Reward-Seeking and Discrimination Deficits Displayed by Hypodopaminergic Mice Are Prevented in Mice Lacking Dopamine D4 Receptors

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ABSTRACT The dopamine D4 receptor (D4R) is predominantly expressed in the prefrontal cortex, a brain area that integrates motor, rewarding, and cognitive information. Because participation of D4Rs in executive learning is largely unknown, we challenged D4R knockout mice ($Drd4^{-/-}$) and their wild-type (WT) littermates, neonatally treated with 6-hydroxydopamine (6-OHDA; icv) or vehicle in two operant learning paradigms. A continuous reinforcement task, in which one food-pellet was delivered after every lever press, showed that 6-OHDA-treated mice (hypodopaminergic) WT mice pressed the reinforcing lever at much lower rates than normodopaminergic WT mice. In contrast, $Drd4^{-/-}$ mice displayed increased lever pressing rates, regardless of their dopamine content. In another study, mice were trained to solve an operant two-choice task in which a first showing lever was coupled to the delivery of one food pellet only after a second lever emerged. Interval between presentation of both levers was initially 12 s and progressively shortened to 6, 2, and finally 0.5 s. Normodopaminergic WT mice obtained a pellet reward in more than 75% of the trials at 12, 6, and 2 s, whereas hypodopaminergic WT mice were severely impaired to select the reward-paired lever. Absence of D4Rs was not detrimental in this task. Moreover, hypodopaminergic $Drd4^{-/-}$ mice were as efficient as their normodopaminergic $Drd4^{-/-}$ siblings in selecting the reward-paired lever. In summary, hypodopaminergic mice exhibit severe impairments to retrieve rewards in two operant positive reinforcement tasks, but these deleterious effects are totally prevented in the absence of functional D4Rs. **Synapse 63:991–997, 2009.**

INTRODUCTION

In mammals, the ability to organize complex sets of actions in time and space has developed together with the increase in size and functions of the most anterior part of the neocortex: the prefrontal cortex (PFCx; Fuster, 1997; Schoenemann, 2005). This brain area receives a prominent terminal field of dopaminergic neurons from the ventral tegmental area, which provides the highest concentration of dopamine (DA) along the entire mammalian cortex (Fuster, 1997). There is extensive literature indicating that the mesocortical DA pathway participates in the integration of motivational and sensory stimuli to coordinate motor planning in positive reinforcement tasks (Ito, 2000; Porrini, 2004; Everitt and Robbins, 2005). In particular, DA has played a remarkable adaptive role throughout vertebrate evolution to consolidate the reinforcing properties of food consumption by coupling the intake of valuable calories to an increase in the individual's hedonic state (Cannon and Bseikri, 2004). In fact, midbrain DA neurons' activity

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correlate with the presentation of reward and its predicting cues (Schultz, 1997) and encode information regarding both the magnitude and probability of occurrence of reward (Fiorillo, 2003, 2008). The ability to predict the outcome of a rewarding event requires real-time categorization of salient environmental cues. Although the importance of PFCx DA in cognitive functions used in instrumental learning such as working memory, preparatory set and inhibitory control has been demonstrated by pharmacological and lesion studies in human and nonhuman primates and in rodents (Kringelbach, 2005), knowledge about the role that the different DA receptors play in these functions is sparse. To study the neurobiology of reward-driven executive function is of fundamental importance to understand how frontocortical dysfunctions arise during drug abuse (Jentsch and Taylor, 1999).

Previous research has focused on the participation of cortical DA D1 and D2 receptors in DA-mediated functions (Callier, 2003; Deng, 2006; Gerfen, 2006; Khan, 2000; Lidow, 1998). In contrast, little is known about the participation of the D4 receptor (D4R) despite the fact that it is mainly expressed in the PFCx (Noaín, 2006). In vitro electrophysiological studies have shown that this G-protein-coupled receptor can stimulate hyperpolarizing, inwardly rectifying potassium channels (Werner, 1996; Wedemeyer, 2007). In vivo, the inhibitory modulation that D4Rs play in the PFCx was demonstrated at the pharmacological level and also using D4R deficient mice (Rubinstein, 2001). Because D4Rs are localized in both excitatory glutamatergic pyramidal neurons and inhibitory GABAergic interneurons of the PFCx (Mrzljak, 1996), it is conceivable that over or understimulation of D4Rs may alter the fine tuning of PFCx circuits involving DA. For example, D4R blockade using the selective antagonist L-745,870 improves working memory at low doses in below-average subjects, while diminishes performance at high doses in above-average animals (Zhang, 2004). The hypothesis that D4Rs participate in PFCx-dependent cognitive and executive functions has been strengthened by the fact that some human allelic variants of the DRD4 gene are more frequently found in patients with attention-deficit hyperactivity disorder (ADHD; Biederman and Faraone, 2005; Faraone, 2001). The current study aims to investigate the participation of the D4R in different aspects of instrumental learning by analyzing the performance of D4R knockout mice (Rubinstein, 1997) and their wild-type (WT) littermates in two operant learning paradigms: a reward-seeking and a two-choice task. To gain further insight into the possible actions of the D4R in these operant tasks, we investigated the effect of the genetic ablation of D4Rs in mice rendered hypodopaminergic by neonatal administration of 6-OHDA (Avale, 2004a).

MATERIALS AND METHODS

Subjects and neonatal lesions with 6-OHDA

All mice tested in this study were male *Drd4*$^{-/-}$ (Rubinstein, 1997) and their WT littermates obtained by mating *Drd4*$^{+/-}$ mice backcrossed for more than 10 generations to the CF-1 outbred line (Avale et al., 2004a). Neonatal injections with 6-OHDA or vehicle were performed as described in Avale et al. (2004a). Briefly, on postnatal day 2 (P2), male pups received the norepinephrine transporter blocker desipramine hydrochloride (20 mg/kg, sc; Sigma-Aldrich, MO). After 30 min, pups were anesthetized by hypothermia and subjected to an intracerebroventricular injection of 25 μg of 6-hydroxydopamine hydrobromide (6-OHDA, Sigma-Aldrich, MO) dissolved in 3 μl of ascorbic acid 0.1% into one of the lateral ventricles, at 1.5 μl/min. Control mice received vehicle. Typically, this lesion reduced DA levels to 20% compared to mice treated with vehicle. However, at the completion of behavioral experiments, the neurotoxic effect of 6-OHDA was confirmed in each animal by measuring striatal DA contents by high-performance liquid chromatography (Avale, 2004a). Mice were genotyped at weaning and housed in groups of four to six with ad libitum access to food and water. At 12 weeks old, mice were individually housed. It is noteworthy to mention that at this age, 6-OHDA-lesioned mice display normal locomotor activity (Avale, 2004a). All animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals, United States Public Health Service (USA).

Operant behavior procedures

Continuous reinforcement task

Habituation to experimental conditions started with food access restriction during 5 days to maintain each animal near 85% of their original body weight. To avoid food neophobia during the experiments, mice were habituated to eat regular chow food mixed with 20-mg food pellets (PJAI-0020, Research Diets, NJ) that were later used as food reinforcers in the operant chambers. Following habituation, mice were trained during 1 h per day for 5 consecutive days to obtain food pellets by pressing one of the two retractable levers that were introduced at either the right or left side of the delivery tray of a mouse-size operant chamber (ENV-307A, Med Associates, VT). During this period, mice received one food pellet per lever press and one extra pellet every minute that passed without responses. Finally, mice were trained to receive one food pellet for each lever press (fixed ratio of 1; FR1). During this test, either the right or left levers were selected following a pseudorandom and balanced sequence. Operant chambers were illuminated by a house light during all sessions.

Two-choice task

Mice were trained along a series of consecutive rounds. In the first training lap, carried out during 1 week, the pressed lever was retracted for a random interval of 15–25 s after which it became available again. On the following week, mice were trained to press on the lever to which they had not been previously exposed. Finally, mice were trained to press either the right or the left lever, randomly introduced in each trial. After this set of training stages, mice were challenged in a two-choice test during 1-h daily sessions that included as many trials as possible. Each trial started when one of the two levers was introduced (see details in Fig. 1). The first lever was designed as the *signal* lever and was coupled to the delivery of one food pellet only after the second *distracting* lever was introduced. The *signal time* between the presentation of the first and second-lever exposure was initially set at 12 s. Signal times were progressively shortened every 16 days: first to 6 s, then to 2 s, and finally to 0.5 s. Performance along the last 4 days of each stage was used for statistical analyses. Once both levers were exposed, the first response on a lever determined the outcome of the trial. At that point, both levers were immediately retracted (Fig. 1). Mice pressing first on the signal lever received a food pellet but those pressing first on the distracting lever did not. After retraction of both levers, a randomized inter trial interval of 56–76 s followed until the next trial commenced. The ratio of rewarded versus total number of trials was taken as an index of performance.

Statistical analyses

For the FR1 stage, two-way ANOVAs were performed with the number of lever presses and interresponse times (IRTs; transformed to $1/x$ to meet ANOVA assumptions). Between factors included genotype (with $Drd4^{-/-}$ and WT as levels) and treatment (with 6-OHDA and vehicle as levels). For the choice task, unless otherwise specified, repeated measures ANOVAs were performed, with between factors as before and within factor: signal time (12, 6, 2, and 0.5 s as levels), using data from the last 4 days of each stage. When post hoc comparisons were needed, Fisher's LSD method was used.

RESULTS

To investigate the participation of D4Rs in reward-seeking behavior, we first evaluated the motivational salience of the lever acting as a conditioned stimulus in a continuous reinforcement task, in which reward was delivered after each response (fixed ratio-1 or FR1). Responding rates showed a significant genotype $\times$ treatment interaction ($F_{(1,12)} = 7.664$, $P < 0.02$).

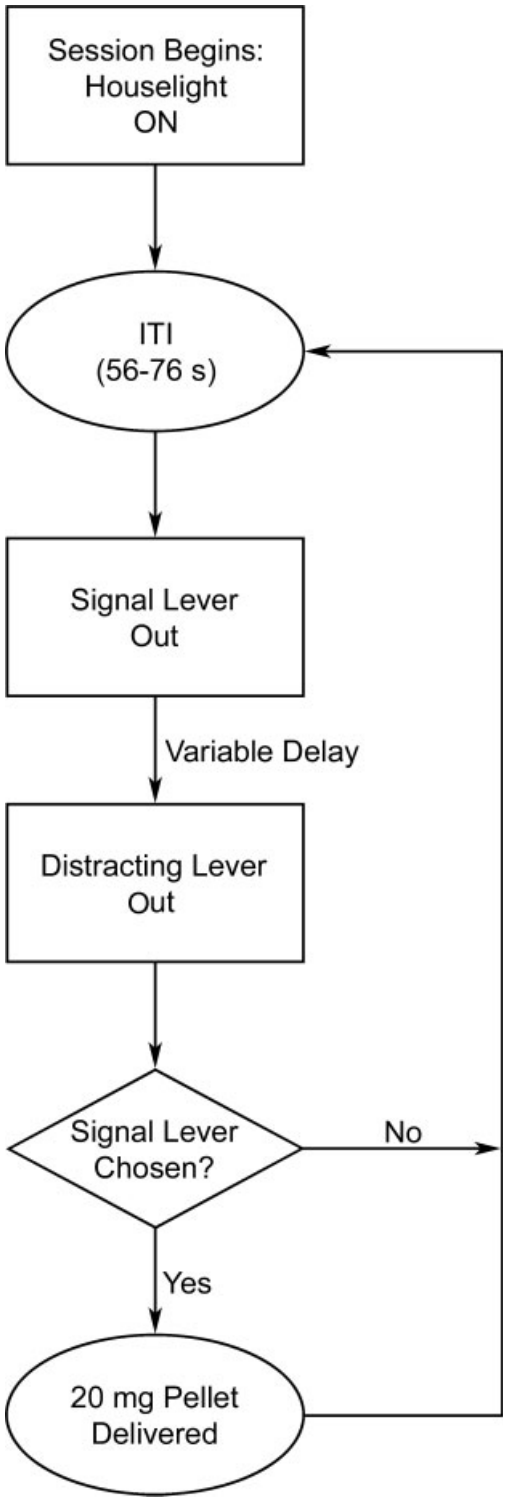

Fig. 1. Schematic of the two-choice operant task. Mice are placed in an operant chamber equipped with two retractable levers at the left and right side of the food delivery tray. After a random intertrial interval of 56–76 s, one of the two levers is presented: the signal lever. Pressing the signal level at this point does not deliver food pellets. After a signal time of 12, 6, 2, or 0.5 s, the second lever comes out (the distracting lever). Once both levers are out, a mouse receives a food pellet only after pressing on the signal lever. Once the mouse makes its choice both levers are retracted and another intertrial interval begins.

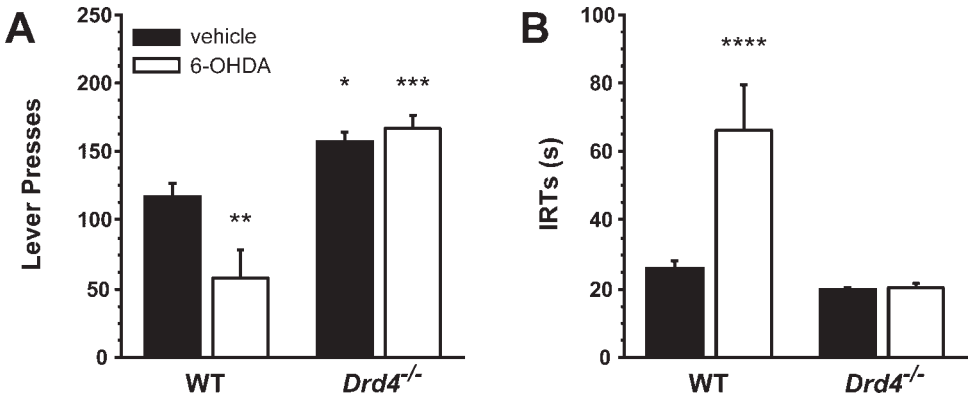

Fig. 2. Operant lever responses for food pellet reward in a fixed ratio-1 schedule. **A:** Number of lever presses and **B**: interresponse times exhibited by wild-type and $Drd4^{-/-}$ mice, treated with vehicle or 6-OHDA. *$P < 0.05$ versus WT vehicle; **$P < 0.01$ versus WT vehicle; ***$P < 0.0001$ versus WT 6-OHDA. ****$P < 0.005$ versus WT vehicle. Black bars represent vehicle-treated animals and white bars represent 6-OHDA-treated animals. Bars represent the mean of four mice per group ± SEM.

Fisher's LSD post hoc comparisons demonstrated that WT mice treated with 6-OHDA pressed the reinforcing lever at much lower rates than WT mice receiving vehicle (Fig. 2A). This difference was not observed in $Drd4^{-/-}$ mice (Fig. 2A) despite the fact that 6-OHDA-treated mice showed similar striatal hypodopaminergic levels to WT mice. In addition, $Drd4^{-/-}$ mice displayed increased rates of lever pressing compared to WT mice, regardless of the treatment (Fig. 2A). However, the higher rate of lever pressing exhibited by $Drd4^{-/-}$ mice did not correlate with increased food consumption, as $Drd4^{-/-}$ mice left a significant number of pellets in the food tray or the waste pan of the operant chamber (below the grid floor). An analysis of IRT showed a significant genotype × treatment interaction ($F_{(1,12)} = 6.541$, $P < 0.05$; genotype: $F_{(1,12)} = 28.739$, $P < 0.001$; treatment: $F_{(1,12)} = 6.939$, $P < 0.05$; Fig. 2B). WT mice treated with 6-OHDA showed longer interresponse intervals than vehicle-treated WT mice, whereas 6-OHDA-treated $Drd4^{-/-}$ mice did not. Altogether, these results demonstrate that WT mice rendered hypodopaminergic by a neonatal lesion with 6-OHDA show a severe reduction (∼50%) in response rate in a continuous reinforcement task and that this effect is absent in mice-lacking D4Rs.

The second test performed in this study evaluated the ability of $Drd4^{-/-}$ and WT mice with normal or lesioned DA neurons to perform a food-reinforcement task that depends on formation of response-outcome association, motor performance, and, with increasing difficulty, proper attention to salient environmental cues. To this end, mice were trained to perform an operant two-choice task as described in Materials and Methods section. In this study, WT mice pressed the reinforcing lever more than 80% of the trials in sessions where the signal time was 12 or 6 s (Fig. 3A). At signal time of 2 s, performance of WT mice receiving vehicle was still high (>0.75), whereas it reached chance (0.5) at the very short signal time of 0.5 s. These results indicate that for WT normodopaminergic mice the distracting lever does not act as a confounding factor at long or intermediate signal times. In contrast, WT mice neonatally lesioned with 6-OHDA showed severe difficulties to differentially press the reward-paired lever. Only at signal times of 12 and 6 s these mice showed a ratio of rewarded versus total trials slightly greater than 0.5. At lower signal times, performance was no different from chance levels. It would seem that, despite having received an exhaustive number of trials across all phases, 6-OHDA-treated WT mice did not form an association between the signal lever and reward outcome. Lack of functional D4Rs was not detrimental to the performance in this two-choice task since $Drd4^{-/-}$ chose the rewarding lever as efficiently as their WT siblings (Fig. 3A). It is worth noting that normodopaminergic animals of both genotypes significantly reduced their performance when signal time was reduced from 12 to 6 s ($F_{(1,6)} = 9$, $P < 0.05$, comparing data from the last 4 days of the 12 s stage and the first 4 days of the 6 s stage). During the following three blocks of 6 s performance increased to high levels ($F_{(3,18)} = 9.167$, $P < 0.01$; post hoc tests indicate that during the first 6-s block performance is lower than in the following three blocks of 6 s for both genotypes, $P < 0.05$).

Interestingly, $Drd4^{-/-}$ mice neonatally lesioned with 6-OHDA were also highly efficient in the selection of the reward-paired lever, indicating that the deficit in this two-choice task elicited by a drastic reduction in central DA levels is not observed in mice lacking functional D4Rs. Repeated measures ANOVA showed a genotype × treatment × stage interaction: ($F_{(3,36)} = 7.586$, $P < 0.0005$, observed power: 0.977; genotype: $F_{(1,12)} = 12.589$, $P < 0.005$; treatment: $F_{(1,12)} = 2.792$, $P > 0.1$; genotype × treatment: $F_{(1,12)} = 12.987$, $P < 0.005$; stage: $F_{(3,36)} = 176.366$, $P < 0.0001$; Fig. 3A).

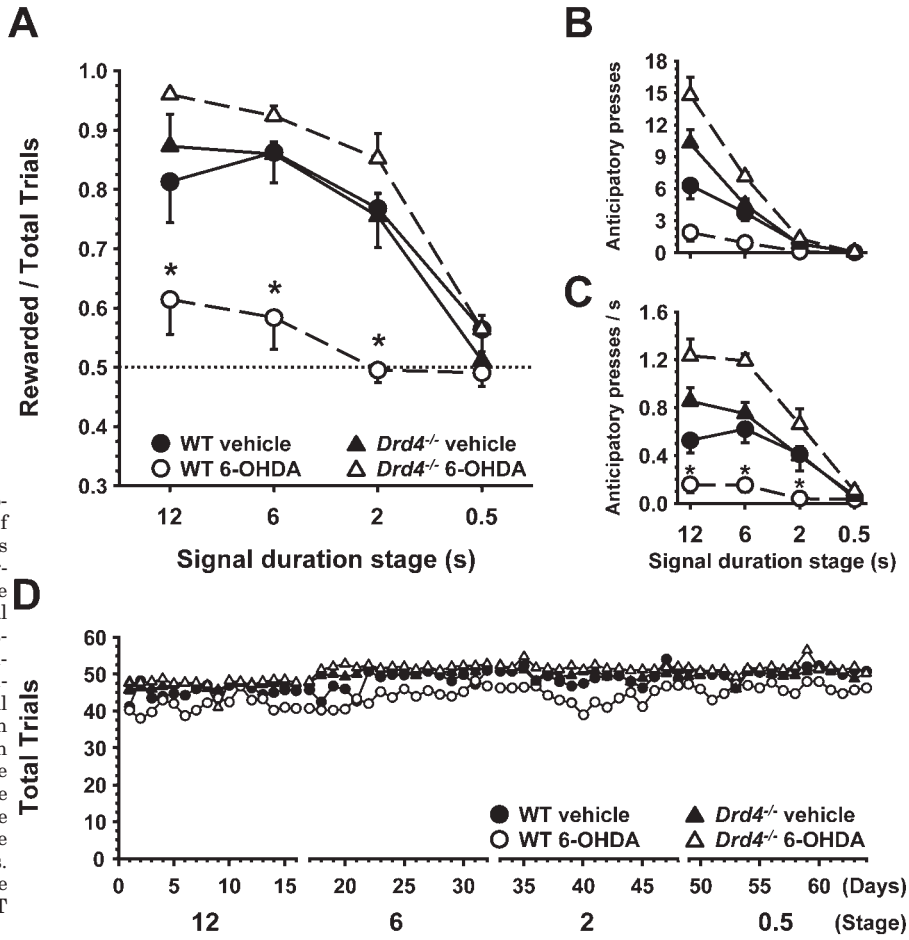

Fig. 3. Mouse performance in the two-choice operant task. **A:** The ratio of rewarded versus total trials was taken as an index of efficiency in this test and determined at the four different signal time stages (12, 6, 2, and 0.5 s). The horizontal dotted line denotes the 0.5 chance of pressing the right or left lever. **B:** Number of anticipatory presses and **C**: number of anticipatory presses per second executed by all mouse groups at the four signal duration stages. **D:** Total trials performed within each session of the task. Wild-type mice are represented with circles and $Drd4^{-/-}$ mice with triangles. Mice receiving vehicle are represented with black symbols while mice treated with 6-OHDA with white symbols. Symbols represent the mean of four mice per group ± SEM. *$P < 0.005$ versus WT vehicle.

Analysis of the number of anticipatory presses that each group of mice executed during the *signal time* (the number of lever presses on the *signal lever* executed before the *distracting* lever came out) revealed a significant genotype × treatment × stage interaction (Wilk's multivariate approach: $F_{(3,10)} = 8.247$, $P < 0.005$; genotype: $F_{(1,12)} = 54.465$, $P < 0.0001$; treatment: $F_{(1,12)} = 0.006$, $P > 0.5$; genotype × treatment: $F_{(1,12)} = 21.2$, $P < 0.001$; stage: $F_{(3,10)} = 76.785$, $P < 0.0001$). Again, this significant interaction was mainly due to the fact that $Drd4^{-/-}$ mice lesioned with 6-OHDA showed no signs of behavioral impairment in comparison to WT lesioned mice (Fig. 3B). Correspondingly to what we observed in the FR1 experiment for the IRTs, $Drd4^{-/-}$ normodopaminergic mice behaved similarly to their WT littermates and only showed higher levels of anticipatory lever pressing at the 12-s signal time (LSD comparison: $P < 0.01$; Fig. 3B). In general, there seems to be a positive linear correlation between the number of anticipatory presses and performance in the two-choice task. Normalization of the number of anticipatory presses with the duration of each stage showed curve patterns almost indistinguishable from those obtained in this operant test (cf. Figs. 3A and 3C). During the choice stages, and despite slower motor performance due to 6-OHDA treatment in WT mice, all four groups were able to complete a similar number of trials per daily session at all signal times (Fig. 3D). It is tempting to speculate that anticipatory pressing behavior ultimately facilitates reducing the distracting effect of the other lever. To test this hypothesis, we performed a correlation analysis for all mice at each stage, combining all genotypes and treatments. The results showed in Table I indicate that mice that executed more anticipatory presses obtained more rewarded trials in the two-choice task. This analysis indicates that motor responses on the signal lever could help in maximizing the number of rewarded

TABLE I. Correlation analysis between performance and number of anticipatory presses

Stage (s)	R^2	$F_{(1,14)}$	Confidence
12	0.41841	10.07211	$P < 0.01$
6	0.50074	14.04134	$P < 0.005$
2	0.378544	8.527740	$P < 0.05$
0.5	0.000270	0.003787	$P = 0.95$

trials by providing a means for sustaining attention on the signal lever.

DISCUSSION

In a previous study, we showed that mice neonatally lesioned with 6-OHDA displayed increased locomotor activity and impaired behavioral inhibition (Avale, 2004a) and that these phenotypes were dependent on functional D4Rs. The results presented here demonstrate that hypodopaminergic mice exhibit severe impairments to retrieve rewards in two operant positive reinforcement tasks. Strikingly, the deficits described here were not present in mice lacking D4Rs despite the fact that the 6-OHDA neonatal treatment induced identical levels of DA depletion in WT and *Drd4*$^{-/-}$ mice. The deficits in operant performance exhibited by DA-depleted WT mice could be due to a neurodevelopmental impairment of neuronal circuits involved in the evaluated tasks or to the lack of normal DA neurotransmission during the task. Therefore, the lack of functional D4Rs either protected such neuronal circuits during development in DA-depleted mice or facilitated the recruitment of an alternative mechanism through secondary adaptation. It has been shown in rodents that during the first 2 weeks of postnatal development, DA is critical for the final maturation of corticostriatal excitatory synapses, because it reduces the probability of glutamate release (Choi, 1997). Selective disruption of central DAergic pathways during this time frame increases glutamatergic transmission (Tang, 2001). Because D4Rs are expressed in cortical GABAergic neurons (Mrzljak, 1996), it is plausible that the absence of hyperpolarizing D4Rs in *Drd4*$^{-/-}$ mice leads to an increased GABAergic tone which compensates the decreased DAergic transmission by reducing the exaggerated glutamatergic input into the basal ganglia and limbic system and allowing the expression of normal locomotor and instrumental behaviors. Complying with the possibility of the recruitment of an alternative mechanism, it has been shown that acute serotonin depletion lowers hyperactivity of lesioned mice to normal levels (Avale, 2004b). Another possibility that may account for this compensation is that *Drd4*$^{-/-}$ mice showed a 9.9-fold increase in D2R^{high} in the striatum (Seeman, 2005), therefore increasing DA binding efficacy in a situation of low DA levels produced by the 6-OHDA lesion. There is also evidence that *Drd4*$^{-/-}$ mice show higher levels of D1R expression (Gan et al., 2004), and it has been shown that DA depletion in the striatum lowers D1R levels (Gerfen, 2000). Therefore, a last possible interpretation of the present results points toward compensatory deregulations of D1R expression levels.

The impaired responses to salient-rewarding stimuli observed in mice with neonatal lesions of 6-OHDA are in agreement with those obtained with mice incapable of synthesizing DA, which are deficient to direct their behavior towards appropriate goals (Cannon and Bseikri, 2004). In addition, the poor operant performance exhibited by the 6-OHDA neonatally treated WT mice is consistent with the idea that DA transmission is involved in the encoding of signals that predict reward (Schultz, 1997). The absence of performance deficits in 6-OHDA-treated *Drd4*$^{-/-}$ mice suggests that the D4Rs contribute to the fine tuning of central circuits involved in motivational and cognitive aspects of food-seeking behavior. This developmental function of the D4R may play an important role in major psychiatric conditions such as ADHD, schizophrenia, and drug abuse. In addition, our results using operant-learning paradigms as the continuous reinforcement schedule and two-choice attentional task strengthen the idea that mice rendered hypodopaminergic by a neonatal lesion with 6-OHDA constitute a useful mouse model to study not only locomotor and motor aspects of behavior but also reward-seeking and discrimination functions.

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REFERENCES

Avale ME, Falzone TL, Gelman DM, Low MJ, Grandy DK, Rubinstein M. 2004a. The dopamine D4 receptor is essential for hyperactivity and impaired behavioral inhibition in a mouse model of attention deficit/hyperactivity disorder. Mol Psychiatry 9:718–726.

Avale ME, Nemirovsky SI, Raisman-Vozari R, Rubinstein M. 2004b. Elevated serotonin is involved in hyperactivity but not in the paradoxical effect of amphetamine in mice neonatally lesioned with 6-hydroxydopamine. J Neurosci Res 78:289–296.

Biederman J, Faraone S. 2005. Attention-deficit hyperactivity disorder. Lancet 366:237–248.

Cannon CM, Bseikri MR. 2004. Is dopamine required for natural reward? Physiol Behav 81:741–748.

Callier S, Snapyan M, Le Crom S, Prou D, Vincent JD, Vernier P. 2003. Evolution and cell biology of dopamine receptors in vertebrates. Biol Cell 95:489–502.

Choi S, Lovinger DM. 1997. Decreased probability of neurotransmitter release underlies striatal long-term depression and postnatal development of corticostriatal synapses. Proc Natl Acad Sci USA 94:2665–2670.

Deng YP, Lei WL, Reiner A. 2006. Differential perikaryal localization in rats of D1 and D2 dopamine receptors on striatal projection neuron types identified by retrograde labeling. J Chem Neuroanat 32:101–116.

Everitt BJ, Robbins TW. 2005. Neural systems of reinforcement for drug addiction: From actions to habits to compulsion. Nat Neurosci 8:1481–1489.

Faraone SV, Doyle AE, Mick E, Biederman J. 2001. Meta-analysis of the association between the 7-repeat allele of the dopamine D_4 receptor gene and attention deficit hyperactivity disorder. Am J Psychiatry 158:1052–1057.

Fiorillo CD, Tobler PN, Schultz W. 2003. Discrete coding of reward probability and uncertainty by dopamine neurons. Science 299:1898–1902.

Fiorillo CD, Newsome WT, Schultz W. 2008. The temporal precision of reward prediction in dopamine neurons. Nat Neurosci 11:966–973.

Fuster JM. 1997. The prefrontal cortex. Philadelphia: Lippincott-Raven Publishers.

Gan L, Falzone TL, Zhang K, Rubinstein M, Baldessarini RJ, Tarazi FI. 2004. Enhanced expression of dopamine D_1 and glutamate NMDA receptors in dopamine D_4 receptor knockout mice. J Mol Neurosci 22:167–178.

Gerfen CR. 2000. Molecular effects of dopamine on striatal-projection pathways. Trends Neurosci 23:S64–S70.

Gerfen CR. 2006. Indirect-pathway neurons lose their spines in Parkinson disease. Nat Neurosci 9:157–158.

Ito R, Dalley JW, Howes SR, Robbins TW, Everitt BJ. 2000. Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. J Neurosci 20:7489–7495.

Jentsch JD, Taylor JR. 1999. Impulsivity resulting from frontostriatal dysfunction in drug abuse: Implications for the control of behavior by reward-related stimuli. Psychopharmacology (Berl) 146:373–390.

Khan ZU, Gutierrez A, Martin R, Penafiel A, Rivera A, de la Calle A. 2000. Dopamine D5 receptors of rat and human brain. Neuroscience 100:689–699.

Kringelbach ML. 2005. The human orbitofrontal cortex: Linking reward to hedonic experience. Nat Rev Neurosci 6:691–702.

Lidow MS, Wang F, Cao Y, Goldman-Rakic PS. 1998. Layer V neurons bear the majority of mRNAs encoding the five distinct dopamine receptor subtypes in the primate prefrontal cortex. Synapse 28:10–20.

Mrzljak L, Bergson C, Pappy M, Huff R, Levenson R, Goldman-Rakic P. 1996. Localization of dopamine D4 receptors in GABAergic neurons of the primate brain. Nature 381:245–248.

Noaín D, Avale ME, Wedemeyer C, Peper M, Rubinstein M. 2006. Identification of brain neurons expressing the dopamine D4 receptor gene using BAC transgenic mice. Eur J Neurosci 24:2429–2438.

Porrino LJ, Lyons D, Smith HR, Daunais JB, Nader MA. 2004. Cocaine selfadministration produces a progressive involvement of limbic, association, and sensorimotor striatal domains. J Neurosci 24:3554–3562.

Rubinstein M, Phillips TJ, Bunzow JR, Falzone TL, Dziewczapolski G, Zhang G, Fang Y, Larson J, McDougall J, Chester J, Saez C, Pugsley T, Gershanik O, Low M, Grandy D. 1997. Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and metamphetamine. Cell 90:991–1001.

Rubinstein M, Cepeda C, Hurst RS, Altemus KL, Ariano MA, Falzone TL, Kozell LB, Meshul CK, Bunzow JR, Low MJ, Levine MS, Grandy DK. 2001. Dopamine D4 receptor-deficient mice display cortical hyperexcitability. J Neurosci 21:3756–3763.

Schoenemann PT, Sheehan MJ, Glotzer LD. 2005. Prefrontal white matter volume is disproportionately larger in humans than in other primates. Nat Neurosci 8:242–252.

Schultz W, Dayan P, Montague PR. 1997. A neural substrate of prediction and reward. Science 275:1593–1599.

Seeman P, Weinshenker D, Quirion R, Srivastava LK, Bhardwaj SK, Grandy DK, Premont RT, Sotnikova TD, Boksa P, El-Ghundi M, O'Dowd BF, George SR, Perreault ML, Mannisto PT, Robinson S, Palmiter RD, Tallerico T. 2005. Dopamine supersensitivity correlates with $D2^{High}$ states, implying many paths to psychosis. Proc Natl Acad Sci USA 102:3513–3518.

Tang K, Low MJ, Grandy DK, Lovinger DM. 2001. Dopamine-dependent synaptic plasticity in striatum during in vivo development. Proc Natl Acad Sci USA 98:1255–1260.

Wedemeyer C, Goutman JD, Avale ME, Franchini LF, Rubinstein M, Calvo DJ. 2007. Functional activation by central monoamines of human dopamine D_4 receptor polymorphic variants coupled to GIRK channels in *Xenopus oocytes*. Eur J Pharmacol 562:165–173.

Werner P, Hussy N, Buell G, Jones K, North R. 1996. D2. D3, and D4 dopamine receptors couple to G protein-regulated potassium channels in *Xenopus oocytes*. Mol Pharmacol 49:656–661.

Zhang K, Grady CJ, Tsapakis EM, Andersen SL, Tarazi FI, Baldessarini RJ. 2004. Regulation of working memory by dopamine D4 receptor in rats. Neuropsychopharmacology 29:1648–1655.