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Dopamine Scales Performance in the Absence of New Learning

Report

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Summary

Learning and motivation are integral in shaping an organism's adaptive behavior. The dopamine system has been implicated in both processes; however, dissociating the two, both experimentally and conceptually, has posed significant challenges. We have developed an animal model that dissociates *expression* or *scaling* of a learned behavior from learning itself. An inducible dopamine transporter (DAT) knockdown mouse line has been generated, which exhibits significantly slower reuptake of released dopamine and increased tonic firing of dopamine neurons without altering phasic burst firing. Mice were trained in experimental tasks prior to inducing a hyperdopaminergic tone and then retested. Elevated dopamine enhanced performance in goal-directed operant responses. These data demonstrate that alterations in dopaminergic tone can scale the performance of a previously learned behavior in the absence of new learning.

Introduction

Both learning and motivation shape an organism's response to its environment. Adaptive behavior requires assigning significance to environmental stimuli and associating appropriate behavioral responses. Equally important, an organism must scale a learned response in relation to its current needs. Although the dopamine (DA) system is strongly implicated in each phenomena, a primary difficulty is dissociating the two, both experimentally and conceptually (Dickinson and Balleine, 2002).

There are two broad perspectives on DA function. In the first, DA facilitates reinforcement learning by providing emphasis or "stamping in" stimulus-reward associations (Wise, 2004). In this context, Schultz et al. have demonstrated that DA cells fire phasically in response to unexpected rewards/events (Mirenowicz and Schultz, 1994; Schultz et al., 1993; Schultz et al., 1997). This work provided the empirical basis for the prediction-error theory of DA (Montague et al., 1996), which posits that DA facilitates learning by signaling discrepancies between predictions and actual events.

In the second perspective, DA's root function is to facilitate motivation. The incentive salience hypothesis, for example, suggests that DA enhances the energizing effect of reward or reward-predicting cues (Robinson and Berridge, 1993; Berridge and Robinson, 2003). Salamone et al. hypothesize that DA maintains behavior when response costs are high (Cousins and Salamone, 1994; Salamone and Correa, 2002). Thus, DA modulates the *expression* of learned behavior by effectively scaling the response generated by previously established associations.

We have designed a genetic approach to manipulate DA signaling to address the question of whether DA can directly scale performance of a learned task in the absence of new learning. An inducible DA transporter (DAT) knockdown mouse line was developed. When DAT expression is reduced, tonic DA levels are enhanced without affecting phasic DA activity. The inducible knockdown allows us isolate the putative performance-scaling effects of DA from learning effects by training our subjects prior to inducing the genetic alteration. We report here the clearest evidence to date that DA directly scales behavioral performance in the absence of new learning.

Results

Generation of Inducible DAT Knockdown

The tetracycline inducible system (Gossen and Bujard, 1992) was used (Figure 1A). A DAT-tTA line was generated by gene-targeting the tetracycline responsive transactivator (tTA) to the 5′-UTR of DAT, placing tTA under the transcriptional control of the DAT promoter and limiting expression to DA neurons. This line is a DAT knockout. A second line, tetO-DAT, was generated by targeting the tetO promoter to the 5′-UTR of DAT, placing DAT under the transcriptional control of tetO. This line is a constitutive DAT knockdown with approximately 5% of wild-type expression levels and the same expression pattern. The residual DAT expression is due to the minimal promoter activity in tetO. We generated DAT-tTA/tetO-DAT compound heterozygotes by crossing these two lines. DAT protein expression in these mice is lower than that in wild-type mice with the same expression pattern (Figure 1B). This expression is mediated by tTA binding to the tetO promoter, as doxycycline (Dox, a tetracycline analog, 200 mg/kg food) decreases expression (Figure 1B). We used Western blotting to examine the time course of Dox treatment effect. DAT protein was almost completely absent after 6 weeks of Dox treatment (Figure 1C). We quantified DAT levels in each sample and used the formula $N(t) = N(0) \times (1/2)^{t/tau}$ to estimate that the half-life (tau) of DAT is approximately 7 days.

Similar strategies of knocking-in elements of the tetracycline inducible system have been published (Bond et al., 2000; Gross et al., 2002). The present study represents a strategy first proposed by Hen et al.

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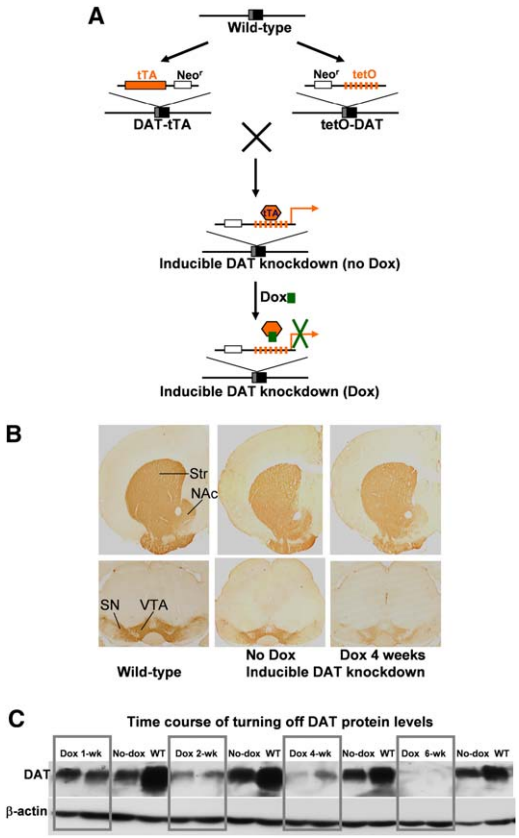

Figure 1. Generation of the Inducible DAT Knockdown Transgenic Mice

(A) The inducible DAT knockdown mice were generated by crossing two knockin lines: DAT-tTA and tetO-DAT. (B) Immunohistochemical straining indicated that 4 weeks of Dox treatment decreased DAT expression while preserving the expression pattern. (C) Western blot analysis of striatal DAT protein indicated that DAT protein was gradually decreased by Dox. Str: striatum, NAc: nucleus accumbens, SN: substantia nigra, VTA: ventral tegmental area.

(Lucas and Hen, 1995), which allows independent use of two knockin lines.

DA Release and Uptake Parameters in Mutant Mice

We characterized DA release and uptake in mutant mice during stages of Dox treatment. In brain slices, stimulation in the nucleus accumbens (NAc) shell elicited oxidation currents that were identified as DA using fast-scan cyclic voltammetry (FSCV), allowing us to employ amperometry to increase temporal resolution. The rise time, peak amplitude, and decay parameters for the evoked DA signals did not differ between wild-type and mutant mice not fed Dox (Figure 2). Mutant mice treated with Dox, however, exhibited a marked reduction in DA clearance rates, as illustrated by the longer decay times of the DA oxidation currents [Figure 2B; $F(4,96) = 93.25$, $p < 0.0001$]. The effect was time-dependent, with the slowest decay after more than 8 weeks of Dox exposure. The current decay was best fit by two exponentials. The first exponential showed considerable variability that was not related to Dox treatment. The second exponential was greatly prolonged with Dox treatment (Figure 2B). The peak levels of DA release

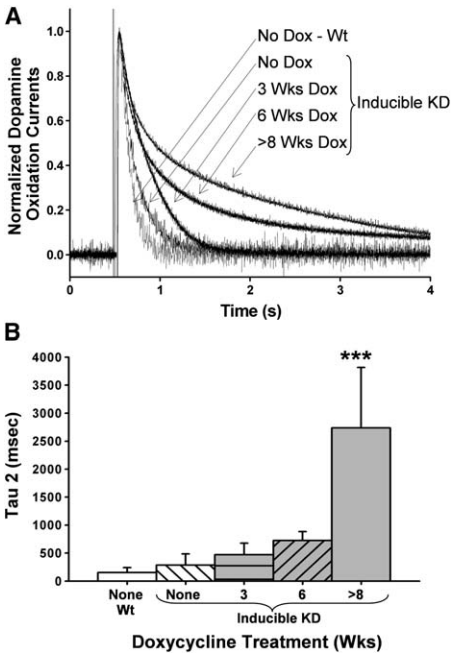

Figure 2. Dopamine Release and Uptake Parameters

(A) Normalized representative amperometric traces of dopamine efflux in the NAc shell. (B) Second order decay time constant ($\tau 2$) of evoked dopamine release. Decreasing DAT by Dox significantly reduced dopamine reuptake (***$p < 0.0001$). Error bars = SEM.

were not significantly different between groups [not shown; $F(4,96) = 1.98$, $p = 0.07$]. Interestingly, DAT expression was suppressed after 6 weeks, but functional elimination of DAT took more than 6 weeks of treatment, suggesting that minimal DAT expression can have a large functional impact.

Action Potential Activity of DA Neurons in Mutant Mice

We evaluated action potential activity of DA neurons in the ventral tegmental area (VTA). Suppressing DAT expression by Dox produced an increase in overall firing rate of DA neurons (Figure 3A, $T = 2.2$, $p < 0.04$). Mice treated with Dox also exhibited a high prevalence of neurons with firing rates >4 Hz (73%) compared with mice that were not fed Dox (30%; $Chi^2 = 7.44$, $p < 0.01$; Figure 3C).

We performed separate analyses on the bursting and nonbursting components of neuronal activity. As Figure 3D shows, *nonbursting* firing rates were higher in mice treated with Dox versus those not exposed to Dox ($T = -2.38$, $p < 0.04$). However, no differences were observed in the quantity or characteristics of bursts (Table 1). Thus, DAT knockdown mice treated with Dox exhibit a chronically elevated DA tone without changes in phasic DA activity.

Downregulation of D2 autoreceptors may explain elevated tonic firing. We conducted radioligand binding autoradiography using [3H]-spiperone. There was no change in D2 autoreceptor expression levels (212 ± 16 fmol/mg in untreated group and 203 ± 21 fmol/mg in >8 week Dox-treated group; $n = 5$, $T = 0.38$, $p = 0.70$).

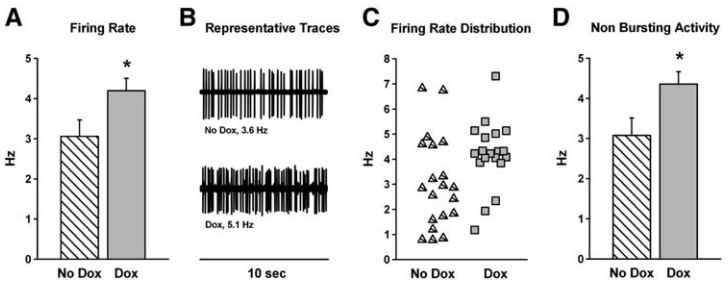

Figure 3. Action Potential Activity of VTA Dopamine Neurons

(A) Mutant mice fed with Dox (five mice, 19 cells) showed higher dopamine neuron firing rates when compared with untreated mice (No Dox, five mice, 20 cells). (B) Representative 10 s traces. (C) Mutant mice fed with Dox exhibited a majority of cells with fast (>4 Hz) firing rates compared with untreated mice. (D) Mutant mice fed with Dox show enhanced nonbursting activity of dopamine neurons compared with untreated mice (*$p < 0.05$). Error bars = SEM.

Induced Knockdown of DAT Expression Scales Performance

Mice were trained and tested on progressive ratio (PR) schedules, which measure the work that animals are willing to do for a reward (Hodos, 1961). We tested all mice on a PR7 schedule (the work requirement increases by 7 lever presses after each reward), food deprived (1 week, ~10% below baseline body weight) and then not food deprived (1 week). No mice were on Dox during this portion of the experiment. There was no performance difference between groups in either food-deprived [Figure 4A, $F(2,25) = 1.4$, $p = 0.26$] or not food-deprived conditions [Figure 4B, $F(2,25) = 0.31$, $p = 0.74$]. We then treated half of the mutant and all the wild-type mice with Dox for 8 weeks. We again tested all mice on a PR7 schedule, food-deprived and then not food-deprived (1 week each). Inducible DAT knockdown on Dox (Inducible: No Dox → Dox) mice displayed more lever pressing than wild-type (WT: No Dox → Dox) mice or DAT knockdown not on Dox (Inducible: No Dox → No Dox) mice when food deprived [Figure 4A, $F(2,25) = 5.7$, $p = 0.009$]. Increased response in the Dox-treated mice cannot be attributed to nonspecific hyperactivity as this elevated responding was not observed on the inactive lever [data not shown, $F(2,25) = 0.61$, $p = 0.55$], under a fixed ratio schedule (Figure 4D, discussed below), or when the mice were sated [Figure 4B, $F(2,25) = 0.046$, $p = 0.96$].

We next tested all mice (under food deprivation) in the concurrent choice task (Cousins and Salamone, 1994). Mice had a choice between lever pressing (FR30) for a preferred food (chocolate-flavored 20 mg pellet) or consuming a less preferred standard rodent chow that was freely available on the floor of the operant box. This setup ("choice" condition) was used on days 1, 3, and 5 of each week. On days 2 and 4, only FR30 was available ("no choice" condition). Before Dox treatment, there was no difference in performance between groups either under the choice [Figure 4C, $F(2,25) = 0.53$, $p = 0.60$] or no choice [Figure 4D, $F(2,25) = 0.78$, $p = 0.47$] conditions. After Dox treatment, the Dox group lever-pressed more and earned more pellets in the choice [Figure 4C, $F(2,25) = 7.0$, $p = 0.004$] but not in the no choice conditions [Figure 4D, $F(2,25) = 3.3$, $p = 0.052$]. Moreover, in the choice condition, the Dox group showed a greater preference than wild-type controls for lever pressing for pellets [Figure 4E, $F(2,25) = 6.3$, $p = 0.006$]. There was no difference in total food consumed [not shown, $F(2,25) = 1.2$, $p = 0.32$]. Thus, DAT knockdown mice showed enhanced motivation for the preferred reward, but not an enhanced appetite for food in general.

To ensure that the above differences were not due to contamination by learning during the testing week, we analyzed day 1 results from the PR7 food-restricted condition separately (since this was the first session after Dox). It is clear that the Dox group displayed more lever pressing compared with all other groups [Figure 4F, $F(2,25) = 3.6$, $p = 0.04$].

Induced Knockdown of DAT Expression Does Not Affect Learning

The above studies allowed us to isolate performance-scaling effects of DA from learning effects by training our subjects prior to inducing changes in dopaminergic tone. To directly test the effect of elevated DA on learning, we used Pavlovian conditioning with the above three groups of mice (inducible DAT knockdown treated with Dox, inducible DAT knockdown not treated with Dox, and wild-type treated with Dox). Mice were presented with a cue light that lasted for 12 s followed by food pellet delivery (unconditioned stimulus; US) at the offset of the light. The auditory cue from the pellet dropping and the light together represent a compound conditioned stimulus (CS). We assessed the acquisition of Pavlovian association between the CS and US with the conditioned response (CR), which was head entry in the feeder. Figure 4G represents head entries during the presentation of the light (0 to 12 s, 2 s bins) and after the presentation of the light (12 to 24 s, 2 s bins) in the last

Table 1. Induced Knockdown of DAT Does Not Affect the Quantity or Characteristics of Bursts

	Firing activity		Amount of bursting			Characteristics of bursts		
	Overall firing rate (Hz)	Nonbursting activity (Hz)	Bursting spikes (%)	Time spent bursting (%)	Burst event frequency (Hz)	Spikes/burst	Burst duration (ms)	Intraburst frequency (Hz)
No Dox	3.06 ± 0.41	3.07 ± 0.43	17.45 ± 5.45	4.17 ± 1.99	0.28 ± 0.10	2.43 ± 0.16	92.06 ± 15.20	18.09 ± 1.24
Dox	4.20 ± 0.31*	4.35 ± 0.31*	20.99 ± 4.71	4.35 ± 1.37	0.37 ± 0.08	2.47 ± 0.10	100.69 ± 9.24	17.06 ± 0.75

For firing activity and amount of bursting: n = 20 (No Dox) and n = 19 (Dox). For characteristics of bursts, only cells showing bursting activity were considered: n = 15 (No Dox) and n = 17 (Dox) (*$p < 0.04$).

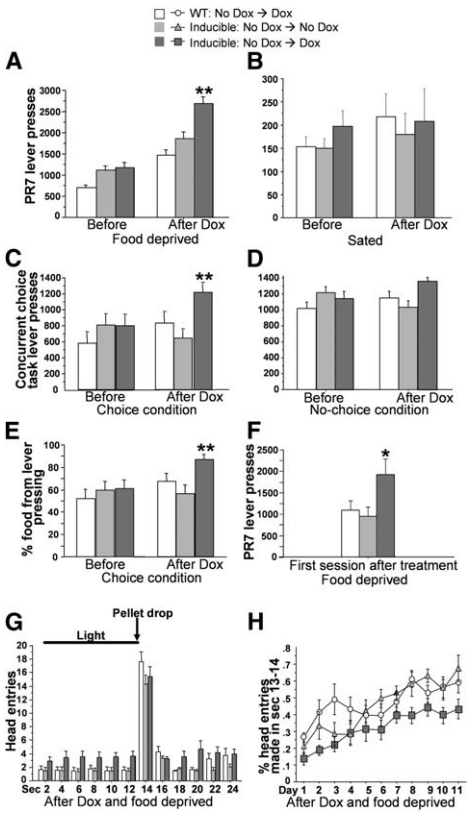

Figure 4. Induced Hyperdopaminergic Tone Scales Performance in the Absence of Learning

Wild-type and mutant mice were tested in a PR7 schedule first before, then after, Dox treatment (WT: No Dox → Dox and Inducible: No Dox → Dox, respectively). A third mutant group was never treated with Dox (Inducible: No Dox → No Dox). Before Dox treatment, there was no difference between any groups in PR7 performance either under food deprivation (A) or no food deprivation (B). Dox treatment of mutant mice significantly enhanced PR7 performance under food deprivation ([A], $p = 0.009$) but not under no food deprivation (B). In the concurrent choice task, before Dox treatment, there was no difference in performance between groups either under the choice (C) or no choice (D) conditions. Dox treatment of mutant mice resulted in more lever-presses under the choice ([C], $p = 0.004$), but not under the no choice, condition ([D], $p = 0.052$). Under the choice condition, Dox-treated mutant mice also showed a higher preference than control mice for lever pressing for the chocolate-flavored pellets ([E], $p = 0.006$). The above difference was not due to contamination by learning during the testing week as indicated by day 1 results (F). Dox-treated mutant mice displayed higher lever pressing than control groups ($p = 0.04$). To examine whether induced hyperdopaminergic tone affects reinforcement learning, the acquisition of Pavlovian association between a CS (the light cue and the auditory cue from the food pellet drop) and the US was assessed with the conditioned response (head entry in the feeder). High levels of head entries at pellet delivery indicate that all mice learned the task (G). Analysis of head entries occurring in the 2 s bin following the CS across training sessions indicates that there was no genotype difference in the acquisition curve (H) (*$p < 0.05$; **$p < 0.01$). Error bars = SEM.

session. We found very few head entries during the 12 s cue light. All groups showed high levels of head entries at the occurrence of the auditory cue, indicating that they learned to associate the CS with the US. Notably, mutant mice on Dox showed significantly more head entries throughout sessions [$F(2,25) = 4.2$, $p = 0.026$], consistent with elevated motivation. However, the pattern and timing of CR were identical between the three groups; that is, all groups discriminated between CS and non-CS. To examine learning, we focused on those head entries occurring in the 2 s bin following the CS (as percentage of head entries during 12 s cue light and 12 s post-cue) across training sessions. Although the auditory cue occurring simultaneously with a lever press during earlier operant tasks was associated with reward, low initial rates of CR and the clear acquisition curve indicate that prior experience in operant tasks transferred little to this new task. There was no genotype difference in the acquisition curve [Figure 4H, group X session interaction $F(20,210) = 0.926$, $p = 0.55$], indicating that all animals learned the task equally.

Discussion

Here we report a genetically altered mouse line in which DAT activity can be virtually eliminated by the administration of Dox, resulting in reduced DA reuptake, increased tonic firing of DA neurons, and unaltered phasic firing. By training mice prior to inducing the above changes, we were able to isolate the effects of increased dopaminergic tone on the expression of an already learned behavior. Thus, we show that alterations in DA transmission can scale the performance of a previously learned behavior in the absence of new learning.

The performance-scaling effects of DA were food deprivation dependent. Increased response due to elevated DA was only observed in deprived, not sated, animals. Thus, elevated DA had no impact on behavior in the absence of pronounced motivational drive. This highlights the importance of considering DA in the context of goal-directed behavior. In both the Bindra/Toates incentive motivation model (Bindra, 1974) and the incentive salience model of Berridge and Robinson (Robinson and Berridge, 1993; Berridge and Robinson, 2003), physiological state interacts with conditioning to produce incentive motivation, with the deprivation state essentially multiplying the incentive value of relevant reward cues. Our data indicate that dopaminergic tone does not simply modulate sensitivity to food deprivation or appetite. Rather, it modulates the performance/deployment of learned behaviors to satisfy motivational states.

The choices made in a PR schedule (to continue to lever-press) and in the concurrent choice task (to work for more desirable food) ensure that these are goal-directed behaviors. Our data do not address the role of DA in scaling habitual behavior. It has been demonstrated that habitual behaviors are less sensitive to motivational state (Balleine and Dickinson, 1998; Dickinson and Balleine, 2002; Yin et al., 2004). Interestingly, using an extinction-reinstatement paradigm with rats overtrained to traverse a runway, Ettenberg et al. (Ettenberg and Horvitz, 1990; Horvitz and Ettenberg, 1988) report that DA blockade has no immediate effect on performance. The insensitivity of habits to motivational state may render them less amenable to scaling by DA, which was further demonstrated in a recent study (Choi et al., 2005). This is consistent with the present findings that in the absence of motivational drive (e.g., sated animals), DA does not scale learned behavioral responses.

Although tonic DA exhibits performance-scaling effects without altering reinforcement learning, DA may contribute to reinforcement learning through phasic activity, which is unaltered in our study. Phasic DA release provides the temporal resolution necessary to represent the contingencies in reinforcement learning (Schultz, 2002). Our data are consistent with the differential assignment of learning and performance-scaling functions to phasic and tonic activity, respectively. Alternatively, recent studies by the Palmiter group (Cannon and Palmiter, 2003; Hnasko et al., 2005) suggest that DA may not be as critical to reinforcement learning as generally believed.

Acute inhibition of DAT and the resulting elevation in extracellular DA may activate DA autoreceptors and decrease DA neuron firing (Lacey et al., 1987); however, chronically reduced DAT and elevated DA may have very different effects. Elevated DA neuron firing arising from reduced DAT has been observed previously in the constitutive DAT knockout mice, and it was speculated that this might be due to the lack of D2 autoreceptor function in these mice (Gainetdinov et al., 1998; Jones et al., 1999). However, we did not find any change in D2 autoreceptor expression with induced DAT knockdown, suggesting that downregulation of D2 autoreceptors is unlikely to mediate the increased tonic activity observed in the present study. Recently, chronic activation of D2 autoreceptors was implicated in increasing tonic DA activity, with downregulation of A-type K+ channels as a potential mechanism (Hahn et al., 2006). Alternatively, changes in afferent control of DA activity may underlie the observed increased tonic firing (Marinelli et al., 2006; Floresco et al., 2003).

Our results highlight the power of genetic approaches for independently manipulating phasic and tonic DA function. Traditional pharmacological approaches have laid a critical foundation, demonstrating that DA blockade or depletion decreases animals' willingness to work for food reward (Cousins and Salamone, 1994; Salamone and Correa, 2002). However, similar approaches have also supported the competing reinforcement learning hypothesis. When DA function is impaired or blocked pharmacologically, it interferes with both acquisition of learned responses (Wise and Schwartz, 1981) and maintenance of reinforced behaviors (McFarland and Ettenberg, 1995; Wise et al., 1978).

Pharmacological manipulations will necessarily alter both tonic and phasic DA transmission. If DA is important for both learning and motivation, drug manipulations will induce alterations in both. Moreover, an acute drug challenge initiates a cascade of acute, dynamic physiological events, compounding the difficulty of assigning any observed behavioral effect to alterations in DA per se. Most drugs are also promiscuous in their molecular targets; e.g., amphetamine acts on the serotonin and norepinephrine transporters in addition to the DA transporter (Wall et al., 1995). Behaviorally, many drugs that target the monoamine systems are themselves potent stimuli, which may become a confounding factor in Pavlovian or instrumental conditioning studies based on stimulus control of conditioned responses. The inducible DAT knockdown mice, in contrast, are in a sustained and stable state at the time of behavioral testing.

The controversy between the reinforcement learning and motivational hypotheses of DA, as well as conflicting data, likely arise from the limitations in pharmacological approaches outlined above. The predominant agent for elevating DA is amphetamine, which has been variously reported to decrease PR responding (Caul and Brindle, 2001; Wiley and Compton, 2004), have no effect, decrease response at low doses but increase it at high doses (Mobini et al., 2000), or increase response at low doses but decrease it at high doses (Mayorga et al., 2000). More precisely targeted genetic manipulations will complement pharmacological approaches and help arbitrate competing interpretations and conflicting results.

In the present study, using animals with a sustained alteration in tonic, but not phasic, DA activity, we show that DA can scale the performance of a previously learned behavior in the absence of new learning. If DA also plays a critical role in learning, it may be that the DA system evolved as an interface between motivational and learning processes, providing a mechanism to integrate these two crucial adaptive behavioral systems. In a recent theoretical work, it was suggested that tonic DA may underlie a learned average reward value for a given environment or context (Niv et al., 2005; see also McClure et al., 2003). This expected average reward, in turn, reflects an opportunity cost for inaction and consequently serves to establish response *vigor*. The present study provides an initial step toward exploring empirically how DA may serve to integrate motivation and learning.

Experimental Procedures

Generation of Inducible DAT Knockdown

Construction of DAT genomic DNA for gene targeting is described elsewhere (Zhuang et al., 2001). The genetic strategy is described in the results section and Figure 1A. 129/SvJ ES cells (Specialty Media) were used. Male chimeras were mated with C57BL6/J females. PGK-neo (floxed) in DAT-tTA was deleted by the germline deleter EIIa-cre. DAT-tTA/+ and tetO-DAT/+ mice were mated to obtain DAT-tTA/tetO-DAT (inducible DAT knockdown) and wild-type littermates. For electrophysiology and electrochemistry experiments, knockdown of DAT expression was achieved by replacing regular rodent chow with rodent chow mixed with 200 mg Dox per kg food (Bio-Serv). For the behavior experiments, knockdown of DAT expression was achieved by replacing water with Dox water (0.4 mg/ml in 5% sucrose solution and 5% sucrose solution for control). This was necessary since the sugar contents in Dox food and regular food were different and could affect behavior experiments using food reward. All animal procedures were approved by the Institutional Animal Care and Use Committee at The University of Chicago.

Western Blots

Mouse brains were lysed. Fifty micrograms of protein was subjected to SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membrane. Nonspecific sites were blocked with 5% nonfat dry milk. Membranes were incubated with rat anti-mouse DAT antibody (1:1000, Chemicon) in TBS with 2% nonfat dry milk. Signals were detected by horseradish peroxidase-conjugated secondary antibodies (ICN) and enhanced chemiluminescence (Pierce). β-actin antibody was used to confirm equal sample loading.

Immunohistochemistry

Animals were transcardially perfused with 4% paraformaldehyde. Brains were equilibrated in 30% sucrose. Frozen sections (50 μm) were blocked with normal goat serum and incubated with rat anti-mouse DAT antibody (1:10,000, Chemicon) over two nights at 4°C.

Immunoreactivity was visualized with the ABC method (Vector) and diaminobenzidine.

Radioligand Binding

We used 50 pM [3H]-spiperone (NEN) and 100 nM ketanserin in 50 mM Tris buffer containing (in mM) 120 NaCl, 5 KCl, 2 $CaCl_2$, and 1 $MgCl_2$ (pH 7.4). Nonspecific binding was determined by adding 1 μM spiperone. After 6 week exposure to BioMax MS film, optical density was quantified using NIH Image and compared to 3H standards (American Radiolabeled Chemicals).

Electrochemistry Recordings

Mice were decapitated; brains were removed into cold, sucrose-artificial cerebrospinal fluid (ACSF) containing the following (in mM): 200 sucrose, 25 $NaHCO_3$, 20 glucose, 10 ascorbic acid, 2.5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, and 1 NaH_2PO_4 (pH 7.4), saturated with 95% O_2 and 5% CO_2. Coronal slices 250 μm thick were prepared with a vibratome (VT100S, Leica). Slices were incubated for 1 hr in bath circulated at 20 ml/min with 32°C ACSF containing (in mM) 125 NaCl, 25 $NaHCO_3$, 20 glucose, 2.5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 1 $NaHCO_3$, and 1 ascorbic acid (pH 7.4), saturated with 95% O_2 and 5% CO_2. For recording, slices were perfused (2 ml/min) with this same 32°C ACSF without ascorbic acid.

Carbon fiber recording electrodes were placed in the NAc shell ~150 μm from a bipolar stimulating electrode with a 250 μm tip separation. DA release was evoked by single-pulse stimulations (400 μA, 1 ms) delivered every 2 min. Currents were recorded using an Axopatch 200B amplifier with a DigiData 1200 interface and pCLAMP 8 software (Axon Instruments). For FSCV, the electrode voltage was ramped from −400 mV to +1000 mV and then back at 200 V/s at 100 ms intervals. Current was filtered at 10 kHz and digitized at 50 kHz. Background-subtracted FSCV allowed for identification of the oxidized substance and for calibration with 5 μM DA at the end of the experiment. For amperometry, a constant voltage of +400 mV was applied. Amperometric traces were filtered at 1 kHz, digitized at 2.5 kHz, and digitally filtered at 100 Hz.

Extracellular Single-Unit Recordings of DA Neurons

All mice were naïve to behavioral testing. Dox exposure was >8 weeks for the Dox group. Mice were anesthetized with choloral hydrate and neurons were recorded in the VTA (0.4–1.0 AP, 0.2–0.6 L, and 4.5–5.5 V mm from brain surface) as previously described (Mathon et al., 2005; White and Wang, 1984).

Signals were recorded using a Fintronics amplifier with a DigiData 1200 interface and axoscope software (Axon Instruments). DA cells were identified according to standard physiological criteria (Grace and Bunney, 1983, 1984b). This included a triphasic (+/−/+) wave form with >2.5 ms duration from start to end (at 400 Hz to 0.5 kHz, White and Wang, 1984) and >1.1 ms from start to trough of negative peak (at 300 Hz to 0.8 kHz, Ungless et al., 2004).

Data were analyzed as follows. Firing rate: total number of spikes over time. Bursts: clusters of spikes occurring at high frequencies, with interspike interval <80 ms at start and >160 ms at end of burst (Grace and Bunney, 1984a). Tonic firing rate: firing activity with bursts subtracted (Mathon et al., 2005).

Behavioral Tests

All experiments were carried out during the light period (06:00–18:00). The same mice were used in the following three behavioral tests in the order described. All tests were conducted in mouse operant conditioning chambers that have two retractable levers, a house light, two signal lights above levers, a signal light and a nose-poke hole on the back wall, and a feeder with photobeam (Med Associates). Twenty milligram chocolate flavored pellets (Bio-Serv) were used as reinforcers. For behavioral effects of DAT knockdown, mice were treated for at least 8 weeks with Dox water.

In the PR operant task, mice were first trained under a fixed ratio 1 (FR1) schedule with only the active lever (right lever) extended. When mice reached a criterion of 30 lever presses in less than 45 min on two consecutive days, they were shifted to a PR7 schedule. Two parameters were recorded: the breakpoint and number of lever presses on the active and inactive levers. For breakpoint, we used two commonly employed criteria: (1) no *active lever press* for 5 min (built into the operant program) and (2) no *reinforcement* for 5 min (post hoc analysis). In the 45 min session time, many mutant mice treated with Dox did not reach breakpoint with either criterion; consequently, we focused our analysis on total lever presses.

In the concurrent choice task, under food deprivation, mice had the choice between lever pressing (FR30 in 30 min session) for a more preferred food (chocolate-flavored 20 mg pellet) or consuming a less preferred, standard rodent chow that was concurrently and freely available on the floor of the operant box. This choice condition schedule was used on day 1, 3, and 5 of each week; and on days 2 and 4, only the no choice condition FR30 was available. Testing lasted for 3 weeks.

In Pavlovian associative learning, mice were habituated to the conditioning chambers for 15 min on two consecutive days, during which four food pellets were placed in the feeders. All animals ate the pellets by the end of the second session. Mice were then trained for 11 days with 20 daily trials (180 s variable intertrial interval). In each trial, mice were exposed to a 12 s illumination of the signal light on the back wall, followed by a single 20 mg food pellet. Interruptions of a feeder photobeam indicated magazine entries.

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