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Fluprazine Hydrochloride: No Influence on the Odor Detection Performance of Male Rats

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DOTY, R. L., C. LI AND J. M. RISSER. *Fluprazine hydrochloride: No influence on the odor detection performance of male rats.* PHARMACOL BIOCHEM BEHAV **35**(3) 699–703, 1990.—Fluprazine hydrochloride (DU 27716) decreases copulatory and offensive attack behaviors of male rats and increases their latency to locate buried food in an open field. Since such behaviors are mediated to some degree by the olfactory system, several investigators have hypothesized that this drug may produce an overall impairment in olfactory sensitivity. To test this hypothesis, the influences of five doses of fluprazine hydrochloride (1.0, 2.5, 5.0, 7.5 and 10.0 mg/kg IP) on the odor detection performance of 12 adult male Long Evans rats was assessed, relative to saline, using high precision olfactometry and a go/no-go operant odor detection task. Treatments were administered every 3rd day in counterbalanced order, with the drug or saline injections occurring 30 minutes before the 260-trial test sessions. No significant influence of fluprazine was observed on odor detection performance, as measured by the nonparametric signal detection sensitivity index SI and the percentage of correct trials. These results indicate that fluprazine does not induce generalized olfactory impairment.

Aggression Fluprazine hydrochloride Odor detection Olfaction Olfactory threshold Rat Signal detection

THE development of anxiolytic drugs that act centrally without blocking sensory input is a fundamental goal of psychopharmacology. Fluprazine hydrochloride (DU 27716), an agent that has been termed an antiaggressive compound (1), appears to have anxiolytic properties at nonsedative doses. Interestingly, low doses of this drug inhibit offensive, but not defensive, attack behaviors of male rats directed towards conspecifics (1, 11, 16) and decreases copulatory responses directed towards females (9).

Although the physiologic basis of this drug's influence on such behaviors is unknown, Thornton and Kemble (15) have proposed that general olfactory impairment may be involved. This hypothesis was based largely on the fact that the aforementioned behaviors are influenced, at least to some degree, by olfactory input, as well as on the observation that fluprazine-treated rats, relative to controls, sniff and nose conspecifics more frequently (12–14) and take longer to find cookies buried in an open field (15). This drug does not produce total anosmia, however, since treated male rats evidence preferences for odors from estrous females, males, and food relative to a blank control (11).

To date, quantitative assessment of the influences of fluprazine on the olfactory sensitivity of the rat has not been made. If general olfactory impairment is present, as suggested by Thornton and Kemble (15), then one would expect a lessening of detection performance to a test odorant. Hence, in the present study we used high precision olfactometry and a go/no-go operant signal detection task to assess the influence of fluprazine on the odor detection performance of adult male rats to ethyl acetate. As will be noted, the results do not support the hypothesis that fluprazine induces general impairment in olfactory sensitivity.

METHOD

Subjects

Twelve adult male Long-Evans rats, 5 months of age at the time of testing, served as subjects. The average weight of the rats over the period of testing was 375 grams (SD = 5 grams). The rats were individually housed in 24 w × 21.5 h × 45 l centimeter polystyrene laboratory cages in which Purina lab chow was available ad lib. A 12:12 hour light:dark schedule was maintained in the colony room.

The rats were placed on a 23.5-hour deprivation schedule two weeks before the beginning of operant training and maintained on

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this schedule throughout the experimental period. Immediately following testing, they were given access to water for 10 minutes. The remainder of their water regimen was obtained during the experimental session.

Stimulus Control and Delivery

The five odorant concentrations were generated using an air-dilution olfactometer and delivered to an animal operant testing chamber described in detail elsewhere (4,5). Ethyl acetate (EA) was chosen as the stimulus since it is sensitive to olfactory alterations induced by other drugs and fulfills a number of criteria important in olfactometry (4,7). At the air intake end of the system, filtered room air was drawn through an oiless compressor at 20 psi and passed through two polycarbonite filters into a refrigerant dryer. The dehumidified and filtered airstream was then split. Each segment of the split airstream was passed through a set of seven interconnected 19-cm-long × 2.5-cm-diameter glass tubes. These tubes were immersed in a water bath maintained at 24 ± 1°C. One set of these tubes was filled with 250 ml of ethyl acetate and served as an over-the-surface saturator (8). The other set contained no odorant and served as a clean air line. The saturated and clean air lines were then channeled through five stages of an eight-stage olfactometer which consisted of a series of Porter flowmeters, mixing chambers, and Teflon™ needle valves. The air from a given stage was delivered to a final mixing manifold via a computer-activated three-way Teflon™ solenoid valve immediately before and during a test trial. A continuous stream of nonodorized air always ran through the final mixing manifold. This manifold was directly connected to the common port of a final three-way Teflon™ solenoid valve with a delivery line leading from its normally closed port into the test chamber. As explained later in the paper, on a given trial either a blank (nonodorized air) or one of the following odorant concentrations (relative to saturation) was presented to a subject: $10^{-5.5}$, $10^{-5.0}$, $10^{-4.5}$, $10^{-4.0}$, and $10^{-3.5}$.

Test Chamber

The animal test chamber consisted of a 10.2-cm-diameter glass funnel fused to a 19-cm-long tube of the same diameter. This chamber was housed in a thermostatically controlled enclosure maintained at 20 ± 1°C. A photocell and light were positioned across the body of the funnel to detect the nose of the animal and initiate the trial sequence described in the next section. An 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 the cup while standing on a stainless steel floor plate, completed a high resistance circuit. In addition to signaling the operant licking response, this cup served as the drinking spout from which the rat received water reinforcement from a solenoid-controlled water reservoir. Air from the chamber was continuously exhausted to the outside of the building by a series of muffin fans connected to the wide end of the chamber by flexible plastic hose.

Both the stimulus delivery contingencies and the subject responses were controlled and monitored by Apple IIe™ computers (one for each of the two test boxes). Response data were compiled on-line and automatically printed to hard copy after each test session.

Operant Testing Procedures

The training procedures are described in detail elsewhere [e.g., (4)]. To initiate a trial, the rat positioned its snout at the neck of the chamber. This broke a photobeam which resulted in a one-second diversion (the "final valve period") of the airstream from the chamber to exhaust and simultaneously activated either the odor or blank air delivery valve, thereby directing either an odor (S+) or an air (S−) 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 together prior to delivery into the test chamber. Any response by the rat during this period aborted the trial. After this one-second diversion, the stimulus was delivered to the sniffing port for five seconds. Lick responses made during the initial two seconds of this five-second period were not reinforced. In the remaining three-second period, a lick response under the S+ condition resulted in the immediate termination of the trial and delivery of a 0.02 ml water reward. Such a response under the S− condition did not result in the delivery of water and immediately terminated the trial. If no responses were made during the three-second response period, the trial was automatically terminated and a two-second intertrial interval intervened before another photobeam break would initiate the start of a new trial.

A daily test session consisted of a total of 260 trials per subject, the first ten of which consisted of five S+ ($10^{-3.5}$) and five S− warm-up trials not used in the performance calculations. Following these warm-up trials, blocks of five S+ and S− trials were presented in a descending series of concentrations (i.e., $10^{-3.5}$, $10^{-4.0}$, $10^{-4.5}$, $10^{-5.0}$, and $10^{-5.5}$). The order of presentation of the five S+ and five S− trials at a given concentration was random, with the restriction that no more than three trials of a kind occurred in succession. After this descending series of 50 trials, two ascending and two descending 50-trial series were instituted, resulting in a total of 50 trials at each of the five concentrations. All testing was performed during the first half of the light phase of the L:D cycle.

Performance Measures

Six performance measures were computed. The first was the nonparametric sensitivity index (SI), which was determined from the proportion of hits (i.e., drinking spout contacts under the S+ condition) and false alarms (FA; drinking spout contacts under the S− condition) (10). The second was the proportion of the session trials on which correct performance occurred. The third was a measure of the general tendency of the animal to perform the operant lick response during a trial, independent of whether air or odor was presented [termed the responsivity index or RI; (10)]. The fourth was the time required for the animal to respond following the presentation of an odor trial, i.e., the S+ response latency. Since the S− condition was automatically terminated at five seconds and since the lack of a response was the correct operant under this condition, S− latencies were not similarly used. The fifth measure was the total time required by a rat to complete a test session, and the sixth was the number of trials which were aborted because the rat touched the cup during the final valve period.

Experimental Design and Injection Protocol

The fluprazine (Duphar B.V., Weesp, Holland) was dissolved in saline and administered IP 30 minutes before testing, in accord with other studies (11,15). The dose range evaluated encompassed doses known to influence a number of behavioral measures without producing sedative effects (9,11). The dose presentation order was counterbalanced within each subject across test days using Latin squares (17). The subjects were tested daily to insure the maintenance of stable performance levels, with the drug tests confined to every third day in order to allow for the dissipation of potential effects of prior drug injections.

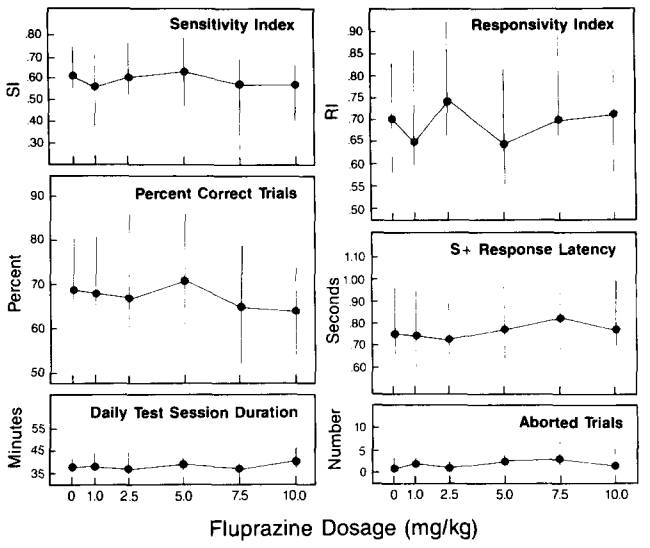

FIG. 1. Overall medians and interquartile ranges for dependent variables across all odorant concentrations as a function of fluprazine dose.

Since the odorant concentrations and numbers of trials were constant across the drug conditions, median values for each dependent measure were first calculated on the data collapsed across all EA odorant concentrations (i.e., across all 250 test trials). Subsequently, these measures were calculated separately for each odorant concentration (50 trials per session) to determine whether the drug differentially altered the performance measures as a function of odor intensity.

RESULTS

The medians and interquartile ranges for each of the six dependent variables across all odorant concentrations combined are presented in Fig. 1. Analogous measures are presented as a function of odorant concentration for the two olfactory sensitivity measures and for RI in Tables 1 and 2, respectively. It is apparent from these data that no consistent overall drug-related alterations were present for any of the behavioral measures at any odorant concentration, with the exception of RI at the lowest odor concentration (10^{-5}) (Friedman ANOVAs). A Wilcoxon signed ranks test on the data at this concentration level showed that the RI value for the 2.5 mg/kg dose was significantly higher than the RI values of saline and of the 1.0, 5.0, and 7.5 mg/kg doses ($p<0.05$, 0.05, 0.01, and 0.05, respectively). Given the number of statistical analyses that were performed, however, this effect is likely due

TABLE 1

MEDIAN AND INTERQUARTILE RANGE OF SI AND PERCENT CORRECT VALUES AS A FUNCTION OF FLUPRAZINE DOSE AND ETHYL ACETATE (EA) ODORANT CONCENTRATION

EA Concentration	Fluprazine Dose (mg/kg) 0	1.00	2.50	5.00	7.50	10.00	p
				Sensitivity Index			
$10^{-3.50}$	0.76 (0.64–0.89)	0.69 (0.58–0.86)	0.82 (0.69–0.96)	0.77 (0.66–0.87)	0.73 (0.63–0.90)	0.66 (0.60–0.77)	ns
$10^{-4.00}$	0.69 (0.66–0.83)	0.69 (0.53–0.86)	0.76 (0.65–0.85)	0.76 (0.67–0.86)	0.64 (0.67–0.78)	0.63 (0.51–0.75)	ns
$10^{-4.50}$	0.60 (0.57–0.68)	0.63 (0.45–0.73)	0.63 (0.57–0.69)	0.57 (0.57–0.77)	0.59 (0.00–0.76)	0.59 (0.45–0.65)	ns
$10^{-5.00}$	0.54 (0.51–0.58)	0.53 (0.23–0.58)	0.53 (0.34–0.56)	0.52 (0.14–0.60)	0.39 (0.00–0.58)	0.53 (0.42–0.58)	ns
$10^{-5.50}$	0.36 (0.00–0.53)	0.00 (0.00–0.49)	0.00 (0.00–0.42)	0.18 (0.00–0.49)	0.39 (0.00–0.52)	0.18 (0.00–0.43)	ns
				Percent Correct Trials			
$10^{-3.50}$	84 (75–94)	80 (72–92)	90 (77–98)	85 (75–92)	81 (71–94)	81 (69–85)	ns
$10^{-4.00}$	82 (75–90)	80 (66–92)	85 (75–91)	86 (74–92)	72 (67–86)	74 (64–85)	ns
$10^{-4.50}$	69 (63–80)	70 (56–82)	74 (62–80)	74 (62–85)	65 (50–84)	68 (61–73)	ns
$10^{-5.00}$	57 (53–63)	56 (54–69)	55 (52–60)	58 (52–69)	54 (51–64)	61 (55–64)	ns
$10^{-5.50}$	55 (49–58)	50 (50–57)	50 (50–52)	52 (50–55)	52 (50–57)	52 (50–55)	ns

Column of p values refers to Friedman two-way ANOVAs performed on the data within each EA concentration across all fluprazine doses.

TABLE 2

MEDIAN AND INTERQUARTILE RANGE OF RI VALUES AS A FUNCTION OF FLUPRAZINE DOSE AND ETHYL ACETATE (EA) ODORANT CONCENTRATION

	Responsivity Index (RI)						
	Fluprazine Dose (mg/kg)						
EA Concentration	0	1.00	2.50	5.00	7.50	10.00	p
$10^{-3.50}$	0.58 (0.52–0.65)	0.53 (0.27–0.65)	0.53 (0.28–0.65)	0.58 (0.52–0.63)	0.58 (0.15–0.69)	0.63 (0.52–0.73)	ns
$10^{-4.00}$	0.58 (0.53–0.67)	0.52 (0.34–0.65)	0.57 (0.54–0.67)	0.57 (0.53–0.68)	0.67 (0.53–0.72)	0.60 (0.54–0.75)	ns
$10^{-4.50}$	0.73 (0.60–0.81)	0.68 (0.55–0.85)	0.68 (0.61–0.81)	0.65 (0.56–0.81)	0.70 (0.60–0.86)	0.70 (0.68–0.78)	ns
$10^{-5.00}$	0.86 (0.77–0.94)	0.81 (0.63–0.91)	0.89 (0.83–0.94)	0.78 (0.68–0.94)	0.83 (0.73–0.98)	0.78 (0.69–0.91)	ns
$10^{-5.50}$	0.87 (0.79–0.92)	0.95 (0.68–1.00)	0.96 (0.88–1.00)	0.85 (0.64–0.94)	0.94 (0.85–1.00)	0.90 (0.69–0.98)	<0.05

p Values refer to Friedman two-way ANOVAs performed on the data within each EA concentration across all fluprazine doses.

to chance. No meaningful changes were present in any of the other behavioral measures.

DISCUSSION

The present study provides strong evidence that the behavioral effects observed in rats injected with fluprazine hydrochloride are unlikely to be the result of generalized olfactory impairment. Although previous studies have noted that fluprazine-treated animals are not totally anosmic [e.g., (10)], this work is the first to demonstrate that such animals fail to evidence alterations in quantitative measures of olfactory sensitivity, even at a drug concentration higher than that used by most other investigators (10 mg/kg). This finding stands in stark contrast to the dose-related effects of such drugs as amphetamine and quinpirole on ethyl acetate odor detection performance in our test paradigm (4,7).

Despite the fact that fluprazine fails to induce a *general* alteration in odor detection performance, it is still possible that its behavioral effects are mediated via selective changes in sensitivity to odorants other than ethyl acetate. Additionally, it is conceivable that this drug influences the perceived quality or hedonicity of odors quite independently of any influence on olfactory sensitivity, per se. An analogy would be the influences of castration on the conspecific odor preferences of sexually experienced male rats. In such animals, estrous odor preferences are markedly depressed even though odor detection performance, per se, is left intact or only marginally altered (2, 3, 6).

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