, both of these regions have anatomically distinct subregions. In the NAc, numerous studies have uncovered functional differences between the shell and core regions [6,15,24]. Because the core is commonly thought of as the sub-region that is involved in locomotor response to various drugs, we chose to focus on virally transducing the core of the NAc to look for restoration of the locomotor response to amphetamine. However, there is some evidence that the shell of the NAc is also involved in the locomotor response to amphetamine [6], making the shell a possible region to target. Similarly, we chose to target the central region of CPu, rather than more lateral or dorsal regions. It is possible that viral restoration of functional DA to regions of the CPu other than the one that we targeted would also lead to restoration of the locomotor response to amphetamine. A comprehensive study of several different regions within the CPu would be necessary to conclude that restoration of DA to the CPu is not

involved in the restoration of the locomotor response to amphetamine.

Only DD- and NAc-injected mice received daily injections of L-dopa, which was necessary to keep them alive, whereas WT and CPu-injected mice did not. The dose of L-dopa we use does not have any acute effect on brain DA levels or behavior in WT mice so it is unlikely that amphetamine-induced behaviors would be affected. Injecting CPu-injected mice with L-dopa would elicit a bout of intense locomotion that would last a few hours, as is seen in DD mice, and any residual DA remaining in the NAc could contribute to the first locomotor response to amphetamine. However, it is unlikely that the second response to amphetamine would be affected. Thus treating only two of the four groups of mice with L-dopa is unlikely to confound interpretation of the results.

We believe that the DA that is released in the NAc and CPu in response to amphetamine is able to bind to and activate post-synaptic dopamine receptors. By co-treating the NAc-injected mice with a cocktail of amphetamine, D1 and D2 receptor antagonists, we have shown that the locomotor response to amphetamine can be blocked through antagonism of DA receptors. This supports the notion that the DA released is acting specifically on DA receptors, and this DA is responsible for the observed locomotor effect. High doses of DA receptor antagonists, especially haloperidol, can induce cataleptic behaviors, which could explain the lack of locomotion [4]. However, a basic catalepsy test confirmed that these mice were capable of initiating movement. Thus, it appears that the attenuation of amphetamine-induced locomotion is due to the actions of the antagonists on DA receptors, as opposed to a non-specific hypolocomotive effect.

Amphetamine is believed to cause the release of DA from pre-synaptic cells through its actions on the DA transporter [20,23]. In WT mice, this release of DA evokes locomotion, as well as leading to the induction of the immediate early gene product c-Fos by acting on post-synaptic DA D1-like receptors [1]. DD mice, which have less than 1% normal DA content, do not show induction of c-Fos following treatment with amphetamine, although they show a hypersensitive c-Fos induction following treatment with direct-acting DA receptor agonists [9]. Although we could detect a locomotor response to amphetamine, presumably caused by the release of virally restored DA, we did not know whether the DA being released was sufficient to activate downstream signaling pathways. We found that both NAc- and CPu-injected mice did express c-Fos in response to amphetamine, and this response was largely limited to the brain regions that had been virally transduced, demonstrating that viral transduction with TH restores functional DA capable of activating DA receptors in specific brain regions.

In animal models, lesioning studies have long been used to determine the necessity of specific brain regions or neurotransmitters for the evocation of various behaviors. Unfortunately, the nature of lesioning studies necessitates the destruction of the cells, and it is therefore impossible to know if the effect being studied is specific to the loss of dopamine. Here, we show that use of a genetic model and a viral restoration system have substantiated a result first shown in lesioning studies—namely that DA production in the NAc is critical for a response to amphetamine. Further, we have shown that DA only in the NAc is sufficient to allow a response to amphetamine similar to that seen in a wild-type animal.

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