

Dopamine D₂ Receptor Activation Modulates Perceived Odor Intensity

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Dopaminergic modulation affects odor detection thresholds and olfactory discrimination capabilities in rats. The authors show that dopamine D₂ receptor modulation affects odor discrimination capabilities in a manner similar to the modulation of stimulus intensity. Performance in a simultaneous odor discrimination task was systematically altered by manipulations of both odorant concentration and D₂ receptor activation (agonist quinpirole, 0.025–0.5 mg/kg; antagonist spiperone, 0.5 mg/kg). Rats' discrimination performance systematically improved at higher odor concentrations. Blockade of D₂ receptors improved performance equivalent to increasing odor concentration by 2 log units, whereas activation of D₂ receptors reduced odor discrimination performance in a dose-dependent manner. Bulbar dopamine release may serve a gain control function in the olfactory system, optimizing its sensitivity to changes in the chemosensory environment.

Keywords: olfaction, dopamine, odor discrimination, concentration, behavior

The olfactory bulb is innervated by multiple neuromodulatory fibers, notably cholinergic fibers from the horizontal limb of the diagonal band of Broca (Le Jeune & Jourdan, 1993; Macrides, Davis, Youngs, Nadi, & Margolis, 1981; Zaborszky, Carlsen, Brashear, & Heimer, 1986; Zaborszky, Cullinan, & Braun, 1991), noradrenergic fibers from the locus coeruleus (McLean & Shipley, 1991; Perez, Hernandez, & Almlil, 1987; Shipley, Halloran, & de la Torre, 1985), and serotonergic fibers from the raphe nucleus (Mamounas, Mullen, O'Hearn, & Molliver, 1991; Moore, Halaris, & Jones, 1978). Receptors for these neuromodulators are found on specific cell types within the olfactory bulb, and experimental manipulation of these neuromodulatory inputs to the olfactory bulb has been shown to modulate bulbar processing, underlying phenomena such as odor generalization acuity (Linster & Cleland, 2002; Linster, Garcia, Hasselmo, & Baxter, 2001), olfactory associative learning (McLean & Harley, 2004; Yuan, Harley, & McLean, 2003), and olfactory short-term memory (Ravel, Elaagouby, & Gervais, 1994; Ravel, Vigouroux, Elaagouby, & Gervais, 1992). In contrast, whereas dopamine is a bulbar neuromodulator, there are no centrifugal dopaminergic projections into the olfactory bulb (McLean & Shipley, 1988; Shipley & Ennis, 1996). Rather, dopamine is synthesized by subclasses of periglomerular and external tufted neurons within the olfactory bulb (Gall, Hendry, Seroogy, Jones, & Haycock, 1987; Halasz, Johansson, Hokfelt, Ljungdahl, & Goldstein, 1981; Toida, Kosaka, Aika, & Kosaka, 2000). Weak staining for dopamine D₁ receptors has been observed in the internal granular and plexiform layers (Levey et

al., 1993) or more broadly throughout the bulb (Nickell, Norman, Wyatt, & Shipley, 1991), although as dopaminergic fibers are not known to project to this layer, their method of activation is in question. It is possible, but has not been demonstrated, that some of the external tufted cells that project to these layers (Liu & Shipley, 1994; Schoenfeld, Marchand, & Macrides, 1985) are dopaminergic. D₂-type dopamine receptors, in contrast, are strongly concentrated in the bulbar periphery—glomerular, olfactory nerve, and external plexiform layers (Levey et al., 1993)—and have been specifically localized to the presynaptic terminals of olfactory sensory neurons within the olfactory bulb (Nickell et al., 1991).

Dopaminergic interactions with olfactory bulb physiology have been demonstrated in several studies. Unilateral olfactory deprivation greatly reduces the levels of both dopamine (Brunjes, Smith-Crafts, & McCarty, 1985) and tyrosine hydroxylase, the rate-limiting enzyme for dopamine synthesis, in the rat and mouse olfactory bulbs (Baker, 1990; Baker, Morel, Stone, & Maruniak, 1993; Brunjes et al., 1985; Cho, Min, Franzen, & Baker, 1996; Stone, Wessel, Joh, & Baker, 1990; Wilson & Sullivan, 1995). Such deprivation also increases dopamine D₂ receptor density in the rat olfactory bulb (Guthrie, Pullara, Marshall, & Leon, 1991) and alters mitral/tufted cell responsiveness to odors (Guthrie, Wilson, & Leon, 1990; Wilson & Sullivan, 1995); the latter effect is mimicked by the administration of the dopamine D₂ receptor antagonist spiperone to nondeprived rats (Wilson & Sullivan, 1995). Furthermore, measures of olfactory bulb metabolism via 2-deoxyglucose uptake have shown that a pretreatment injection of the dopamine receptor agonist apomorphine, but not the dopamine receptor antagonist haloperidol, prevents the formation of an odor-specific pattern of glomerular activity (Sallaz & Jourdan, 1992).

Behaviorally, dopaminergic modulation appears to play a role in altering odor detection thresholds as well as odor discrimination and learning capabilities. When administered the dopamine D₂ receptor agonist quinpirole, for example, rat odor detection performance decreased; this effect was eliminated when pre- or post-treated with the D₂ antagonist spiperone, further illustrating the

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specificity of the effect (Doty & Risser, 1989). In contrast, the administration of the D_1 -selective partial agonist SKF 38393 enhanced odor detection performance, whereas the D_1 receptor antagonist SCH 23390 eliminated this effect (Doty et al., 1998). Similarly, activation of D_1 receptors or blockade of D_2 receptors each have been shown to improve the ability of adult rats to discriminate structurally and perceptually similar odorant pairs, whereas D_1 blockade or D_2 activation impair odor discrimination (Yue, Cleland, Pavlis, & Linster, 2004). Lastly, dopaminergic modulation has been proposed to influence olfactory learning (Coopersmith, Weihmuller, Kirstein, Marshall, & Leon, 1991; Kendrick, Levy, & Keverne, 1992; Keverne, 1995; Rosenkranz & Grace, 2002; Zhang, Okutani, Yagi, Inoue, & Kaba, 2000).

Previous studies have indicated that D_2 receptor activation reduces olfactory discrimination performance (Yue et al., 2004) in a manner similar to the effects of reduced odorant stimulus concentrations (Cleland & Narla, 2003). As the activation of inhibitory presynaptic D_2 receptors on olfactory sensory neuron (OSN) axonal arbors could be expected to mimic the effects of reduced stimulus concentrations by reducing inputs to bulbar neurons, hence reducing perceived odor intensity as suggested by Doty and Risser (1989), we sought to study the relationship between the effects of D_2 receptor activation and odor stimulus concentration. Specifically, we measured rats' capacities to discriminate between odor pairs at three vapor-phase concentrations (0.001, 0.1, 10 Pa) and tested how these capacities were affected by the intraperitoneal administration of either the selective D_2 agonist quinpirole or the selective D_2 antagonist spiperone. We found that (a) rats' odor discrimination performance improves with increasing odor concentration, (b) the reduction of D_2 receptor activation via administration of spiperone improves odor discrimination performance for low concentration odorants to a level comparable to that of control rats tested at higher odor concentrations, and (c) administration of the D_2 agonist quinpirole yielded a dose-dependent reduction in odor discrimination performance. Together, these results suggest that changes in D_2 receptor activation modulate odor discrimination in the same manner as do changes in odor concentration.

Method

Subjects

Nineteen adult male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA), weighing between 435 and 525 g at the onset of the experiment, were used for this study. All rats were previously trained in odor discrimination and habituation tasks during a study ending 1 month prior to the initiation of the present study. Rats were kept on a 12-hr light–dark cycle in an environmentally controlled room and individually housed in transparent, Plexiglas rat cages ($25.5 \times 48.0 \times 20.0$ cm high). Water was continuously available, but a food deprivation schedule was followed to maintain the rats at 85%–95% ad libitum body weight. Daily experimental sessions were conducted 5 days a week, during the late morning and early afternoon (1130–1430). All procedures were performed under the auspices of a protocol approved by the Cornell University Institutional Animal Care and Use Committee.

Behavioral Paradigm

We tested rats' odor discrimination performance in a simultaneous two-choice olfactory discrimination task. Briefly, rats were conditioned to

discriminate between two simultaneously presented odors, one that was rewarded and one that was not rewarded. We have shown that this paradigm can measure graded similarities between aliphatic odorants according to the principle that more similar odorants are more difficult to discriminate than are less similar compounds (Cleland, Morse, Yue, & Linster, 2002). Unlike discrimination studies that use an automated olfactometer, which can require hundreds of repeated trials to reach criterion (Bodyak & Slotnick, 1999; De Rosa, Hasselmo, & Baxter, 2001; Doty et al., 1998; Lu & Slotnick, 1998), this digging paradigm enables the rapid discrimination learning of similar odorants in as few as 8 and rarely more than 15 to 20 trials and is capable of measuring the rate of learning as well as its asymptotic value (Cleland et al., 2002; Linster & Hasselmo, 1999; Linster, Johnson, Morse, Yue, & Leon, 2002).

All behavioral training and testing occurred in modified rat cages ($25.5 \times 48.0 \times 20.0$ cm high), each divided by a sliding, opaque Plexiglas panel to separate the cage into start and test chambers of equal size (see Figure 1). White ceramic dishes (9.0 cm diameter, 4.5 cm high) were used to deposit the odorants and hide the reward. Before each training set began, six dishes were evenly filled with bedding (50 ml; Bed-O-Cobs, 0.125 in. [0.32 cm], The Andersons, Maumee, OH), and 50 μ L of the appropriate diluted odorant was placed on top of the bedding in the center of the appropriate dish. A piece of sweetened cereal (Kellogg's Froot Loops, 0.75 cm long, previously exposed to heat and air to minimize odor) was then placed on top of the bedding in the center of three of the dishes; the remaining three dishes were nonrewarded. An additional 50 ml of bedding was then added to each dish. All dishes, as well as the experimental cages and panels, were cleaned with ethanol after each training set.

After arrival, rats were first handled for 1 week and then shaped to dig in dishes filled with bedding for a reward. Dishes containing bedding and rewards were placed in the home cage overnight daily during the 1st week after arrival to familiarize the animals with the reward. During the subsequent shaping period, rats were food-deprived and trained to retrieve rewards from dishes of bedding by first providing the reward on top of the bedding and subsequently burying the reward at increasing depths. The bedding was not scented during shaping. When all of the rats had learned to retrieve rewards buried in bedding, the experiment began.

Each training set constituted 15 consecutive discrimination trials with the same pair of odorants (one rewarded, one not rewarded) to prevent the effects of overtraining and to maximize the sensitivity of these behavioral measures (Cleland et al., 2002). Each rat was run on only a single set of 15 trials on any given day; consequently, each rat was only trained on each odor pair for a total of 15 trials over the course of this study. For each rat, one of the two odors in a given odor set was randomly selected to be consistently rewarded; hence, half of the rats were rewarded for each of the odors in a pair, counterbalancing the effects of any innate odor preference biases. Each trial was preceded by placing the rat in the start chamber and the two scented dishes in the test chamber in a random orientation, with the

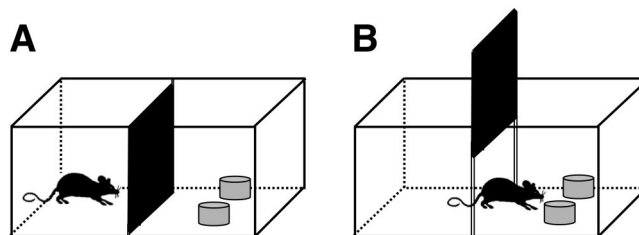


Figure 1. Experimental set-up. All experiments were run in a modified rat cage with a sliding door separating the cage into start and test chambers. (A) Rats remained in the start chamber while the experimenter prepared the odors and reward. (B) At the beginning of each trial, the separation door was removed, and the rat entered the test chamber. Rats were trained to return to the start chamber after they retrieved the reward.

divider panel in place (Figure 1A). Once the divider was lifted, the rat was allowed into the test chamber and the trial began (Figure 1B). For each trial, the dish in which the rat first dug was recorded. Self-correction (after initially digging in the incorrect dish) was permitted, though such trials were scored as incorrect. Rats were permitted to finish eating the reward before being directed toward the start chamber to begin the next trial. The list and sequence of correct and incorrect selections, as well as the total time required to complete the training set, were recorded.

To control for the possibility that rats might identify the rewarded dish on the basis of the spatial location or sounds from manipulating the dishes in the chamber, the experimenters removed both dishes after every trial, added rewards if necessary, and then replaced the dishes in the test chamber at random locations and orientations. After every fifth trial, the two dishes were exchanged for two fresh, identically scented dishes. To control for the possibility of the rat performing the task by learning to directly detect the odor of the reward instead of learning the association between the reward and the test odorant, the rewarded odorant was presented without the reward on every fifth trial (i.e., probe trials 5, 10, and 15). In probe trials, a reward was dropped onto the scented bedding immediately after the rat began to dig in the correct dish to avoid extinguishing the association. Rats that failed to dig during more than one of these probe trials were excluded from data analyses. All training took place in a quiet, dimly lit room to minimize irrelevant stimuli.

Odorants

Most studies to date that have investigated the effects of dopaminergic modulation on olfactory capabilities have used only one or a few odorants and have examined these effects using a single odorant concentration (Doty et al., 1998; Doty & Rissler, 1989; Sallaz & Jourdan, 1992; Wilson & Sullivan, 1995; Yue et al., 2004). In the present study, we used 12 pairs of odorants at three different concentrations. Twenty-four *n*-aliphatic compounds, paired to be highly similar on the basis of shared functional groups and single-carbon differences in aliphatic chain lengths, were used as odorants (see Table 1). These odor pairs were selected on the basis of previous rodent studies demonstrating the perceptual similarity of aliphatic compounds of similar carbon chain lengths (Cleland et al., 2002; Cleland & Narla, 2003; Linster & Hasselmo, 1999; Linster et al., 2002; Wiltrout, Dogra, & Linster, 2003).

Each odor pair was presented at three different vapor-phase concentrations, 0.001, 0.1, and 10 Pa, to enable comparison of the effects of pharmacological intervention and odorant concentration on performance. Vapor-phase partial pressures of odorants (Wiltrout et al., 2003; Yue et al., 2004) were equalized to generate similar intensities for each odorant

presented. Specifically, vapor pressures of pure odorants were estimated with the Hass–Newton equation as implemented in Advanced Chemistry Development/Boiling Point and Vapor Pressure Calculator (Version 4.5; Advanced Chemistry Development, Toronto, Ontario, Canada). Pure odorants were variously diluted in mineral oil according to Raoult's law to achieve theoretically similar vapor phase partial pressures in the headspace (see Table 1 for corresponding volume/volume dilutions). A formula weight estimate of 335 g/mol for mineral oil (Jefo Nutrition, Inc.) was used to calculate mole fractions; because mineral oil is typically heterogeneous, this value should be considered an average. Solvent surface effects and other nonlinearities were neglected. These dilutions should be considered a reduction in the variance of vapor-phase odorant concentrations rather than precise vapor-phase concentration matching as could be achieved by gas chromatographic measurements.

Drug Injections

All drugs were purchased from Sigma-Aldrich (St. Louis, MO). The drugs and dosages used are listed in Table 2. Drug dosages were determined on the basis of the results of previous studies. All drugs were diluted in sterile saline such that the injection of 0.1 ml per 100 g body weight corresponded to the proper dosage. Drugs were administered intraperitoneally 20 min prior to beginning each training set.

Experimental Design

A total of 12 odor sets (see Table 1) were used in this study, each at three concentrations. This large number of odor pairs enabled each rat to be tested under every one of the drug and control conditions (see Table 2) but to encounter each odor set only once. Hence, the rats were always naïve to the test odors at the beginning of each training/testing series. Saline-injected control rats and rats injected with the D₂ antagonist spiperone (0.5 mg/kg) were tested on all three odor concentrations equally, but to maintain their naïvete, were not tested on the same odors at different concentrations. Tests were administered in increasing order of odor concentrations. Because preliminary results showed that administration of the D₂ agonist quinpirole reduced rats' discrimination performance toward chance when tested on lower odor concentrations, we tested the effect of quinpirole only when presenting the highest odor concentration, 10 Pa. Five dosages of quinpirole were used to determine a dose-response curve (0.025, 0.05, 0.1, 0.2, and 0.5 mg/kg; Table 2); each rat was tested at four of these five dosages. The order of odor set presentations and drug dosages was randomized among rats. Odor concentrations were presented in ascending order for any given rat; however, all other experimental conditions were tested simultaneously at each odor concentration.

Data Analysis

The primary data for these experiments, termed *discrimination performance*, comprised the proportion of correct responses (i.e., digging first in the rewarded dish instead of the nonrewarded dish) made by each rat across 15 training trials. Rats that failed to dig during a probe trial (see above) more than once were excluded from analysis. An analysis of variance (ANOVA) conducted with odor concentration and drug treatment group as main effects was performed to determine to effects of concentration and drug treatment on odor discrimination performance. Multiple comparisons tests (Tukey's honestly significant difference [HSD]) were then completed to determine the odor concentrations for which significant differences between the treatment groups existed. Correlations between odor concentration or drug dosage and performance were calculated with Pearson's correlations where appropriate. All statistical tests were two-tailed with a 5% level of significance ($\alpha = .05$). Data analyses were performed with SPSS statistical software.

Table 1
Odor Sets and Volume/Volume Dilutions Yielding Vapor-Phase Partial Pressures of 10 Pa

Odor set	Odorant dilution in mineral oil	
	Odor A	Odor B
1	Octanoic acid pure	Nonanoic acid pure
2	Propyl acetate 31 μ l/50ml	Butyl acetate 110 μ l/50 ml
3	Propanoic acid 165 μ l/50 ml	Acetic acid 40 μ l/50 ml
4	Octanal 740 μ l/50 ml	Heptanal 350 μ l/50 ml
5	Butyl pentanoate 3 ml/50 ml	Butyl hexanoate 8 ml/50 ml
6	Hexanol 4.2 ml/50 ml	Hexanol 1.3 ml/50 ml
7	Ethyl butyrate 90 μ l/50 ml	Propyl butyrate 260 μ l/50 ml
8	Butanoic acid 640 μ l/50 ml	Pentanoic acid 2.3 ml/50 ml
9	Butyl butyrate 830 μ l/50 ml	Butyl propanoate 302 μ l/50 ml
10	Amyl acetate 360 μ l/50 ml	Hexyl acetate 1.1 ml/50 ml
11	Pentyl butyrate 2.8 ml/50 ml	Hexyl butyrate 8.1 ml/50 ml
12	Hexanoic acid 7.4 ml/50 ml	Heptanoic acid 23 ml/50 ml

Table 2
Experimental Groups, Defined by Drug Dosages and Odor Stimulus Concentrations

Experimental group	Dosage (mg/kg)	Concentrations tested (Pa)
Saline		0.001, 0.1, 10
Spiroperone (D ₂ antagonist)	0.5	0.001, 0.1, 10
Quinpirole (D ₂ agonist)	0.025	10
	0.05	10
	0.10	10
	0.20	10
	0.50	10

Results

Effect of Odor Concentration and D₂ Receptor Blockade on Odor Discrimination Performance

We plotted the discrimination performance of control and spiperone-injected rats with respect to odorant concentration and analyzed the results in an ANOVA with odorant concentration and drug treatment group (saline or 0.5 mg/kg spiperone) as main effects. There was a strong effect of odor concentration, $F(2, 108) = 16.01$; $p < .001$, demonstrating that rats performed at significantly different levels depending on the concentration of the presented odor. There was also a strong effect of treatment group, $F(1, 108) = 13.99$; $p < .001$, indicating that saline injected and spiperone injected rats performed differently overall. There was no interaction between odor-concentration and drug effects, $F(2, 108) = 0.84$, $p > .1$, implying that the potency of the drug treatment did not depend significantly on stimulus concentration. Among control (saline-injected) rats, further tests demonstrated significant differences in discrimination performance as a function of odor concentration, $F(2, 78) = 20.40$, $p < .001$. Additionally, the number of correct discrimination trials was significantly correlated with odor concentration (Pearson's $R = .53$, $p < .01$; Figure 2, *saline*).

Significant differences in discrimination performance with respect to presented odor concentrations were also observed in spiperone-treated rats, $F(2, 30) = 5.11$; $p < .001$, and the number of correct trials was also correlated with odor concentration (Pearson's $R = 0.468$; $p < .01$; Figure 2, *spiperone*). Additionally, post hoc tests demonstrated significant differences in discrimination performance between control and spiperone-injected rats at the two lower odor concentrations (0.001 Pa: $F(1, 36) = 8.2$; $p < .001$; 0.1 Pa: $F(1, 38) = 5.23$; $p < .05$), although this difference was not significant at the highest odor concentration (10 Pa: $F(1, 34) = 1.59$; $p > .1$). These data demonstrate that blockade of D₂-type dopamine receptors improves rats' discrimination performance between odors at low and moderate concentrations (Figure 2, *asterisks* denote significant differences).

Effect of D₂ Receptor Activation on Odor Discrimination Performance

Rats were injected with the D₂ receptor agonist quinpirole at one of five dosages (0.025, 0.05, 0.1, 0.2 and 0.5 mg/kg) and tested, along with a saline-injected control group, using only the highest

concentration of odors (10 Pa). ANOVA revealed a significant effect of drug treatment group ($F(5, 84) = 3.58$; $p < .01$), indicating that rats performed differently depending on the dosage of quinpirole. Further post hoc tests demonstrated significant differences in performance between control rats and those injected with the two highest doses of quinpirole (0.2 and 0.5 mg/kg; $p < .05$). There was also a significant negative correlation between discrimination performance and quinpirole concentration in this task (Pearson's $R = -0.26$; $p < .05$), indicating that quinpirole injections impaired the rats on the odor discrimination task in a dose-dependent manner (Figure 3).

Comparison of Odor Concentration and D₂ Receptor Modulation Effects

To compare the effects of changes in odor concentration with the blockade of D₂ receptors, we statistically compared the performance of rats injected with the D₂ antagonist spiperone with that of control rats tested at higher odor concentrations. Rats injected with the D₂ antagonist spiperone (0.5 mg/kg) discriminated odorants at 0.001 Pa as well as control rats discriminated odorants at 0.1 Pa ($p > .20$; Figure 4A). When tested at 0.1 Pa, rats injected with spiperone performed similarly to control rats tested with odorants at 10 Pa ($p > .30$, Figure 4A). These results show that the blockade of D₂ dopamine receptors improves rats' odor discrimination performance by a factor comparable to raising the odor concentration by two log units.

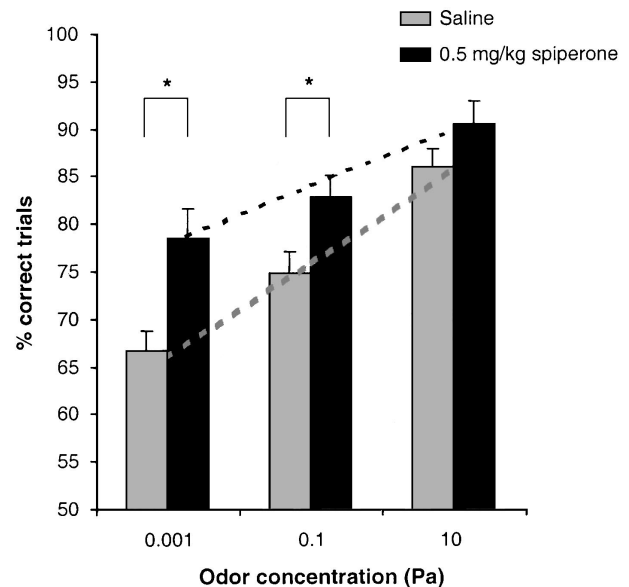


Figure 2. Effect of odor concentration and D₂ receptor blockade on odor discrimination performance. The percentage of correct trials is plotted as a function of odor concentration (0.001, 0.1, and 10 Pa) for saline-injected control rats (Saline) and rats injected with the D₂ receptor antagonist spiperone (0.5 mg/kg spiperone). Error bars depict standard errors. For both groups of rats, discrimination performance increased as a function of odor concentration. Spiperone-treated rats performed significantly better than control rats when tested at the two lower odor concentrations (0.001 and 0.1 Pa). Asterisks indicate significant differences in performance between the spiperone and control groups ($p < .05$).

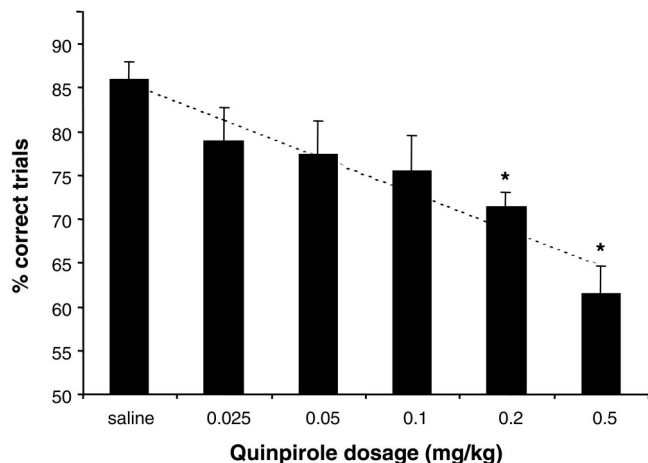


Figure 3. Effect of D_2 receptor activation on odor discrimination performance. Rats treated with various dosages of the D_2 receptor agonist quinpirole were tested on odor discrimination with only the highest concentration of odor stimuli (10 Pa). The percentage of correct trials is plotted as a function of quinpirole dosage. Error bars depict standard errors. Odor discrimination performance systematically declined as a function of quinpirole dosage. There was a significant difference in discrimination performance between rats injected with the two highest dosages of quinpirole and saline-injected control rats (asterisks).

To assess the effects of odor concentration in comparison with the effects of D_2 receptor activation, we compared the discrimination performances of rats injected with one of three dosages of quinpirole (0.025, 0.1 and 0.5 mg/kg), and tested at the highest odor concentration (10 Pa), with those of control rats tested at all three odorant concentrations. Separate *t* tests revealed that there was no significant difference between control rats and rats injected with 0.025 mg/kg quinpirole when both were tested with odorants presented at 10 Pa ($p > .10$; Figure 4B, left). Rats injected with 0.1 mg/kg quinpirole and tested with 10 Pa odorants performed similarly to saline-injected rats presented with 0.1 Pa odorants ($p > .10$, Figure 4B, center), and rats injected with 0.5 mg/kg quinpirole and tested with 10 Pa odorants performed similarly to saline-injected rats presented with odorants at 0.001 Pa ($p > .20$; Figure 4B, right). These results indicate that the D_2 dopaminergic agonist quinpirole affects odor discrimination in a manner similar to the effects of reductions in odor concentration. Specifically, injection of a medium dosage of quinpirole (0.1 mg/kg) reduces discrimination performance to an extent comparable to that induced by decreasing odorant concentration by two orders of magnitude: from 10 to 0.1 Pa. Similarly, injection of a higher dose (0.5 mg/kg) reduces performance comparably to an odor concentration reduced by four orders of magnitude (from 10 to 0.001 Pa).

In summary, blockade of D_2 receptors improved odor discrimination similarly to increasing odor concentration, whereas activation of D_2 receptors decreased odor discrimination similarly to lowering odor concentration. These results suggest that D_2 receptor modulation changes odor perception in ways similar to changes in odor concentration.

Discussion

The results of the experiments presented here show that systemic injections of the dopamine D_2 receptor antagonist spiperone

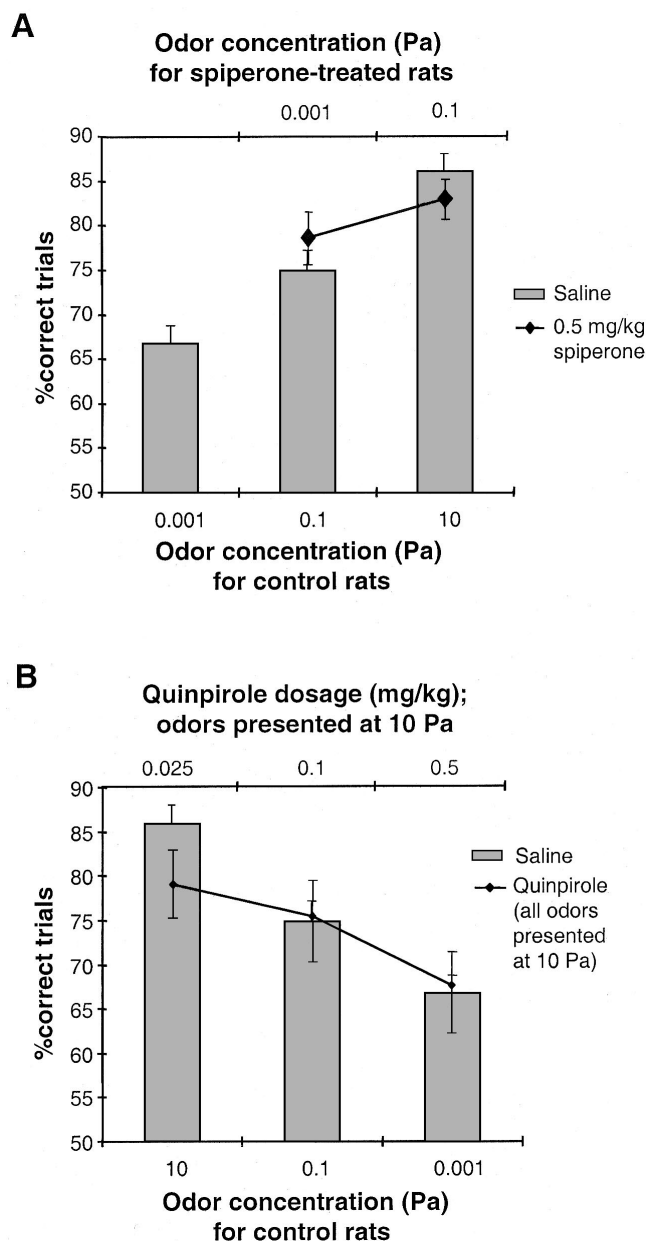


Figure 4. Comparison between the effects of odor concentration and D_2 receptor modulation on odor discrimination performance. (A) Comparison between rats injected with the D_2 antagonist spiperone and control rats, each presented with different odor concentrations. The performance of spiperone-injected rats tested at 0.001 and 0.1 Pa was similar to that of saline control rats tested at 0.1 and 10 Pa. (B) Comparison between the performances of saline-injected control rats (presented with various concentrations of odorants) and rats injected with one of three dosages of quinpirole (presented with odors at 10 Pa only). Rats treated with the lowest dose of quinpirole performed similarly to control rats also tested at 10 Pa. In contrast, rats injected with 0.2 mg/kg quinpirole and tested at 10 Pa performed similarly to control rats tested with the next lower odor concentration, 0.1 Pa. Rats injected with the highest dosage of quinpirole, 0.5 mg/kg, and tested at 10 Pa, performed similarly to control rats tested at the lowest odor concentration, 0.001 Pa. Error bars depict standard errors.

and agonist quinpirole produce opposite effects on rats performing a difficult simultaneous odor discrimination task. Whereas spiperone administration facilitated discrimination performance, quinpirole impaired discrimination performance. The performance improvements observed after injection of the D_2 antagonist were comparable to those obtained by increasing odor stimulus concentration by two log units, whereas the performance impairment observed following D_2 agonist administration was found to depend on the dosage of quinpirole injected: Higher dosages were associated with lower performance levels.

These results, based on systemic injections of dopaminergic D_2 agonists and antagonists, do not identify the pathways responsible for the resulting modulation of odor discrimination performance. Whereas dopaminergic modulation within the olfactory bulb is entirely endogenous, centrifugal dopaminergic fibers do project to the piriform cortex and other secondary olfactory structures as well as to other neural structures involved in the integration of sensory inputs and the generation of behavior. The role of the dopaminergic system in mediating reward-guided behaviors has been studied extensively, and all five known dopaminergic receptor subtypes have been implicated (e.g., see Berridge & Robinson, 1998; Dayan & Balleine, 2002; Eyny & Horvitz, 2003; Ponnusamy et al., 2005; Robbins & Everitt, 1996; Schultz, 2002). Hence, the dopaminergic modulation of attention, synaptic potentiation, and reward associations (reviewed in Berridge & Robinson, 1998; Dayan & Balleine, 2002; Robbins & Everitt, 1996; Schultz, 2002) could influence the olfactory behaviors described herein. However, we focus here on the potential effects of dopaminergic modulation within the olfactory system proper, and particularly the olfactory bulb, in the context of existing studies of dopaminergic mechanisms specifically affecting olfactory perception and learning.

Previous studies have found that olfactory capabilities, particularly difficult odor discriminations, are affected by D_2 receptor activity (Yue et al., 2004) as well as by odor stimulus concentration (Cleland & Narla, 2003). We present in the current study a novel finding suggesting that modulation of D_2 receptor activity may contribute to the processing of olfactory input by adjusting perceived odorant intensities. Consistent with this hypothesis, varying the dosage of a D_2 agonist produced effects on odor discrimination performance comparable to those produced by varying odor stimulus concentration; specifically, increasing concentrations of quinpirole had the same effects on performance as reducing odorant intensities. Correspondingly, blockade of D_2 receptor activation significantly improved rats' discrimination capabilities at low and moderate odor concentrations. Rats injected with spiperone discriminated low-concentration odorants as well as control rats discriminated higher-concentration odorants.

Multiple studies have reported that D_2 receptor activation presynaptically inhibits olfactory nerve terminals (Berkowicz & Trombley, 2000; Davila, Blakemore, & Trombley, 2003; Ennis et al., 2001; Hsia, Vincent, & Lledo, 1999), thus depressing or even blocking synaptic transmission between olfactory receptor neurons and their synaptic targets, including mitral cells and some subclasses of tufted and periglomerular cells (Berkowicz & Trombley, 2000; Davila et al., 2003; Ennis et al., 2001; Hsia et al., 1999; Sallaz & Jourdan, 1992). This weakened synaptic transmission, mimicking the response to lower concentration stimuli, might account for the reduced olfactory discrimination performance observed on injection of the D_2 agonist quinpirole. Likewise, en-

hanced transmission at OSN output synapses via blockade of baseline D_2 receptor activation by spontaneous dopaminergic periglomerular (PG) cell activity (Puopolo, Bean, & Raviola, 2005) may yield synaptic input to mitral cells resembling that generated by higher stimulus concentrations, which would explain the heightened discrimination capabilities observed in rats injected with the dopamine D_2 receptor antagonist spiperone. Indeed, in vivo administration of the D_2 antagonist spiperone increased the responsiveness of rat olfactory bulb mitral cells to odors (Wilson & Sullivan, 1995). Higher odor concentrations have been repeatedly associated with greater olfactory discrimination performance in mice (Cleland & Narla, 2003) and honeybees (Bhagavan & Smith, 1997; Cleland & Linster, 2002; Pelz, Gerber, & Menzel, 1997), and now in rats. Improved discrimination performance may be a simple consequence of higher intensity OSN output to mitral cells, whether resulting from increased odor stimulus concentrations or from reduced D_2 inhibition of this OSN output.

These results, interpreted in light of the underlying olfactory bulb circuitry, suggest that presynaptic D_2 inhibition serves as a homeostatic regulator of olfactory stimulus intensity. Such regulation can maintain optimal responsivity to odor inputs by rendering the olfactory system sensitive to changes in odor intensities, rather than to their absolute levels. Specifically, the direct innervation of dopaminergic PG cells by OSNs and the inhibition of OSN terminals by PG cells form a negative feedback loop that normalizes the degree of excitation provided to mitral/tufted cells. Dopaminergic inhibition of OSN output within a given glomerulus will depend on the activation level of that convergent OSN population such that increased OSN activity presynaptically inhibits its own output. In the present experiments, this presynaptic inhibition was enhanced with a D_2 agonist, hence reducing the odor intensity perceived by the rats. Furthermore, on a longer time scale (hours to days), OSN activity also appears to regulate the dopamine production capacity of PG neurons: Olfactory deprivation systematically reduces bulbar tyrosine hydroxylase (TH) expression (Baker, 1990; Baker et al., 1993; Brunjes et al., 1985; Cho et al., 1996; Stone et al., 1990; Wilson & Sullivan, 1995) as well as bulbar dopamine levels (Brunjes et al., 1985). These altered TH expression levels may reflect the strength of the dopaminergic response to a given intensity of OSN input. That is, deprivation-induced reductions in TH expression may indicate a reduction of OSN terminal inhibition so as to potentiate responses to lower-intensity odorants. This effect was replicated in the present study with D_2 antagonists, reducing the efficacy of OSN terminal inhibition such that rats perceived weak odor stimuli as strong. The net effect of these regulatory mechanisms is to adjust the strength of presynaptic feedback inhibition to correspond to the levels and qualities of background odorants, maximizing the animal's ability to detect both quantitative and qualitative changes in the chemosensory environment.

In summary, bulbar D_2 -type dopamine receptors are important regulators of olfactory processing and perception. Modulation of D_2 receptor activation can enhance or impair the discrimination of odors, presumably by altering the perceived intensity of a given odorant through changes in the effective sensitivity of bulbar neurons to OSN activity. Dopamine release appears to be constitutive and homeostatically regulated and may serve as a form of gain control for the olfactory system, adjusting its sensitivity appropriately in response to the intensity of the milieu of back-

ground odors. Perceptually, D₂ receptor-mediated changes in gain would be similar to genuine changes in odor concentration, with corresponding effects on detection thresholds, discrimination performance, and olfactory associative learning.

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