Dopamine D₂ receptors mediate reversal learning in male C57BL/6J mice

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Dopamine is critical for directing goal-oriented behavior. We investigated dopamine D₂ receptor involvement in reversal learning and reinforcement efficacy in mice lacking functional dopamine D₂ receptors and their heterozygous and wild-type littermates. Mice discriminated between two odors to receive a food reinforcer: One odor signaled a reinforcer (S⁺); the other odor signaled no reinforcer (S⁻). After mice learned the S⁺/S⁻ relationship, we inverted the reinforcement contingencies. The necessary number of trials to relearn the new reinforcement contingencies served as our index of reversal learning. Mice lacking functional dopamine D₂ receptors repeatedly failed to inhibit previously reinforced responses during reversal trials. In a separate experiment, mice responded for reinforcers on a progressive ratio schedule of reinforcement. Mice lacking functional dopamine D₂ receptors earned significantly fewer reinforcers than did heterozygous mice. Our results suggest that dopamine D₂ receptors regulate reversal learning and influence the reinforcing efficacy of natural rewards.

The ability of an organism to redirect behaviors when reinforcement contingencies change is especially important for adaptive responding to a fluctuating environment (Slotnick, 2001). Reversal learning paradigms, where a subject must inhibit a previously reinforced behavior and emit responses that originally were not reinforced provide valid indices of this skill. Recent evidence from excitotoxic lesion studies demonstrates that the ventral striatopallidal pathway mediates reversal learning (Ferry, Lu, & Price, 2000). This finding is of major importance, because certain debilitating neuropsychiatric disorders, such as schizophrenia (Crider, 1997) and attention deficit/hyperactivity disorder (Itami & Uno, 2002), are associated with disrupted signaling in striatopallidal and mesocortical pathways (Ongur & Price, 2000).

Dopamine D₂ receptors (D₂Rs) are located predominantly in dorsal and ventral striatum and olfactory tubercle and midbrain (Civelli, Bunzow, & Grandy, 1993). A burgeoning body of literature demonstrates that dopamine D₂Rs within the striatopallidal pathway affect reversal learning. Indeed, disruption of D₂R signaling impairs reversal learning performance in humans (Mehta, Sahakian, McKenna, & Robbins, 1999) and mice (Gasbarri et al., 1997). However, caution is warranted when interpreting these results since commercially available agonists and D₂R antagonists are known to have nonselective effects

in vivo (Neve & Neve, 1997). A complementary approach to investigating the role of D₂Rs in reversal learning is to utilize mice that are completely or partially deficient in functional D₂Rs (D₂R^{-/-} and D₂R^{+/-}, respectively).

We have previously demonstrated that female D₂R^{-/-} mice are significantly impaired in our reversal learning paradigm and that their deficiencies are not attributable to locomotor or olfactory deficits (Kruzich & Grandy, 2004). However, a comparable evaluation of male wild-type (+/+), heterozygous (+/-), and knockout (-/-) mice has not been reported. The reinforcement efficacy of alternative natural rewards (e.g., sucrose) in our D₂R-deficient mice has not been reported, although Phillips et al. (1998) did report that a preference for saccharin over quinine was intact in mice lacking half (heterozygous; +/-) or all (knockout; -/-) of their normal complement of D₂Rs. Such studies may inform regarding the question of whether or not impaired acquisition of reversal learning is mediated by altered D₂R-mediated reward perception. In the present context, it is worth noting that humans with histories of chronic drug abuse display decreased orbitofrontal D₂R density, hypofrontal cortical activity, enhanced responsiveness to drug-related cues, and diminished drive for natural rewards (Thanos et al., 2005; Volkow, Fowler, & Wang, 2002; Volkow et al., 1999).

The availability of heterozygous (D₂R^{+/-}) male mice that express ~50% of the D₂Rs present in wild-type (D₂R^{+/+}) mice (Kelly et al., 1997) are a valuable resource with which to investigate gene-dosage relationships across reversal learning and reinforcement efficacy tasks. Thus, further behavioral characterization of D₂R^{+/-} mice may reveal a role for dopamine D₂Rs in reversal learning and the reinforcement efficacy of a naturally rewarding nutritional substance (sucrose). We therefore sought to test the hypotheses that dopamine D₂Rs mediate reversal learning in a gene-dosage-dependent manner and that

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D₂R density is correlated with reinforcement efficacy as measured by a progressive ratio schedule of reinforcement in drug-naïve mice.

METHOD

Subjects

Male mice (8–10 weeks old) were congenic offspring of the original F₂ hybrid (129/Sv × C57BL/6J; Kelly et al., 1997) which were backcrossed to inbred C57BL/6J stock (Jackson Laboratories, ME) for 20 generations. The sex of the donor/mutant was alternated for each generation. All experimental subjects were genotyped according to previously published methods (Kelly et al., 1997). Littermates from all three genotypes, *wild-type* (D₂R^{+/+}), *heterozygous* (D₂R^{+/-}), and *knockout* (D₂R^{-/-}), were used for all experiments. All mice were maintained at 90% of their ad lib weight except during the free-feeding portion of the sucrose reinforcement studies.

Procedure

Two-odor discrimination. Mice were trained according to previously published methods (Kruzich & Grandy, 2004), with some exceptions. They were trained to ambulate the distance of a clear Nalgene animal container (30 × 30 cm) to retrieve a 20-mg food pellet (Formula A/I; Research Diets, New Brunswick, NJ) located within a 3-cm dish attached to the cage floor with Velcro. The latency to ambulate the distance from the start area to the dish and begin eating the food pellet was timed with a standard stopwatch. After they had completed 10 successful trials, the animals were trained to dig through sterile playground sand for pellets during the same daily testing session. During the first three digging trials, the animals were presented with a dish filled with sand. Three pellets were contained within the dish. One pellet was completely buried, the second pellet was partially buried (a portion was visible to subjects), and the third rested on the surface of the sand. The latencies to locate and consume all three pellets were recorded separately. Subsequent to the first three digging trials, the animals received seven additional food reinforced trials in which they had to dig for one completely buried pellet.

On the next day, each mouse was presented with two dishes, each containing a different scent. Sand was scented to mask the scent of the reinforcer by the addition of 0.80 g of either cinnamon or dill weed (dill) cooking spice, and 0.40 g of ground food pellets was then added to 98.80 g of sterile, scented sand. Previous studies had demonstrated that these sand mixtures are not palatable to our mice (Kruzich & Grandy, 2004). One of the scents (cinnamon or dill) signaled that a pellet was buried at the bottom of the dish (S⁺). The other scent signaled that no pellet was buried within that separate dish (S⁻). The determination of which scent (cinnamon or dill) served as the S⁺ or S⁻ was counterbalanced for each subject. A trial terminated when the animal dug in the S⁺ dish and consumed the food pellet or began digging in the S⁻ dish or if 3 min elapsed before any response occurred. Digging was defined as the actual displacement of sand with the forelimbs and/or nose. Sniffing or resting forepaws on top of the sand was not considered digging and was not penalized. Trials were separated by 20 sec, and they were run in blocks of 10. We considered successful discrimination to be 8 out of 10 correct responses in a block. After they had demonstrated successful odor discrimination, the animals were returned to their home cages.

Reversal learning. The reversal trials began the following day. During reversal learning trials, the reinforcement contingencies from the previous day were inverted—the scent that formerly served as the S⁺ now signaled no food pellet, while the former S⁻ now signaled reinforcer availability. This manipulation measured the animal's ability to inhibit previously reinforced response patterns and redirect its behavior to stimuli that originally signaled no reinforcer. The number of trials necessary to demonstrate 8 out of 10 accurate

responses in a single block served as our index of reversal learning. If a subject failed to make a response within 3 min, the trial ended, no pellet was given, and an error of omission was recorded. Multiple blocks were run during each session until our criterion for reversal learning was met by each subject. Reversal testing occurred during just one session.

Progressive ratio responding for sucrose. Mice weighing ~90% of their free-feeding weight were trained to nosepoke for 10% sucrose solutions in standard mouse operant chambers (Med Associates, St. Albans, VT) during daily 30-min sessions on a continuous schedule of reinforcement. The testing apparatus were 12 identical Med Associates modular mouse test chambers (ENV-307A: 21.6 × 17.8 × 12.7 cm) placed within Melamine sound-attenuating cabinets. Houselights were mounted outside each chamber. In the center of the front wall, 1.27 cm above the grid floor, there was a nosepoke response unit that could be illuminated when required (ENV-313M). On each side of the center poke response unit there was a stimulus light (ENV-321M), 4.45 cm above the grid floor. A liquid food receptacle (ENV-303LP) with head entry detectors (ENV-303HDLP) was located 1.91 cm below the left and right stimulus lights and 0.63 cm above the grid floor. Sucrose solution rewards (10% w/v) were delivered to the receptacles via syringe pumps (PHM-100). Following two sessions of consistent responding (defined as an animal's earning at least 50 reinforcers), the response requirement was increased by two starting at a fixed ratio (FR)2 and eventually ending at an FR10. Once responding at the FR10 schedule stabilized (≤10% variability in intake across at least two consecutive sessions), we tested the reinforcement efficacy of the 10% sucrose in our mouse lines with a progressive ratio (PR) schedule of reinforcement (Hodos, 1961). During the progressive ratio sessions, the response output increments for each subsequent schedule of reinforcement were increased by two responses. The pattern of response requirements was therefore 2, 4, 6, 8, 10, etc. The final completed schedule served as the breakpoint for the PR experiment. The average of at least two testing sessions was used to determine the breakpoint for each individual animal. When subjects provided data from at least two PR sessions, they were allowed unrestricted access to food in their home cages and were then retested in the PR paradigm at free-feeding weights. This manipulation assessed whether reinforcement efficacy is differentially affected in our mouse lines at different levels of motivation.

Data Analysis

The latencies to retrieve pellets during the pellet retrieval and digging trials were analyzed separately with two-way repeated measures ANOVAs with genotype and trials as the factors. The numbers of trials needed to demonstrate accurate odor discrimination, reversal learning, and number of errors committed were measured with a 3 × 2 repeated measures ANOVA (genotype × test). Errors were further categorically defined as errors of omission (failing to dig in either dish within 3 min) or of commission (digging in the incorrect dish) and analyzed with a Kruskal–Wallis ANOVA on ranks.

The number of days to acquire nosepoke responses for sucrose was measured with a two-way repeated measures ANOVA. Breakpoints were also analyzed with a two-way repeated measures ANOVA with genotype and feeding condition as the independent variables.

Significance was set at $p < .05$. Following a significant ANOVA, post hoc testing, utilizing the Bonferroni t test, which corrects for multiple pairwise comparisons, was used.

RESULTS

Odor Discrimination and Reversal Learning

The mice demonstrated no differences in learning pellet retrieval, digging through sand, or discriminating between two odors (data not shown). In contrast, the

three genotypes were significantly different with respect to their performance in our reversal learning paradigm (see Figure 1). There were significant effects of genotype [$F(2,30) = 10.622, p < .001$] and test [$F(1,30) = 40.633, p < .001$], and a significant interaction of genotype $\times$ test [$F(2,30) = 8.503, p < .01$]. Post hoc analyses revealed that the $D_2R^{-/-}$ mice performed significantly worse during the reversal than did either wild-type ($D_2R^{+/+}$) or heterozygous ($D_2R^{+/-}$) mice ($p < .05$ for all comparisons). The $D_2R^{+/-}$ mice required more trials than did the $D_2R^{+/+}$ mice to attain our reversal learning criterion ($p < .05$). Last, the $D_2R^{+/-}$ and $D_2R^{-/-}$ mice required more trials to achieve the reversal learning criterion as opposed to two-odor discrimination ($p < .05$ for all comparisons; the comparison between two-odor discrimination and reversal learning within the $D_2R^{+/+}$ mice yielded $p = .055$), replicating previous reports for other species in which it has been shown that reversal learning requires more trials to be learned than does the simple odor discrimination (e.g., Ferry et al., 2000).

Errors

When behavior was parceled into errors of commission or errors of omission, significant differences were found for commission errors [$F(2,30) = 17.31, p < .001$] but not for errors of omission (data not shown). The $D_2R^{-/-}$ mice committed significantly more errors of commission than did the $D_2R^{+/-}$ and $D_2R^{+/+}$ mice ($p < .05$ for all comparisons; see Figure 2).

Progressive Ratio Responding for Sucrose

Significant differences in responding along PR schedules of reinforcement for 10% sucrose were found across genotype [$F(2,25) = 3.88, p < .05$] and feeding condi-

Figure 1. Two-odor discrimination and reversal learning performance. Each genotype required significantly more trials to demonstrate reversal learning as opposed to two-odor discrimination. Both $D_2R^{+/-}$ ($n = 14$) and $D_2R^{-/-}$ mice ($n = 9$) required significantly more trials than did $D_2R^{+/+}$ mice ($n = 10$) to demonstrate reversal learning. $D_2R^{-/-}$ mice required significantly more trials to demonstrate accurate reversal learning than did $D_2R^{+/-}$ mice. * $p < .05$.

Figure 2. Errors of commission. The $D_2R^{-/-}$ mice demonstrated a significant inability to suppress responding to stimuli previously signaling reinforcement availability in comparison with both $D_2R^{+/+}$ and $D_2R^{+/-}$ mice. * $p < .05$.

tion [$F(1,25) = 37.9, p < .001$], yet the genotype $\times$ feeding condition interaction was not significant [$F(2,25) = 1.23, p = .31$]. A separate t test between the $D_2R^{+/-}$ and $D_2R^{-/-}$ during food-restricted testing showed that the $D_2R^{-/-}$ mice responded for significantly fewer infusions than did the $D_2R^{+/-}$ mice ($p < .05$). There was a trend for $D_2R^{+/-}$ mice to engage in greater behavioral output for sucrose under food-restricted conditions than that for $D_2R^{+/+}$ ($p = .10$). All groups responded significantly less during the free-feeding phase than during the food-restricted testing ($p < .05$ for all comparisons; see Figure 3).

DISCUSSION

The present study supports and extends our previous work showing that D_2Rs mediate reversal learning in mice. Although we fully acknowledge that the use of genetically engineered mice is not without caveat (e.g., developmental compensation, where an animal grows and matures in the absence of a gene; functional systems may or may not counteract the lack), we advanced our previous work by demonstrating that female mice from our engineered line show disrupted reversal learning in a gene-dosage-dependent manner (Kruzich & Grandy, 2004). Now we provide evidence here that the gene dosage of *drd2* can affect the reinforcement efficacy of 10% sucrose solutions across feeding manipulations in C57BL/6J mice.

The consensus of genetic studies investigating the involvement of rodent dopamine D_2Rs in learning tasks indicate that D_2Rs are involved in the behavioral suppression of previously reinforced responses as well as the animal's ability to sculpt behavior when reinforcement contingencies change (Liu et al., 2004; Robinson, Sandstrom, Denenberg, & Palmiter, 2005). A potential mechanism involving the dopamine D_2Rs in learning tasks is to serve as a brake (Carlsson et al., 2001) or error signal (Schultz, 2000) in real time during ongoing behavior.

Figure 3. Progressive ratio responding for sucrose reinforcement. The D₂R^{+/-} mice ($n = 10$) responded for significantly more reinforcers than did the D₂R^{-/-} mice ($n = 10$) ($p < .05$) and showed a trend toward increased responding in comparison with D₂R^{+/+} mice ($n = 8$) ($p = .10$) during the food-deprived phase. Each genotype earned significantly fewer reinforcers during the free-feeding manipulation than during the food-deprived phase of the experiment ($p < .05$).

Carlsson et al. have postulated that D₂Rs affect behavior by halting excitatory cortically driven behavioral output when unexpected outcomes are encountered. In support of this view are recent findings that dopaminergic neurons signal differences in anticipated reward and actual outcome of behavior (Schultz, 2000). Our present results fit well within the developing framework of these hypotheses. It is interesting that all dopamine D₂R genotypes are equally competent with respect to their associative learning and odor discrimination abilities. The performance deficiencies displayed by the D₂R^{-/-} manifest during tasks requiring inhibition of previously reinforced behaviors, a response thought to be mediated by striatothalamic circuitry (Ferry et al., 2000).

We previously demonstrated that female dopamine D₂R^{-/-} mice display disruptions in reversal learning and two-odor discrimination (Kruzich & Grandy, 2004). Interestingly, in the present study we failed to find a statistically significant difference in the ability of male mice of any genotype to acquire the ability to discriminate between two odors. Previous reports have demonstrated that male C57BL/6J mice learn odor discrimination tasks faster than female C57BL/6J mice do (Mihalick, Langlois, & Krienke, 2000). In addition, in our previous report, animals received a total of 10 trials per day. In the present study, the animals received as many 10-trial blocks as were necessary to demonstrate two-odor discrimination and reversal learning in 1 day. Possibly, sex of the subjects and number of trials differentially interacted, accounting for this difference between the results of the two studies.

The progressive ratio experiments were conducted in an effort to address the question of whether or not dopamine D₂R density is correlated with motivation and reinforcement efficacy (Thanos et al., 2005; Volkow et al., 1999). What is not clear from the otherwise elegant studies published by Thanos et al. (2005) and Volkow et al.

(1999) is whether or not diminished drive for natural rewards and dopamine D₂R binding density is a product of chronic substance abuse, a genetic predisposition, or some combination of both. In this context, it may be worth noting that a mouse's genotype failed to predict food-reinforced pellet retrieval, digging, or two-odor discrimination performance—which in our estimation most likely reflects the acute reinforcing properties of food pellets within our mouse line. Intriguingly, results from human subjects indicate that subjects with ~50% of normal DA binding sites (i.e., dopamine D₂Rs; analogous to our D₂R^{+/-} mice that have ~50% D₂R relative to D₂R^{+/+} littermates) show a reduced motivation for natural rewards (Thanos et al., 2005; Volkow et al., 1999). Therefore, it was surprising to discover that our D₂R^{+/-} mice demonstrated an *enhanced* behavioral output for natural rewards during food-deprived testing in comparison with D₂R^{-/-} and D₂R^{+/+} mice, which is the exact opposite of the human findings (Volkow et al., 1999). It may very well be that human subjects display diminished reinforcement efficacy of natural rewards and associated decreased density of dopamine D₂Rs as a result of previous psychostimulant abuse.

An alternative interpretation of the existing data is that the heterozygous genotype confers an advantage on C57BL/6J mice—that is, variability in behavior that might, in fact, be influenced by nucleotide sequence polymorphisms in the DNA surrounding the *drd2* locus (Falconer & Mackay, 1996). In this context, it is worth recalling that the heterozygous (D₂R^{+/-}) mice used in this study possessed dopamine D₂R alleles that were derived from both 129SvEv and C57BL/6J strains of mice. It is also a possibility that certain combinations of alleles surrounding the targeted locus for the dopamine D₂R allele (*drd2*) in the donor strain and our mutant line could have contributed to the differences in breakpoints displayed by the D₂R^{+/-} mice in our progressive ratio study (Foley et al., 2003), although the congenicity (backcrossed for more than 20 generations) of our mutant line diminishes this likelihood.

In conclusion, the results of the present study demonstrate that D₂Rs can exert a significant role in behavioral plasticity. Mice lacking functional dopamine D₂Rs readily learned the initial odor discrimination task. However, when the reinforcement contingencies were inverted, the D₂R^{-/-} mice were impaired in their ability to alter their response patterns and redirect behavior to stimuli previously indicating no reinforcement. We also determined that dopamine D₂Rs contribute to reinforcement efficacy of sucrose in progressive ratio schedules of reinforcement in C57BL/6J mice. Again, the D₂R^{-/-} mice demonstrated no impairments in the ability to acquire operant responding for sucrose along standard fixed-ratio schedules of reinforcement. Nonetheless, the D₂R^{-/-} mice showed a significant decrease in behavioral output (breakpoints) when responding was reinforced according to a progressive ratio schedule of reinforcement. These findings lend themselves to the hypothesis that disrupted dopamine D₂R neurotransmission underlies cognitive deficits associated

with many psychiatric disorders and thereby represents an additional target for the development of pharmacotherapies that could attenuate the perseveration and inflexibility of compulsive ideation and behavior.

REFERENCES

- CARLSSON, A., WATERS, N., HOLM-WATERS, S., TEDROFF, J., NILSSON, M., & CARLSSON, M. L. (2001). Interactions between monoamines, glutamate, and GABA in schizophrenia: New evidence. *Annual Review of Pharmacology & Toxicology*, **41**, 237-260.
- CIVELLI, O., BUNZOW, J. R., & GRANDY, D. K. (1993). Molecular diversity of the dopamine receptors. *Annual Review of Pharmacology & Toxicology*, **33**, 281-307.
- CRIDER, A. (1997). Perseveration in schizophrenia. *Schizophrenia Bulletin*, **23**, 63-74.
- FALCONER, D. S., & MACKAY, T. F. C. (1996). *Introduction to quantitative genetics*. Harlow, U.K.: Prentice Hall.
- FERRY, A. T., LU, X. C., & PRICE, J. L. (2000). Effects of excitotoxic lesions in the ventral striatopallidal-thalamocortical pathway on odor reversal learning: Inability to extinguish an incorrect response. *Experimental Brain Research*, **131**, 320-335.
- FOLEY, A. G., HEDIGAN, K., ROULLET, P., MORICARD, Y., MURPHY, K. J., SARA, S. J., & REGAN, C. M. (2003). Consolidation of memory for odour-reward association requires transient polysialylation of the neural cell adhesion molecule in the rat hippocampal dentate gyrus. *Journal of Neuroscience Research*, **74**, 570-576.
- GASBARRI, A., SULLI, A., PACITTI, C., PUGLISI-ALLEGRA, S., CABIB, S., CASTELLANO, C., ET AL. (1997). Strain-dependent effects of D₂ dopaminergic and muscarinic-cholinergic agonists and antagonists on memory consolidation processes in mice. *Behavioural Brain Research*, **86**, 97-104.
- HODOS, W. (1961). Progressive ratio as a measure of reward strength. *Science*, **134**, 943-944.
- ITAMI, S., & UNO, H. (2002). Orbitofrontal cortex dysfunction in attention-deficit hyperactivity disorder revealed by reversal and extinction tasks. *NeuroReport*, **13**, 2453-2457.
- KELLY, M. A., RUBINSTEIN, M., ASA, S. L., ZHANG, G., SAEZ, C., BUNZOW, J. R., ET AL. (1997). Pituitary lactotroph hyperplasia and chronic hyperprolactinemia in dopamine D2 receptor-deficient mice. *Neuron*, **19**, 103-113.
- KRUZICH, P. J., & GRANDY, D. K. (2004). Dopamine D2 receptors mediate two-odor discrimination and reversal learning in C57BL/6 mice. *BMC Neuroscience*, **5**, 12.
- LIU, Z., RICHMOND, B. J., MURRAY, E. A., SAUNDERS, R. C., STEENROD, S., SUBBLEFIELD, B. K., ET AL. (2004). DNA targeting of rhinal cortex D2 receptor protein reversibly blocks learning of cues that predict reward. *Proceedings of the National Academy of Sciences*, **101**, 12336-12341.
- MEHTA, M. A., SAHAKIAN, B. J., MCKENNA, P. J., & ROBBINS, T. W. (1999). Systemic sulpiride in young adult volunteers simulates the profile of cognitive deficits in Parkinson's disease. *Psychopharmacology*, **146**, 162-174.
- MIHALICK, S. M., LANGLOIS, J. C., & KRIENKE, J. D. (2000). Strain and sex differences on olfactory discrimination learning in C57BL/6J and DBA/2J inbred mice (*Mus musculus*). *Journal of Comparative Psychology*, **114**, 365-370.
- NEVE, K. A., & NEVE, R. L. (1997). *The dopamine receptors*. Totowa, NJ: Humana Press.
- ONGUR, D., & PRICE, J. L. (2000). The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys, and humans. *Cerebral Cortex*, **10**, 206-219.
- PHILLIPS, T. J., BROWN, K. J., BURKHART-KASCH, S., WENGER, C. D., KELLY, M. A., RUBINSTEIN, M., ET AL. (1998). Alcohol preference and sensitivity are markedly reduced in mice lacking dopamine D2 receptors. *Nature Neuroscience*, **1**, 610-615.
- ROBINSON, S., SANDSTROM, S. M., DENENBERG, V. H., & PALMITER, R. D. (2005). Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. *Behavioral Neuroscience*, **119**, 5-15.
- SCHULTZ, W. (2000). Multiple reward signals in the brain. *Nature Reviews Neuroscience*, **1**, 199-207.
- SLOTNICK, B. M. (2001). Animal cognition and the rat olfactory system. *Trends in Cognitive Sciences*, **5**, 216-222.
- THANOS, P. K., RIVERA, S. N., WEAVER, K., GRANDY, D. K., RUBINSTEIN, M., UMEGAKI, H., ET AL. (2005). Dopamine D2R DNA transfer in dopamine D2 receptor-deficient mice: Effects on ethanol drinking. *Life Sciences*, **77**, 130-139.
- VOLKOW, N. D., FOWLER, J. S., & WANG, G. J. (2002). Role of dopamine in drug reinforcement and addiction in humans: Results from imaging studies. *Behavioural Pharmacology*, **13**, 355-366.
- VOLKOW, N. D., WANG, G. J., FOWLER, J. S., LOGAN, J., GATLEY, S. J., GIFFORD, A., ET AL. (1999). Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. *American Journal of Psychiatry*, **156**, 1440-1443.