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No Influence of Scopolamine Hydrobromide on Odor Detection Performance of Rats

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Abstract

Despite speculation that the muscarinic cholinergic antagonist, scopolamine, may influence the olfactory sensitivity of rats, there have been no definitive studies on this point to date. In this study, we examined the influence of a range of doses of scopolamine hydrobromine (namely, 0.10, 0.125, 0.15 and 0.20 mg/kg i.p.) on the odor detection performance of 15 adult male Long–Evans rats to ethyl acetate. Air-dilution olfactometry and a go/no-go operant signal detection task were employed. The drug conditions and a saline control were administered to each animal in an order counterbalanced by Latin squares, with 2 day intervals interspersed between tests. Scopolamine had no significant influence on odor detection performance *per se*, as measured by percent correct S+ and S– responses and a non-parametric signal detection measure of sensitivity. This is in contrast to the relatively large effects previously observed in the same test paradigm for such drugs as the D-1 agonist SKF 38393 and the D-2 agonist quinpirole. These data suggest that scopolamine has no meaningful influence on a well-practiced odor detection task.

Key words: odor detection, olfaction, psychophysics, rat, scopolamine hydrobromide, sensory threshold, signal detection theory

Introduction

Whether olfactory sensitivity is influenced by drugs which affect cholinergic systems is not clear. Skouby and Zilstorff-Pedersen (1954) reported that, in humans, unilateral intranasal installation of small amounts of acetylcholine hydrochloride (0.1–10 µg/ml in saline) and acetyl-beta-methyl-choline hydrochloride (10 ug/ml in saline) increased olfactory sensitivity on the side of the nose where they were applied, a phenomenon that has also been reported for very low doses of cocaine and strychnine (Zwaardemaker, 1895). However, direct application of a drug to a region of the olfactory epithelium need not be representative of the drug's influence on olfactory processing when administered systemically. More recently, Serby *et al.* (1989) reported that sc administration of the muscarinic antagonist scopolamine hydrobromide at 0.22, 0.43 or 0.65 mg/70 kg doses in humans decreased odor detection sensitivity to the odorant geraniol. The d' measure of overall sensitivity was 2.60 ± 0.20 (mean ± SEM) for placebo-treated humans and 1.62 ± 0.21 for humans treated with scopolamine; no dose effects were observed. However, scopolamine had no significant influence on the ability to identify odors, as measured by the University of Pennsylvania Smell Identification Test (UPSIT).

Although alterations in odor-mediated learning and memory tasks following the administration of cholinergic drugs have been reported in animals (Mollenauer *et al.*, 1976; Cheal, 1981; Soffie and Lamberty, 1988; Hunter and Murray, 1989; Ravel *et al.*, 1992, 1994), odor detection *per se* has not been measured. Conceivably a number of such effects could reflect decreased smell function, rather than alterations in memory encoding. Limited support for this notion came from early observations that scopolamine reduced the fear response of rats to cats (Plotnik *et al.*, 1974) and that olfactory bulbectomy had a similar effect (Mollenauer *et al.*, 1974). The possibility that olfactory sensitivity was altered by scopolamine was tangentially supported by evidence that ether-soaked cotton balls failed to induce a typical head-turning response in scopolamine-treated rats (Mollenauer *et al.*, 1976).

In light of these findings, Mollenauer *et al.* (1976) trained 13 rats to perform a two-choice odor discrimination task in which responding to the odor of wet Purina lab chow (versus no odor) was reinforced using sucrose pellets; subsequently, six of the rats were administered 0.8 mg/kg scopolamine hydrobromide and their performance compared with the remaining rats, who received saline alone. Although the

scopolamine-treated rats evidenced decreased performance on the first 10 of 20 test trials, their performance on the last 10 trials was normal. An analysis of the first 10 trials revealed that the rats typically explored both odor stations repeatedly without remaining long enough near either to score a response, precluding the expression of choice errors and suggesting the presence of an attentional deficit. In a second study, these authors trained rats in an analogous test, except that the apparatus only allowed access to the stimulus and reinforcement area through 3.8 cm holes located over the odor stations. Despite the fact that the scopolamine-treated rats approached the holes repeatedly, they were reluctant to poke their heads through them, resulting in poor performance on the task. The major conclusion of these investigators, i.e. that scopolamine-treated rats are not anosmic, is in accord with observations of others (e.g. Cheal, 1981; Soffie and Lamberty, 1988; Hunter and Murray, 1989), although none of these studies addressed the issue of whether scopolamine causes at least some alteration in olfactory sensitivity. The relatively recent observation that physostigmine, a muscarinic agonist, enhances odor discrimination performance on difficult discrimination tasks in a dose-related manner begs the question as to whether cholinergic processes are involved in the modulation of basal olfactory sensitivity (Doty *et al.*, 1999).

The present study sought to determine whether IP injections of scopolamine hydrobromide influence odor detection performance in rats, as measured by an operant go/no-go conditioning paradigm known to be sensitive to a number of drug effects (e.g. Doty and Ferguson-Segall, 1987; Doty and Risser, 1989; Doty *et al.*, 1998). To achieve this end, the influences of saline and four doses of that drug known to produce minimal response stereotypy were evaluated (0.10, 0.125, 0.15 and 0.20 mg/kg).

Methods

Subjects

Fifteen adult Long–Evans male rats (Charles River, Wilmington, MA) were selected from a larger number on the basis of stable performance on an operant odor detection task described in the next section. The subjects were trained specifically for this study and had not been used in other work. They were maintained on a 12:12 light:dark cycle and housed in pairs in $24 \times 21.5 \times 45.1$ cm polystyrene laboratory cages with Purina lab chow and water available *ad libitum*. Two weeks prior to operant training, they were placed on a 23.5 h water deprivation schedule and maintained on this schedule throughout the experimental period. In addition to the water received during the test session, water was available *ad libitum* to each rat for 30 min following each test session. Testing was performed within the light phase of the light:dark cycle.

Odor detection test equipment and procedures

The odorant concentrations were generated using an air-dilution olfactometer (Slotnick and Nigrosh, 1974; Doty and Ferguson-Segall, 1987). Ethyl acetate, which has a fruity smell to humans, was used as the test stimulus since it: (i) has a relatively high vapor pressure (73.84 mm Hg at 20°C), thereby ensuring appropriate odor saturation within the over-the-surface saturator; (ii) has little tendency, relative to many other odorants, to adhere to and collect on olfactometer surfaces, thereby minimizing the potential for contamination; and (iii) has been used in a number of previous rat odor detection studies (Doty and Ferguson-Segall, 1987; Doty *et al.*, 1988).

Each test chamber was made of a 9.5-cm-diameter, 22-cm-long Plexiglas tube. The airflow output from the olfactometer passed through a vertical wind tunnel located at one end of the chamber. The volume flow through the tunnel was 2.7 l/min during the intertrial interval and 3 l/min during an odor trial. The diameter of the wind tunnel was 10 cm, and its cross-sectional area was 78.5 cm^2. The temperature and relative humidity of the air was held constant. A 3-cm-diameter sampling port in the side of the wind tunnel allowed the rat to smell the incoming airstream. The test chamber and wind tunnel were housed in a sound-attenuated enclosure maintained at 20 ± 1°C. A photocell and light were positioned at the sampling port to detect the nose of the animal and to initiate the trial sequence. A 8-mm-diameter, 6-mm-deep stainless steel cup projecting through the floor of the chamber served as the response cup. The subject, by licking this cup while standing on a stainless steel floor plate, completed a high resistance circuit, thereby signaling his or her response. In addition to mediating the operant response, this cup served as a drinking port from which the rat received water reinforcement from a solenoid-controlled water reservoir. Air from the test chamber was continuously exhausted to the outside of the building via a muffin fan.

The training procedures are discussed in detail elsewhere (Doty and Ferguson-Segall, 1987). A trial sequence was initiated when a rat positioned its snout in the sampling port, triggering a photobeam signal to divert (to exhaust) the airstream for 1 s and simultaneously releasing an odor or the blank stimulus into the terminal mixing manifold. The airstream diversion at the beginning of the trial served as a warning signal for stimulus presentation and, more importantly, provided an interval for the odorant and carrier streams to mix. Any lick response by the rat during this period aborted the trial. After this diversion, the odor (S+) or blank (S–) airstream was delivered into the wind tunnel for 5 s. A lick response made during the initial 2 s of the 5 s S+ stimulus period was not reinforced, although it was recorded to establish the S+ response latency measure. A lick during the remaining 3 s of this period resulted in the immediate termination of the trial and delivery of a 0.02 ml water reward. A lick response made during the initial 2 s of the 5 s S– stimulus period was ignored and did not terminate

the trial, whereas one in the remaining 3 s did so. This reinforcement contingency was designed to delay the response long enough to ensure an adequate period for sampling the stimulus. If no responses were made during the remaining 3 s of stimulus delivery, the trial was automatically terminated and a 4 s intertrial pause occurred before another photobeam break could initiate a trial.

A daily test session consisted of a total of 160 trials per subject, the first 10 of which consisted of five S+ ($10^{-3.5}$ log concentration relative to saturation) and five S– warm-up trials not included in the performance calculations. Following these warm-up trials, blocks of five S+ and five S– trials were presented in a descending series of concentrations (i.e. $10^{-3.5}$, $10^{-5.0}$, $10^{-6.5}$, $10^{-7.0}$ and $10^{-7.5}$ relative to saturation). The order of the five S+ and five S– trials at a given concentration was random, with the restriction that no more than three of a kind occurred in succession. Two analogous series of 50 trials followed this first series, resulting in a total of 30 trials at each of the five-odorant concentrations. Both the stimulus delivery contingencies and the subject responses were controlled and monitored by computer. Response data were compiled on-line and automatically printed to hard copy after each test session.

Dependent measures

Two measures of odor detection performance were used. The first was the traditional percent correct trials measure (i.e. the percent of the S+ and S– trials on which a correct response occurred). The second was the nonparametric sensitivity index (SI) of Frey and Colliver (1973), which is calculated from the proportion of hits (licks under the S+ condition) and false alarms (licks under the S– condition). The SI measure, whose formula is

$$\mathrm{SI} = \frac{P(\mathrm{Hit}) - P(\mathrm{False\ Alarm})}{2[P(\mathrm{Hit}) + P(\mathrm{False\ Alarm})] - [P(\mathrm{Hit}) + P(\mathrm{False\ Alarm})]^2}$$

ranges in value from 0 (no detection) to 1.00 (perfect detection). In addition to not requiring the parametric assumptions of homogeneity of variance and distribution normality of the parametric signal detection sensitivity index d', SI can be calculated when the hit rate is 1.00 and the false alarm rate zero.

Two measures of the rats' general behavior were also assessed. The first, the responsivity index (RI) of Frey and Colliver (1973), reflects the general tendency of the rat to provide responses. The RI index is calculated from the formula

$$\mathrm{RI} = \frac{P(\mathrm{Hit}) + P(\mathrm{False\ Alarm}) - 1}{1 - [P(\mathrm{Hit}) - P(\mathrm{False\ Alarm})]^2}$$

and can range in value from –1.00 (very conservative response criterion) to +1.00 (very liberal response criterion). RI, like SI, can be calculated when the hit rate is 1.00 and the false alarm rate zero. The second measure is the duration of the overall test session in minutes. The latter measure provides an index of whether the drug influences the speed of overall performance of the rat in the test situation and alerts the investigator to competing responses (e.g. grooming).

Experimental and statistical design

Each rat received five drug treatment conditions [0 (saline), 0.10, 0.125, 0.15 and 0.20 mg/kg], each on a separate day, in an order counterbalanced using three 5×5 Latin squares. The test days were separated from one another by two intervening days, on which saline was administered, but no sensory testing occurred. On the test days, the drug or saline was administered IP 15 min before the rat was placed in the test chamber. The data for each dependent measure were assessed using a drug dose by odorant concentration analysis of variance (ANOVA).

Results

Percent correct

No significant influence of drug dose or its interaction with odorant concentration was observed for the percent correct measure [$F(4,56) = 0.267$, $P = 0.898$ and $F(16,224) = 1.087$, $P = 0.369$, respectively]. However, significant odorant concentration effects were present [$F(4,56) = 102.40$, $P < 0.0001$], reflecting the fact that detection performance generally increased as odorant concentration increased (Figure 1).

Sentivity index

As in the case of percent correct, no significant influence of drug dose or its interaction with odorant concentration was observed for the SI detection measure [$F(4,56) = 0.232$, $P = 0.919$ and $F(16,225) = 1.194$, $P = 0.274$, respectively]. However, as with the percent correct detection measure, a significant odorant concentration main effect was present [$F(4,56) = 101.3$, $P < 0.0001$], reflecting the aforementioned relationship with odorant concentration (Figure 1).

Responsivity index

RI was not significantly influenced by either drug dose or its interaction with odorant concentration; a significant odorant concentration effect reflected a general decrement as a function of increased odorant concentration [$F(4,56) = 54.12$, $P < 0.0001$] (Figure 2).

Session duration

Although the session duration was not significantly related to drug dose [$F(4,56) = 1.08$, $P = 0.37$], the duration data for the 0.20 mg/kg condition was 7–10 min longer than those of the other drug conditions, which averaged 58.83 min.

Discussion

The present data suggest that systemic administration of scopolamine hydrobromide, a central antagonist of the

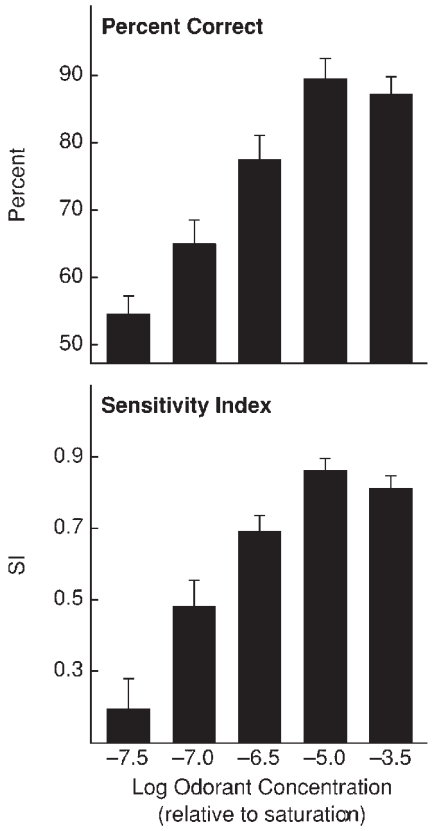

Figure 1 **(A)** Percent of trials correct under all drug treatment conditions as a function of odorant concentration. **(B)** SI values under all drug treatment conditions as a function of odorant concentration.

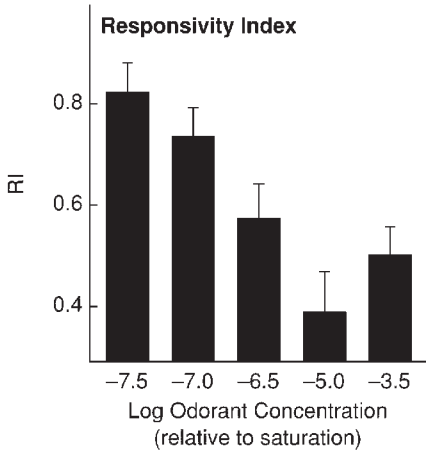

Figure 2 **(A)** General tendency of the rat to provide responses (RI) under all drug treatment conditions as a function of odorant concentration.

muscarinic acetylcholine receptor, has no meaningful influence on the odor detection performance of rats. These findings are in general accord with the observations of Ravel *et al.* (1992), who noted no effect of scopolamine at the 0-duration retention interval in a delayed odor matching task, and contrasts with notions that this drug conceivably alters such sensitivity (Mollenauer *et al.*, 1974, 1976). These data imply that the well-established adverse effects of scopolamine on odor memory and related tasks are unlikely the result of alterations in olfactory sensitivity *per se* (e.g. De Rosa and Hasselmo, 2000; Fletcher and Wilson, 2002).

That being said, it should be noted that the drug dose range used in this study was comparatively low and was designed to minimize response stereotypy. Thus, one cannot totally exclude the possibility that alterations in detection performance may occur at higher drug concentrations, although behavioral assessment at such concentrations is problematic. Nevertheless, the drug doses employed in this study are known to influence odor memory and learning, mitigating to some degree such an argument. For example, Ravel *et al.* (1992) reported that 0.125 and 0.0625 mg/kg doses of scopolamine impaired short-term odor memory performance of rats without meaningfully impairing behavioral task performance. A dose of 0.50 mg/kg did, however, impair such non-olfaction-related performance. Recently, Fletcher and Wilson (2002) reported that 0.2 mg/kg of scopolamine, a dose used in this study, injected before a conditioned associative learning task blocked the acquisition of a learned enhancement in olfactory acuity, although olfactory function was intact. This observation, as well as evidence that cholinergic pathways are largely involved in central modulation of odor memory and discrimination (Kendrick *et al.*, 1997), suggests that muscarinic receptor –mediated processes may not be a major contributor to the modulation of circuits involved in the mediation of odor sensitivity, per se.

The present data are seemingly in conflict with the those of Serby *et al.* (1989), who reported that scopolamine at very low doses (e.g. 0.009 mg/kg compared with 0.10–0.20 mg/kg doses in the present study) decreased perithreshold sensitivity to geraniol in humans. It is not immediately apparent why this difference is present. It seems counterintuitive that doses lower than those used in our study would be more disruptive of cholinergic pathways responsible for mediating olfactory sensitivity. The lack of an effect on UPSIT scores noted by Serby *et al.* would be in concurrence with the present work, in that typically UPSIT scores and threshold measures are correlated. Additional research is needed to determine whether odorant type, drug dosage, or species effects are responsible for these disparities.

In the present paradigm, RI was inversely related to SI, a phenomenon that we and others have previously observed (e.g. Doty and Ferguson-Segall, 1987). This seems to reflect the point made by Frey and Colliver (1973) that an increase in cue similarity (i.e. an increase in difficulty of the discrimination task) results in poorer differential responding and an increase in general responsiveness, i.e. an increase in RI. According to these authors, RI generally differs from the traditional signal detection index of β in reflecting a general responsivity, rather than a perceptual responsivity. The latter reflects the amount of 'signalness' a subject requires

before responding, whereas the former reflects the actual number of responses made by the subject. Hence, there is precedence for such an inverse association although, theoretically, RI can be independent of SI, as indicated by data on eyelid conditioning in rabbits provided by Frey and Colliver (1973). While, in the present study, scopolamine had no noticeable influence on RI, it did tend to increase the time required by an animal to complete the testing, demonstrating at least some non-sensory drug-related influence on the behavior of the rats.

By employing a well-validated computerized olfactometer and a go/no-go odor detection paradigm, the present study puts to rest speculations that a number of the effects of scopolamine on behavioral tasks in rats reflect the blocking of general olfactory sensory input during periods of stimulus exposure, at least at the drug doses employed. Whether this drug alters detection performance to other odorants or at higher dose levels is not known, but would seem unlikely.

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