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Dopamine β-Hydroxylase Knockout Mice have Alterations in Dopamine Signaling and are Hypersensitive to Cocaine

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Multiple lines of evidence demonstrate that the noradrenergic system provides both direct and indirect excitatory drive onto midbrain dopamine (DA) neurons. We used DA β-hydroxylase (DBH) knockout (*Dbh*−/−) mice that lack norepinephrine (NE) to determine the consequences of chronic NE deficiency on midbrain DA neuron function *in vivo*. Basal extracellular DA levels were significantly attenuated in the nucleus accumbens (NAc) and caudate putamen (CP), but not prefrontal cortex (PFC), of *Dbh*−/− mice, while amphetamine-induced DA release was absent in the NAc and attenuated in the CP and PFC. The decrease in dopaminergic tone was associated with a profound increase in the density of high-affinity state D_1 and D_2 DA receptors in the NAc and CP, while DA receptors in the PFC were relatively unaffected. As a behavioral consequence of these neurochemical changes, *Dbh*−/− mice were hypersensitive to the psychomotor, rewarding, and aversive effects of cocaine, as measured by locomotor activity and conditioned place preference. Antagonists of DA, but not 5-HT, receptors attenuated the locomotor hypersensitivity to cocaine in *Dbh*−/− mice. As DBH activity in humans is genetically controlled and the DBH inhibitor disulfiram has shown promise as a pharmacotherapy for cocaine dependence, these results have implications for the influence of genetic and pharmacological DBH inhibition on DA system function and drug addiction.

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INTRODUCTION

Brainstem noradrenergic neurons project both directly and indirectly to midbrain dopamine (DA) neurons, where they regulate firing patterns and DA release (Swanson and Hartman, 1975; Jones and Moore, 1977; Grenhoff *et al*, 1993; Grenhoff and Svensson, 1993; Darracq *et al*, 1998; Ventura *et al*, 2003; Liprando *et al*, 2004). DA β-hydroxylase (DBH) controls norepinephrine (NE) production and the NE/DA ratio in noradrenergic neurons and modulates the activity of the DA system and certain psychostimulant-induced behaviors. This pathway has recently become of particular interest for three reasons: (1) a common, single-base polymorphism in the human *Dbh* gene that controls DBH enzymatic activity has been identified (Zabetian *et al*, 2001); (2) DBH activity affects responses to cocaine in humans, and recent studies indicate that the DBH inhibitor disulfiram has efficacy as a treatment for cocaine dependence (Carroll *et al*, 1998, 2004; Cubells *et al*, 2000; George *et al*, 2000; Petrakis *et al*, 2000); and (3) *Dbh* knockout (*Dbh*−/−) mice have changes in DA receptor signaling and are hypersensitive to the psychomotor effects of amphetamine (Weinshenker *et al*, 2002). Therefore, it is of interest to understand the influence of DBH and the noradrenergic system on DA neuron function.

The psychostimulants cocaine and amphetamine facilitate release and/or block reuptake of DA, NE, and 5-HT, resulting in increased synaptic availability of these neurotransmitters. The mesolimbic and mesocortical DA systems, comprised of projections from the VTA to the nucleus accumbens (NAc) and prefrontal cortex (PFC), respectively, have been primarily implicated in both the reinforcing and aversive effects of these drugs of abuse (Koob *et al*, 1998; Spanagel and Weiss, 1999). While DA pathways and signaling have been the focus of most psychostimulant addiction research, it is also clear that NE plays an important role in modulating responses to psychostimu-

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lants. For example, lesions of noradrenergic neurons, administration of the α_1-adrenoreceptor (α_1AR) antagonist prazosin, or targeted disruption of the α_{1B}AR attenuate the psychomotor effects of psychostimulants in rodents (Snoddy and Tessel, 1985; Mohammed *et al*, 1986; Darracq *et al*, 1998; Drouin *et al*, 2002a, b). Paradoxically, we found that *Dbh* knockout (*Dbh*−/−) mice are hypersensitive to the psychomotor effects of amphetamine (Weinshenker *et al*, 2002), indicating that differences exist between chronic NE deficiency and disruption of a single adrenergic receptor or relatively acute losses of NE signaling. In addition, amphetamine-induced accumbal DA release and place preference are lost in mice with NE depletion in the PFC, suggesting that psychostimulant reward depends on a functional noradrenergic system (Ventura *et al*, 2003).

As the noradrenergic system has a facilitory effect on DA neurons, we hypothesized that DA release would be compromised in *Dbh*−/− mice and that a compensatory increase in DA receptor signaling might underlie the hypersensitivity of *Dbh*−/− mice to amphetamine. To identify neurochemical changes associated with the amphetamine hypersensitivity in *Dbh*−/− mice, we assessed DA release by microdialysis and high-affinity state DA receptors by radioligand binding *in vitro*. To further explore the effects of chronic DBH inhibition and NE deficiency on behavioral responses to psychostimulants, we tested cocaine-induced locomotion, reward, and aversion.

MATERIALS AND METHODS

Mouse Breeding and Genotyping

Dbh−/− mice, maintained on a mixed 129/SvEv and C57BL/6J background, were developed and generated as described (Thomas *et al*, 1995, 1998). *Dbh*−/− males were bred to *Dbh*+/− females. Pregnant *Dbh*+/− mice were given the AR agonists isoproterenol and phenylephrine (20 μg/ml each) + vitamin C (2 mg/ml) from E9.5–E14.5, and L-3,4-dihydroxyphenylserine (DOPS; 2 mg/ml + vitamin C 2 mg/ml) from E14.5 to birth in their drinking water to rescue the embryonic lethality associated with the homozygous *Dbh*−/− mutation. Owing to this treatment, NE and epinephrine were present in *Dbh*−/− animals before but not after birth. *Dbh*−/− mice were identified by the delayed growth and ptosis phenotypes, which are 100% correlated with the *Dbh*−/− genotype. Genotypes were confirmed by PCR. *Dbh*+/− mice were used as controls because they have normal catecholamine levels and are indistinguishable from *Dbh*+/+ mice for all previously tested phenotypes (Thomas *et al*, 1995, 1998). All mice were reared in a specific pathogen-free facility with a 12 h light/dark cycle (lights on at 0700 h, lights off at 1900 h); food and water were available *ad libitum*. Naïve mice between 3 and 6 months of age were used for all experiments. Both male and female mice were used for all experiments. No sex differences were observed, and results were combined.

Experimental protocols were approved by the animal care committee at Emory University, and meet the guidelines of the American Association for Accreditation of Laboratory Animal Care and Italian national law (DL no. 116, 1992) governing the use of animals for research.

Locomotor Activity

Experiments were conducted in an isolated behavior room between 1000 and 1600 h. Ambulations (consecutive beam breaks) were measured in transparent plexiglass cages ($40 \times 20 \times 20\,cm^3$) placed into a rack with seven infrared photobeams spaced 5 cm apart, each end beam 5 cm from the cage wall (San Diego Instruments Inc., LaJolla, CA). Mice were placed in the activity chambers for 4 h, injected with cocaine (5, 10, or 20 mg/kg i.p.; Sigma-Aldrich, St Louis, MO), and ambulations were recorded for an additional 2 h. Data were analyzed by ANOVA followed by Bonferroni *post-hoc* tests. For the antagonist studies, saline, the 5-HT_{1A} antagonist WAY100635 (0.03 mg/kg), the 5-HT_2 antagonist ketanserin (0.3 mg/kg), the D_1 antagonist SCH23390 (0.03 mg/kg), or the D_2 antagonist eticlopride were injected i.p. 30 min prior to cocaine (20 mg/kg). Antagonist doses were chosen based on the literature and our pilot experiments; higher doses were tried, but typically resulted in sedation and ataxia, indicating nonspecific effects. All drugs were purchased from Sigma-Aldrich (St Louis, MO).

Radioligand Binding

Mice were euthanized by CO_2 asphyxiation, and brains were quickly removed and dissected on ice. Dissections were guided by the mouse brain atlas (Paxinos and Franklin, 1997). The frontal cortex was isolated by removing the olfactory bulb and making a cut 1 mm caudal to the beginning of the brain proper. As this region is comprised mostly of PFC and related structures, it will be subsequently referred to as the PFC. The striatum was isolated by making a second cut 1.5 mm caudal to the first cut, then cutting away the cortex, the most medial structures including the septum and diagonal band, and the most ventral structures including the olfactory tubercle and ventral pallidum. Each remaining hemisphere was then cut horizontally just above the anterior commmissure to isolate the dorsal striatum caudate putamen (CP) and NAc. Brain tissue was then placed in microfuge tubes, frozen on dry ice, and stored at −70°C until used. As ~30 mg of tissue was required for each assay, tissue from eight mice per genotype was batched.

D_2 receptor radioligand binding experiments were performed as described (Seeman *et al*, 2002). Briefly, tissue was homogenized in buffer (50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 5 mM KCl, 1.5 mM $CaCl_2$) with or without 200 μM guanilylimidodiphosphate (guanine nucleotide (GN)), and placed in glass test tubes, followed by the addition of [^{3}H]raclopride (76.8 Ci/mmol; 12 final concentrations, from 0.2 to 20 nM). Samples were incubated at room temperature and passed through a filter, which was then rinsed, placed in vials of scintillant, and monitored 6 h later for tritium using a spectrometer. Nonspecific binding for D_2 receptors was defined as that which occurred in the presence of 10 μM S-sulpiride (Ravizza, Milan, Italy). The density (B_{max}) and dissociation constant (K_d) of [^{3}H]raclopride-binding sites were obtained by Scatchard analysis. Scatchard analysis was performed in triplicate on each tissue batch, and the receptor density values were consistently within 5–8% of each other. D_1 receptor density was measured by the same

procedure, except that the ligand was [^{3}H]SCH23390 (75.5 Curies/mmol) and nonspecific binding was defined by 1 μM (+)butaclamol. [^{3}H]raclopride K_d values ranged from 1 to 2.5 nM, and [^{3}H]SCH23390 K_d values ranged from 0.21 to 0.49 nM, similar to published values. The addition of GN consistently elevated the K_d of [^{3}H]raclopride, reflecting the release of endogenous DA, which then competes with [^{3}H]raclopride. The addition of GN did not affect the K_d of [^{3}H]SCH23390. Each binding assay was performed three times on separate days, and the absolute values for the total receptor densities varied 5–8% from day to day. Therefore, it was more reliable to measure the difference caused by GN on the receptor density in the *Dbh*+/− and *Dbh*−/− samples on the same day. This procedure permitted the reliable detection of differences in DA receptor densities which ranged from 0.7 to 11.7 pmol/g for D_1 and from 0.4 to 10.3 pmol/g for D_2. The net difference caused by GN varied by less than 10% for the triplicate experiments.

Microdialysis

Upon their arrival, animals were housed in groups of four in standard breeding cages (27 × 21 × 13.5 cm^3), with food and water ad libitum on a 12/12 h dark/light cycle (light on between 0700 and 1900 h). All mice used were handled and accustomed to the environment where the experiment was to be performed and then randomly assigned to different treatments. All experiments were carried out between 1400 and 1800 h.

Animals were anesthetized with chloral hydrate (450 mg/kg), mounted in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) equipped with a mouse adapter, and implanted unilaterally with a guide cannula (stainless steel, shaft OD 0.38 mm, Metalant AB, Stockholm, Sweden) in the CP, NAc, or PFC. The length of the guide cannula was: 2.5 mm for the CP, 4 mm for the NAc, and 1 mm for the PFC. The guide cannula was fixed with epoxy glue, and dental cement was added for further stabilization. The coordinates from bregma, measured in mm according to the mouse brain atlas (Paxinos and Franklin, 1997), were: +1.0 anteroposterior and +1.4 lateral for the CP, +1.4 anteroposterior and +0.6 lateral for the NAc (mostly including the shell subdivision; Paxinos and Franklin, 1997; Ventura *et al*, 2003), and +2.52 anteroposterior and 0.6 lateral for the PFC. The probe (dialysis membrane length 2 mm for CP and PFC and 1 mm for NAc; o.d. 0.24 mm, MAB 4 cuprophane microdialysis probe, Metalant AB) was introduced 24–48 h after implantation of the guide cannula. The animals were lightly anesthetized with chloral hydrate (225 mg/kg) to facilitate manual insertion of the microdialysis probe into the guide cannula. The membranes were tested for *in vitro* recovery of DA on the day before use in order to verify recovery.

The microdialysis probe was connected to a CMA/100 pump (Carnegie Medicine, Stockholm, Sweden) through PE-20 tubing and an ultra-low torque dual-channel liquid swivel (Model 375/D/22QM, Instech Laboratories, Inc., Plymouth Meeting, PA) to allow free movement. Artificial CSF (147 mM NaCl, 2.2 mM $CaCl_2$ and 4 mM KCl) was pumped through the dialysis probe at a constant flow rate of 2 μl/min. Experiments were carried out 22–24 h after probe placement. Each animal was placed in a circular cage (21.5 cm in height, 17.5 cm in diameter) provided with microdialysis equipment (Instech Laboratories, Inc.) and with home cage bedding on the floor. Dialysis perfusion was started 1 h later. Following the start of dialysis perfusion, mice were left undisturbed for approximately 2 h before the collection of baseline samples. Following baseline sample collection (1 sample every 20 min for 60 min), mice were injected with saline or amphetamine (2.5 mg/kg, i.p.), and dialysate was collected every 20 min for 120 min. Only data from mice with correctly placed cannula (judged by methylene blue staining) were reported. In all, 20 μl of the dialysate samples was analyzed by high-performance liquid chromatography (HPLC). The remaining 20 μl was kept for possible subsequent analysis. Concentrations (pg/20 μl) were not corrected for probe recovery. The mean concentration of the three samples collected immediately before treatment (less than 10% variation) was taken as basal concentration.

The HPLC system consisted of an Alliance (Waters Corporation, Milford, MA) system and a coulometric detector (ESA Model 5200A Coulochem II) provided with a conditioning cell (M 5021) and an analytical cell (M 5011). The conditioning cell was set at 400 mV, electrode 1 at 200 mV, and electrode 2 at −250 mV. A Nova-Pack C18 column (3.9 × 150 mm, Waters) maintained at 33°C was used. The flow rate was 1.1 ml/min. The mobile phase was as described previously (Ventura *et al*, 2003). The assay detection limit was 0.1 pg.

Statistical analyses were performed on raw data (concentrations, pg/20 μl). The effects of amphetamine on extracellular monoamine levels in the CP, NAc, or PFC were analyzed by repeated-measures ANOVA with two between-factor (strain, two levels: *Dbh*+/− and *Dbh*−/−; treatment, two levels: amphetamine and saline) and one within-factor (minutes, seven levels: 0, 20, 40, 60, 80, 100, and 120). Simple effects were assessed by one-way ANOVA for each time point. Individual between-group comparisons, when appropriate, were performed by *post-hoc* test (Duncan's multiple range test).

Conditioned Place Preference

Experiments were conducted in an isolated behavior room between 1000 and 1600 h. Mice were placed in the 'neutral' middle compartment of a three-compartment conditioned place preference chamber (San Diego Instruments, La Jolla, CA) and allowed to freely explore the other two compartments that were distinguishable by floor texture and wall pattern for 20 min, and time spent in each compartment was recorded ('pretest'). After 1–6 days, mice were subjected to 'conditioning' sessions for three consecutive days. Mice were given an injection of saline (10 ml/kg, i.p.) and restricted to one compartment for 30 min in the morning, then given an i.p. injection of saline or cocaine (5, 10, 20, 40, or 60 mg/kg, 10 ml/kg) and restricted to the other compartment for 30 min in the afternoon (~4 h after the morning conditioning session). Mice were designated to receive cocaine on either the 'A' side or 'B' side using an unbiased design (ie for each genotype, equal numbers of mice received cocaine on each side, and equal numbers of mice received cocaine on the 'preferred' side and 'nonpreferred' side based on pretest results). The day following the last

conditioning session, mice were placed in the neutral middle compartment in a drug-free state, allowed to freely explore all compartments for 20 min, and time spent in each compartment was recorded. Testing sessions were begun roughly halfway between the morning and afternoon conditioning sessions of the third day of conditioning. The preference score was calculated by subtracting the amount of time spent on the saline-paired side from the amount of time spent on the cocaine-paired side. Data were analyzed by paired *t*-tests (pretest preference *vs* post-test preference for each group).

RESULTS

Basal and Amphetamine-Induced DA Release is Attenuated in *Dbh*−/− Mice

As the activity of mesolimbic dopaminergic neurons is enhanced by NE, we predicted that striatal DA release would be compromised in *Dbh*−/− mice. We assessed DA release in the NAc, CP, and PFC of awake, behaving *Dbh*+/− and *Dbh*−/− mice by microdialysis. Basal extracellular DA levels were significantly reduced in the NAc and CP, but not the PFC, of *Dbh*−/− mice (NAc: *Dbh*+/− 1.25 ± 0.25 pg/20 μl, *Dbh*−/− 0.56 ± 0.5, $P<0.05$; CP: *Dbh*+/− 1.99 ± 0.27, *Dbh*−/− 1.32 ± 0.11, $P<0.05$; PFC: *Dbh*+/− 0.56 ± 0.03, *Dbh*−/− 0.47 ± 0.03). Basal extracellular NE levels were 1.02 ± 0.13 in the PFC and 0.62 ± 0.09 in the NAc of *Dbh*+/− mice, while NE was undetectable in *Dbh*−/− mice.

The effects of amphetamine on DA release are shown in Figure 1. Extracellular DA levels in the NAc, CP, and PFC of *Dbh*+/− mice were increased dramatically by amphetamine administration (2.5 mg/kg, i.p.), peaking at ~100% maximal increase in the NAc and CP and ~200% maximal increase in the PFC. In contrast, the amphetamine-induced increase in extracellular DA was absent in the NAc, and present but reduced in the CP (50% maximal increase) and PFC (75% maximal increase) of *Dbh*−/− mice. Statistical analyses revealed a significant strain × treatment × minutes interaction for the NAc ($F_{6,226}=3.44$; $P<0.005$), CP ($F_{6,120}=2.23$; $P<0.05$), and PFC ($F_{6,114}=2.30$; $P<0.05$). *Post hoc* analysis revealed significant differences between *Dbh*+/− and *Dbh*−/− mice challenged with amphetamine at multiple time points (Figure 1).

Increased Density of Striatal High-Affinity State DA Receptors in *Dbh*−/− Mice

Reductions in striatal DA availability typically result in the upregulation of striatal DA receptors. In addition, *Dbh*−/− mice share a number of phenotypes with wild-type animals that have undergone psychostimulant sensitization, including greater psychostimulant- and D_2 agonist-induced locomotion, and an increase in striatal high-affinity state D_2 receptors is thought to contribute to these phenotypes in sensitized animals (Seeman *et al*, 2002, 2005; Weinshenker

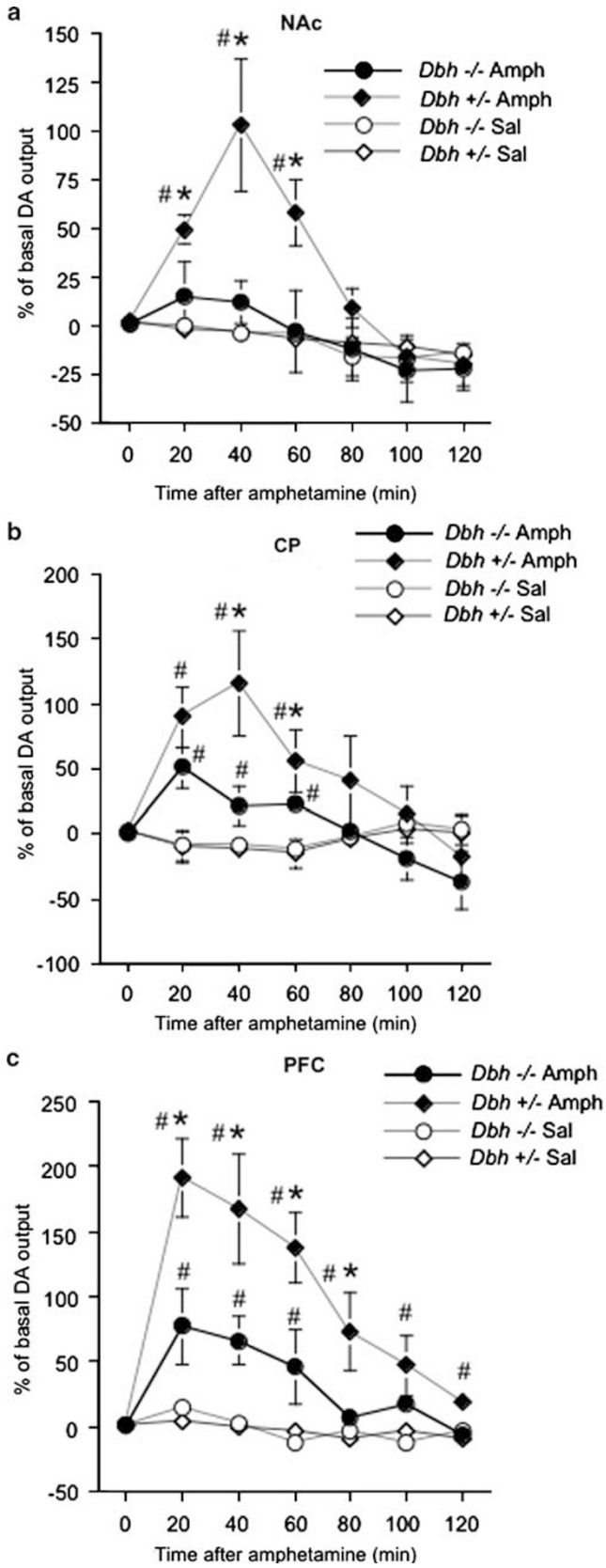

Figure 1 Amphetamine-induced DA release is attenuated in *Dbh*−/− mice. Shown are extracellular DA levels in the (a) NAc, (b) CP, and (c) PFC of *Dbh*+/− and *Dbh*−/− mice after administration of saline (sal) or amphetamine (amph; 2.5 mg/kg, i.p.; $N=5$–11 per genotype and treatment group). Values are expressed as mean ± SEM. *$P<0.05$ compared to amphetamine-treated *Dbh*−/− mice for that time point. #$P<0.05$ compared to saline-treated mice of the same genotype for that time point.

et al, 2002). We measured saturation binding of the D_1 antagonist SCH23390 and the D_2 antagonist raclopride to brain tissue homogenates in the presence and absence of GN. Under normal conditions, antagonist binding to high-affinity state receptors is prevented by endogenous DA, but previous data are consistent with the principle that GN permits the release of receptor-bound DA and allows the binding of the antagonist to all receptors (Seeman *et al*, 1989). Thus, the difference in antagonist binding in the presence and absence of GN represents the density of high-affinity state receptors.

While no large differences in total striatal DA receptors were found between *Dbh*+/− and −/− mice, high-affinity state DA receptors were markedly elevated in *Dbh*−/− mice (D_1 receptors: 1.9 pmol/g for *Dbh*+/− mice, 5.7 pmol/g for *Dbh*−/− mice in the CP; 6.6 pmol/g for *Dbh*+/− mice, 11.7 pmol/g for *Dbh*−/− mice in the NAc; D_2 receptors: 3.7 pmol/g for *Dbh*+/− mice, 10.3 pmol/g for *Dbh*−/− mice in the CP; 0.8 pmol/g for *Dbh*+/− mice, 5.3 pmol/g for *Dbh*−/− mice in the NAc; Figure 2a–d). Similar results were obtained when antagonist binding to total striatal homogenates was examined independently from separate groups of animals and when two other methods of measuring high-affinity state receptors were used (DA/[H^3]raclopride or DA/[^{3}H]domperidone competition; Seeman *et al*, 2005). In contrast, the density of high-affinity state DA receptors in the PFC of *Dbh*−/− mice was similar to that of controls (D_1 receptors: 0.7 pmol/g for *Dbh*+/− mice, 0.7 pmol/g for *Dbh*−/− mice; D_2 receptors: 0.4 pmol/g for *Dbh*+/− mice, 0.6 pmol/g for *Dbh*−/− mice, and total D_2 receptor density was modestly reduced (Figure 2e and f).

Dbh−/− Mice are Hypersensitive to Cocaine-Induced Locomotion

We have previously shown that *Dbh*−/− mice are hypersensitive to amphetamine-induced locomotion and stereotypy (Weinshenker *et al*, 2002). To determine whether this hypersensitivity extends to cocaine, we measured the locomotor response of *Dbh*+/− and *Dbh*−/− mice to cocaine. Cocaine produced a dose-dependent increase in locomotor activity in both *Dbh*+/− and −/− mice (Figure 3). However, as with amphetamine, cocaine-induced locomotion was greater in *Dbh*−/− mice at all doses tested; there was a significant genotype × time interaction for 5 mg/kg ($F_{11,168} = 6.82$, $P < 0.0001$), 10 mg/kg ($F_{11,168} = 8.80$, $P < 0.0001$), and 20 mg/kg cocaine ($F_{11,144} = 5.52$, $P < 0.0001$). *Post hoc* analysis revealed significant differences between

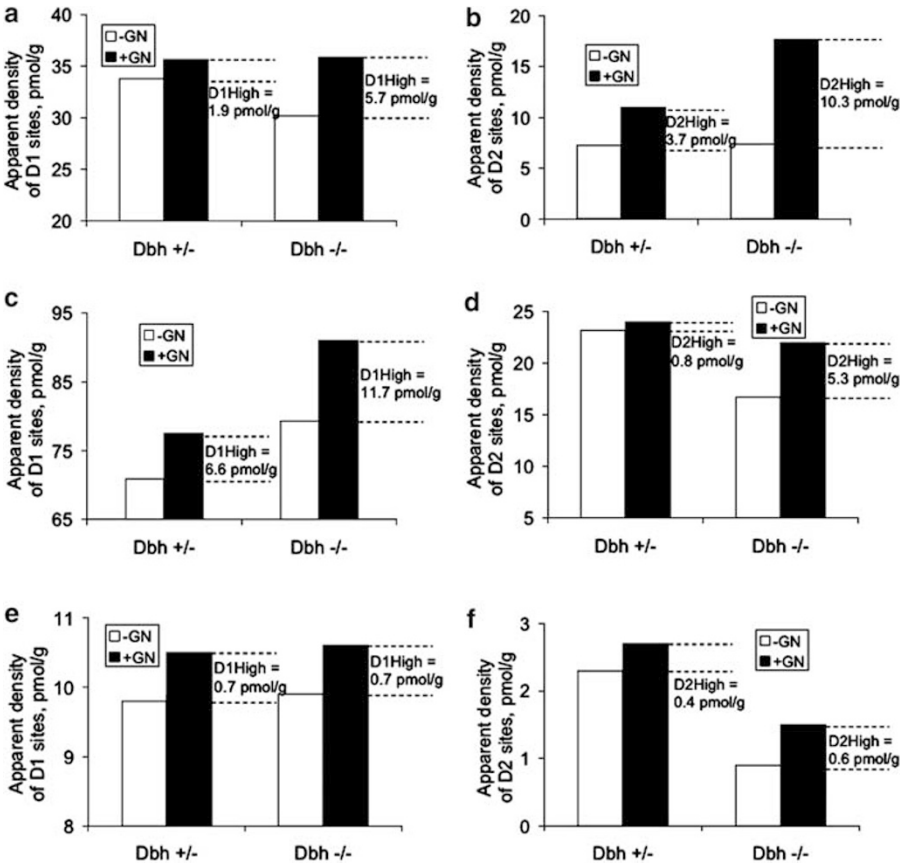

Figure 2 The densities of high-affinity state DA receptors in the NAc and CP are elevated in *Dbh*−/− mice. Shown are the results from Scatchard analysis of saturation curves of [^{3}H]SCH23390 (D_1 receptors; a, c, and e) and [^{3}H]raclopride (D_2 receptors; b, d, and f) binding to homogenized NAc (a and b), CP (c and d), and PFC (e and f) in the presence (total receptors, black bars) and absence (low-affinity state receptors, white bars) of guanilylimidodiphosphate (GN). The difference between the −GN and +GN conditions represents the density of high-affinity state receptors ($D1^{High}$, $D2^{High}$). The two bars on the left side of each graph represent data from *Dbh*+/− mice, and the two bars on the right side of each graph represent data from *Dbh*−/− mice. Tissue from eight animals was pooled for each genotype, and Scatchard analysis was performed in triplicate on each tissue batch. Binding values from the three runs agreed to within 5–8% of each other.

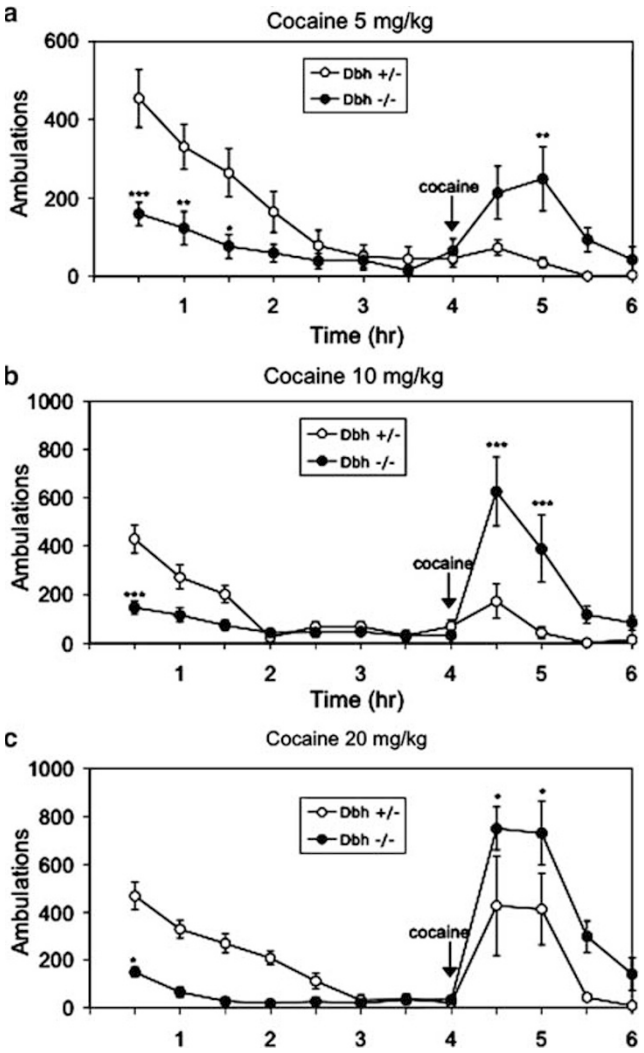

Figure 3 *Dbh*−/− mice are hypersensitive to cocaine-induced locomotion. Mice were placed in activity chambers and injected with cocaine 4 h later (black arrows), and ambulations were recorded via infrared beam breaks for two additional hours. Cocaine doses were (a) 5 mg/kg, (b) 10 mg/kg, and (c) 20 mg/kg. $N = 7$–8 per group. Values are expressed as mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to *Dbh* + /− mice at that time point.

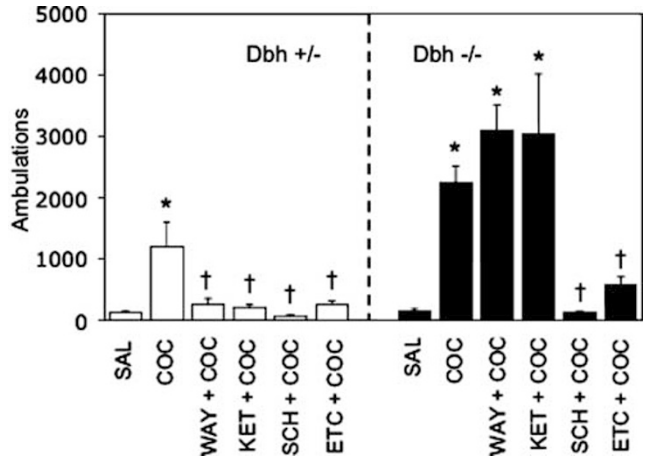

Figure 4 Effects of DA and 5-HT antagonists on cocaine-induced locomotion in *Dbh* mice. Shown are the total ambulations of *Dbh* + /− mice (white bars, left of dashed line) and *Dbh*−/− mice (black bars, right of dashed line) for the 2 h following administration of saline (SAL), cocaine (COC; 20 mg/kg), the 5-HT_{1A} antagonist WAY100635 (0.03 mg/kg) + cocaine (WAY + COC), the 5-HT_2 antagonist ketanserin (0.3 mg/kg) + cocaine (KET + COC), the D_1 antagonist SCH23390 (0.03 mg/kg) + cocaine (SCH + COC), or the D_2 antagonist eticlopride (0.3 mg/kg) + cocaine (ETC + COC). Antagonists were administered 30 min prior to cocaine. $N = 6$–12 per group. Data were analyzed by one-way ANOVA followed by Bonferroni *post-hoc* tests. *$P < 0.05$ compared to saline control for that genotype. †$P < 0.05$ compared to cocaine alone for that genotype.

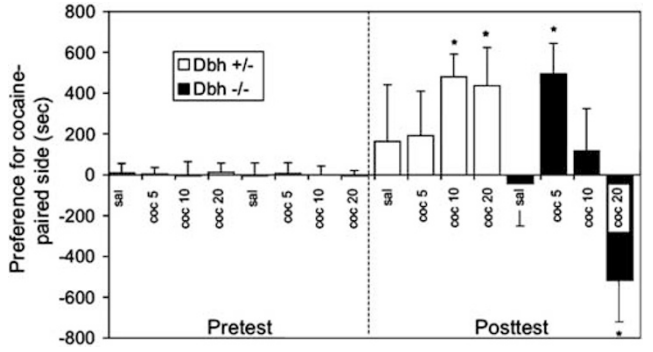

Figure 5 *Dbh*−/− mice are hypersensitive to cocaine reward and aversion. Shown is the preference of *Dbh* + /− mice (white bars) and *Dbh*−/− mice (black bars) in seconds for the 'cocaine-paired' (saline, cocaine 5, 10, or 20 mg/kg) side before (Pretest, left of dashed line) and after (Post-test, right of dashed line) 3 days of pairing. $N = 7$–10 per group. Values are expressed as mean ± SEM. *$P < 0.05$ compared to the pretest for that group.

Dbh + /− and *Dbh*−/− mice at multiple time points following cocaine administration (Figure 3). Locomotor activity in response to a novel environment prior to drug administration was reduced in *Dbh*−/− mice, as described previously (Weinshenker *et al*, 2002; Figure 3).

To determine whether the alterations in DA receptors contributed to the locomotor hypersensitivity of *Dbh*−/− mice to cocaine, saline, the D_1 antagonist SCH23390, or the D_2 antagonist eticlopride were administered to mice 30 min prior to cocaine (20 mg/kg). As serotonergic hyperinnervation of the striatum can occur after DA depletion, we also tested the ability of 5-HT antagonists to prevent the cocaine hypersensitivity of *Dbh*−/− mice. A significant effect of treatment was found for both genotypes (one-way ANOVA: *Dbh* + /− $F_{5,49} = 4.35$, $P < 0.0001$; *Dbh*−/− $F_{5,48} = 12.03$, $P < 0.0001$). *Post hoc* analysis revealed that both SCH23390 and eticlopride attenuated cocaine-induced locomotion in *Dbh* + /− and *Dbh*−/− mice (Figure 4). In contrast, the 5-HT_{1A} antagonist WAY100635 and the 5-HT_2 antagonist ketanserin attenuated cocaine-induced locomotion in *Dbh* + /− mice, but were completely without effect in *Dbh*−/− mice (Figure 4).

Altered Cocaine Reward and Aversion in *Dbh*−/− Mice

To determine whether the alterations of dopaminergic signaling in *Dbh*−/− mice affect psychostimulant reward, we assessed cocaine conditioned place preference in *Dbh* + /− and *Dbh*−/− mice. The side preference of both genotypes before pairing with cocaine and after pairing with saline was essentially random (Figure 5). While *Dbh* + /− mice

expressed a significant place preference to cocaine at the moderate and high dose (10 and 20 mg/kg) but not the low dose (5 mg/kg), *Dbh*−/− mice expressed a significant preference only at the low dose and avoided the cocaine-paired chamber at the high dose (Figure 5). To determine whether the normal dose response to cocaine was shifted in *Dbh*−/− mice, we examined whether higher doses of cocaine produced a place aversion in *Dbh*+/− mice. Conditioned place aversion was not observed in *Dbh*+/− using cocaine doses up to 60 mg/kg, the highest dose tested (Figure 5). *Dbh*+/− mice in fact showed a trend towards a place preference at 40 and 60 mg/kg cocaine, but it was not quite significant in either case (post-test preference for cocaine-paired side at 40 mg/kg: $+327 \pm 178$ s, $P = 0.1$ compared to pretest, $N = 10$; 60 mg/kg: $+248 \pm 245$ s, $P = 0.21$ compared to pretest, $N = 9$).

DISCUSSION

Regulation of DA Release by NE

We found that basal extracellular DA levels were attenuated in the NAc and CP of *Dbh*−/− mice. Consistent with these results, LC lesions decrease striatal DA availability (Russell *et al*, 1989; Lategan *et al*, 1990, 1992; Haidkind *et al*, 2002), resulting in DA receptor supersensitivity (Donaldson *et al*, 1976; Lategan *et al*, 1989; Harro *et al*, 2000). Basal DA release in the PFC was not significantly affected, suggesting that NE is more critical for the basal tone of mesolimbic and nigrostriatal DA neurons than for mesocortical DA neurons. However, it is important to note that the NE transporter (NET) can take up DA, and noradrenergic neurons may release DA as well as NE (Carboni and Silvagni, 2004; Devoto *et al*, 2005). Therefore, it is possible that basal DA levels in the PFC of *Dbh*−/− mice are maintained by the local release of DA from 'noradrenergic' neurons. This 'ectopic' DA could be synthesized *de novo* by the 'noradrenergic' neurons or be released from dopaminergic terminals and taken up into the noradrenergic neurons via the NET.

Amphetamine can increase DA release in an impulse-independent manner by acting directly on DA transporters. However, accumbal DA release following systemic amphetamine appears to be primarily impulse-dependent in some mouse strains, and NE is critical for this aspect of DA outflow (Ventura *et al*, 2004). Amphetamine-induced burst firing of DA neurons is dependent on α_1AR signaling, and amphetamine-induced DA release in the NAc is attenuated in α_{1B}AR knockout mice, mice with depletion of NE in the PFC, and by α_1AR antagonists (Darracq *et al*, 1998; Shi *et al*, 2000; Paladini *et al*, 2001; Auclair *et al*, 2002; Ventura *et al*, 2003). Our data confirm and extend these findings. Amphetamine-induced DA release was completely abolished in the NAc of *Dbh*−/− mice, while DA release persisted in the CP and PFC, although at reduced levels. These results indicate that NE is required for the maximal increase in extracellular DA observed after psychostimulant administration, and that mesolimbic DA neurons absolutely depend on the noradrenergic system for this response.

The LC, A1, and A2 brainstem cell groups project directly to the VTA and exert excitatory control over DA neuron firing (Jones and Moore, 1977; Simon *et al*, 1979; Grenhoff *et al*, 1993, 1995; Grenhoff and Svensson, 1993; Liprando *et al*, 2004; Mejias-Aponte *et al*, 2004). In addition, PFC neurons express α_1ARs (Palacios *et al*, 1987; Pieribone *et al*, 1994), and α_1AR antagonists infused directly into the PFC or NE depletion in the PFC attenuate amphetamine-induced DA release in the NAc (Darracq *et al*, 1998; Ventura *et al*, 2003). Thus, the lack of both 'direct' and 'indirect' (via the PFC and other structures; Taber *et al*, 1995; Tong *et al*, 1996; Marek and Aghajanian, 1999; Carr and Sesack, 2000; Darracq *et al*, 2001) excitatory noradrenergic inputs to the VTA likely contributes to the reduction of accumbal DA release in *Dbh*−/− mice.

DA Receptor Supersensitivity in *Dbh*−/− Mice

A reduction in DA availability typically results in the upregulation of DA receptor signaling in terminal regions. For example, DA receptor hypersensitivity is observed after lesions of DA neurons (Arnt, 1985) or genetic DA depletion (Kim *et al*, 2000). An increase in high-affinity state DA receptors was observed in the NAc and CP, but not the PFC, of *Dbh*−/− mice. As DA availability in the PFC of *Dbh*−/− mice was normal under basal conditions but decreased after amphetamine administration, the increase in striatal high-affinity state receptors correlated with the basal availability of extracellular DA, as opposed to the availability of DA after evoked release. Although the density of both high-affinity state D_1 and D_2 receptors was increased in the striatum, *Dbh*−/− mice were hypersensitive to the behavioral effects of a D_2 agonist, but not a D_1 agonist; in fact, *Dbh*−/− mice are relatively insensitive to D_1 agonist-induced locomotion (Weinshenker *et al*, 2002). As D_1 signaling in the PFC opposes DA release in the NAc and locomotor activation (Vezina *et al*, 1991; Ventura *et al*, 2004), PFC D_1 receptors may be preferentially activated by D_1 agonists in *Dbh*−/− mice, although other explanations are possible.

Taken together, these neurochemical changes in DA signaling may explain the behavioral hypersensitivity of *Dbh*−/− mice to amphetamine. We have previously shown that amphetamine-induced locomotion is modestly enhanced in *Dbh*−/− mice, while amphetamine-induced stereotypy is greatly magnified (Weinshenker *et al*, 2002). DA signaling in the CP is thought to underlie stereotypies. For example, a number of groups have shown that 6-OHDA lesions of the CP abolish amphetamine-induced stereotypy (Kelly *et al*, 1975; Makanjuola and Ashcroft, 1982). Thus, the persistence of some amphetamine-induced DA release in the CP of *Dbh*−/− mice coupled with the profound striatal DA receptor hypersensitivity likely underlies their stereotypy-related phenotypes. In contrast, the locomotor response to amphetamine has been attributed to DA signaling in the NAc (eg Kelly *et al*, 1975; Pijnenburg *et al*, 1975; Makanjuola and Ashcroft, 1982), and it might seem surprising that amphetamine-induced locomotion is intact in *Dbh*−/− mice despite a lack of amphetamine-induced DA release in the NAc. As the locomotor response to amphetamine is blocked by DA antagonists in *Dbh*−/− mice (Weinshenker *et al*, 2002), we speculate that DA release in other brain regions, such as the CP and/or PFC, is responsible. This is consistent with the work of Ventura *et al* (2003), who demonstrated that amphetamine-induced

locomotion can occur in the absence of DA release in the NAc. Alternatively, DA release in the NAc of *Dbh*−/− mice may occur at levels below our limit of detection, yet be sufficient for locomotor behavior due to receptor hypersensitivity.

Chronic *Dbh* Deficiency Affects Cocaine Locomotion, Reward, and Aversion

Our results indicate that, similar to amphetamine, *Dbh*−/− mice are hypersensitive to cocaine-induced locomotion. We assessed the effects of DA and 5-HT antagonists to determine whether the hypersensitivity was mediated by DA and/or 5-HT receptors. WAY100635 and ketanserin were used for three reasons. First, 5-HT_{1A} and 5-HT_2 receptors modulate DA release and cocaine-induced locomotion in rats (De Deurwaerdère and Spampinato, 1999; Carey *et al*, 2001; Broderick *et al*, 2004). Second, serotonergic hyperinnervation of the striatum occurs after DA neuron lesions, and these receptors contribute to the hyperlocomotion observed in monoamine-depleted animals (Stachowiak *et al*, 1984; Mignon and Wolf, 2002; Zhang *et al*, 2002; Bishop *et al*, 2003). Third, the residual locomotor response to psychostimulants in α_{1B} knockout mice is mediated by compensatory 5-HT_{2A} signaling (Auclair *et al*, 2004). We found that cocaine-induced locomotion in *Dbh*+/− mice was attenuated by DA and 5-HT antagonists, but only the DA antagonists effectively eliminated cocaine-induced locomotion in *Dbh*−/− mice. We conclude that the expression of cocaine hypersensitivity of *Dbh*−/− mice is mediated by changes in DA receptors, although changes in 5-HT signaling could contribute to the development of the hypersensitivity.

Interestingly, acute pharmacological inhibition of DBH or α_1ARs has the opposite effect of chronic DBH deficiency; a single injection of the DBH inhibitor disulfiram or the α_1AR antagonist prazosin attenuated psychostimulant-induced locomotion (Maj *et al*, 1968; Darracq *et al*, 1998; Weinshenker *et al*, 2002). In contrast, chronic disulfiram administration increases cocaine sensitization (Haile *et al*, 2003). These observations indicate that the dopaminergic adaptations in response to the chronic absence of NE, and not the acute lack of NE, underlie the psychostimulant hypersensitivity of *Dbh*−/− mice.

Psychostimulants possess both rewarding and aversive properties. The conditioned place preference (CPP) of *Dbh*−/− mice to a low dose of cocaine that does not support a CPP in *Dbh*+/− mice suggests a hypersensitivity to cocaine reward. Perhaps the most striking phenotype we observed was the aversion of *Dbh*−/− mice to a dose of cocaine (20 mg/kg) that produced a place preference in control mice. We attempted to produce a place aversion in control mice by increasing the dose of cocaine used during the conditioning sessions. Even at the highest dose tested (60 mg/kg), *Dbh*+/− mice still tended to prefer the cocaine-paired side. We did not test higher doses because 60 mg/kg is near the threshold for cocaine-induced seizures in mice, which would confound the results (eg Golden *et al*, 2001). Thus, it is not technically correct to say that chronic *Dbh* deficiency produces hypersensitivity to the cocaine place aversion, because the phenotype was never observed in control mice. Rather, there appears to be a novel shift in the balance between cocaine reward and aversion in *Dbh*−/− mice, where the aversive properties of cocaine can become overwhelming and preclude the reward (Ettenberg and Geist, 1991). Importantly, no seizure activity was observed in *Dbh*−/− mice following administration of the cocaine dose (20 mg/kg) that produced the place aversion. To our knowledge, this is one of the only examples of a conditioned place aversion to cocaine in rodents. Both a lack of NE and hyperdopaminergic signaling could contribute to this phenotype. For example, mice specifically lacking NE in the PFC show a similar place aversion to amphetamine (Ventura *et al*, 2003), and *Dbh*−/− mice are hypersensitive to the aversive effects of ethanol (Weinshenker *et al*, 2000). In addition, because DA release occurs in response to aversive as well as rewarding events (Thierry *et al*, 1976; Abercrombie *et al*, 1989; Jensen *et al*, 2003) and *Dbh*−/− mice have hypersensitive DA receptors, excessive DA signaling may also underlie the aversion.

DBH and Cocaine Dependence

DBH activity varies between individuals, and this variation has a strong genetic component that is primarily controlled by a single, common polymorphism (Zabetian *et al*, 2001). Our results suggest that dopaminergic function and psychostimulant responses could be altered in individuals with low DBH activity, a hypothesis that is supported by the human literature. For instance, cocaine-induced paranoia is more prevalent in individuals with genetically low DBH activity (Cubells *et al*, 2000). Furthermore, the DBH inhibitor disulfiram has shown promise as a treatment for cocaine dependence (Carroll *et al*, 2004). These findings suggest that genetic or pharmacological inhibition of DBH may increase the aversive effects of cocaine.

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