Odor detection performance of rats following
d-amphetamine treatment: a signal detection analysis

R.L. Doty and M. Ferguson-Segall
Smell and Taste Center, Department of Otorhinolaryngology and Human Communication, and Department of Physiology,
School of Medicine, University of Pennsylvania, 3400 Spruce Street, Philadelphia, PA 19104, USA

Abstract. The effects of d-amphetamine sulfate (0.2, 0.4, 0.8, and 1.6 mg/kg SC) on the odor detection performance of 16 adult male Long Evans rats was assessed using high precision olfactometry and a go/no-go operant signal detection task. The drug or saline was administered every 3rd day in a counterbalanced order, with the injections occurring 5 min before each 260-trial test session. Relative to saline, enhanced detection performance to the target stimulus (ethyl acetate), as measured by a non-parametric signal detection index (SI), was observed following administration of 0.2 mg/kg of the drug, whereas decreased detection performance was observed following administration of 1.6 mg/kg of the drug. Significant increases in the responsivity index (RI) occurred at the higher drug dosages for the lower odorant concentrations. In addition, small but statistically significant increases in the latency to respond in the presence of the odor (i.e., S+ response latency) were present at the higher drug dosages. Overall, these data suggest that (a) odor detection performance is enhanced by low doses of amphetamine, (b) odor detection performance is depressed by moderate doses of amphetamine, and (c) drug-related alterations in response criteria occur following the administration of moderate doses of amphetamine.

Key words: Amphetamine - Olfaction - Odor - Catecholamines - Norepinephrine - Dopamine - Olfactometry - Signal detection - Olfactory sensitivity - Odor detection - Attention - Arousal

It is well known from animal studies that d-amphetamine enhances neural activity within the brainstem reticular formation, produces electroencephalographic (EEG) arousal, and lowers the threshold for EEG arousal to electrical brain stimulation and to some forms of sensory stimulation (e.g., Bradley and Key 1958; Altshuler and Burch 1975). In addition, there is evidence that amphetamine improves various measures of human visual sensory perception or performance, including visual acuity (Lebensohn and Sullivan 1944), dark adaptation (Yudkin 1941), the flicker fusion threshold (Simonson and Enzer 1942), vigilance to alterations in movement (Mackworth 1965), and binocular imbalance associated with sleepiness (Kleitman and Schreiber 1940). However, paradoxical effects of amphetamine (e.g., drowsiness and lowered electrical brain activity) have been noted in some cases within an hour of its administration (Tecce and Cole 1974), and rodent and primate studies uniformly fail to observe enhanced performance in visual and auditory detection tasks following its administration (e.g., Ridley et al. 1980). Indeed, most animal studies report significant dose-related decreases in such performance (Thurmond 1965; Uehling and Venator 1967; Ahlenius et al. 1975; Delay et al. 1979; Goetsch and Isaac 1983; Koek and Slangen 1983; Hienz et al. 1985).

Although there is some suggestion from the Russian literature that amphetamine may improve the odor-guided tracking performance of dogs (e.g., Myznikov 1958; Krushinsky and Fless 1959), no well-controlled animal studies have quantitatively examined the influences of amphetamine on odor detection performance. Several human studies suggest that olfactory sensitivity may be decreased by oral amphetamine intake (e.g., Goetzl and Stone 1948; Guild 1956). Others, however, find no such effect (e.g., Janowitz and Grossman 1949). Unfortunately these studies are not definitive, since they are based upon the blast injection procedure of Elsberg and Levy (1935), a non-forced-choice procedure which confounds the measure of sensitivity with the response criterion, as well as with stimulus pressure and volume artifacts (see Wenzel 1948).

The purpose of the present study was to establish measures of olfactory sensitivity in rats following the administration of d-amphetamine using high precision olfactometry and a go/no-go operant signal detection task. Despite the lack of enhancement of detection performance in other modalities following the administration of this drug, amphetamine might be expected to improve odor detection performance, since amphetamine releases catecholamines in central olfactory regions such as the olfactory tubercle (e.g., Speciale et al. 1980) and a 0.87 correlation has been noted, in Korsakoff's psychosis patients, between cerebrospinal fluid (CSF) levels of a major metabolite of norepinephrine and scores on an odor identification test known to correlate strongly with odor detection thresholds (Doty et al. 1984; Mair et al. 1986).

Materials and methods

Subjects. Sixteen adult male Long-Evans rats, ranging in age from 7 to 9 months at the time of testing, served as subjects. These animals were selected from a larger number on the basis of adequate performance on an operant task...
Fig. 1. Schematic representation of the nine-stage dynamic air-dilution olfactometer and associated rat testing chambers. The three-way solenoid valves and both the stimulus and response contingencies are controlled by computer. See text for details.

Stimulus control and delivery. Odorant stimuli were generated using an air-dilution olfactometer and delivered to an animal operant testing chamber (Fig. 1). All internal surfaces in contact with flows through the system were constructed of glass or Teflon. Ethyl acetate, which has a fruity-like smell to humans, was chosen as the test stimulus since it (a) has relatively high vapor pressure, (b) has little tendency, relative to other odors, to "cling" or "stick" to olfactometer surfaces, and (c) has been used in a number of previous rat odor discrimination studies (e.g. Nigrosh et al. 1975; Slotnick 1984). At the air intake end of the system, filtered room air was drawn through a Thomas Model 1007CM72 oilless compressor at 20 psi and passed through two Balston Type 93 polycarbonate filters into a Norgen Model D-10-100-0010 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 the ethyl acetate stimulus and served as an over-the-surface saturator (Dravniaks 1979). The other set contained no odorant and served as a clean air line. The saturated and clean air lines were then channeled through a 9-stage olfactometer consisting of a series of Porter flowmeters, mixing chambers, and Teflon needle valves, providing incremental odor dilutions at each stage. 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 non-odorized 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 open port into the test chamber. On a given trial one of the following odorant concentrations (relative to saturation) was presented to a subject, as explained in detail later in the paper: 10^{-5.4}, 10^{-4.8}, 10^{-4.3}, 10^{-3.7}, and 10^{-3.2}.

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 (Fig. 2). 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. A 3 mm diameter, 2.4 cm long stainless steel rod attached through the upper left anterior wall of the chamber served as the response bar. The subject, by touching the bar with its paw while standing on a stainless steel floor plate, completed a high resistance circuit. A stainless steel cup (8 mm diameter, 6 mm deep) at the base of the chamber served as a 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 (e.g., bar presses and licks) 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 training and testing procedures. The animals were initially trained to lick the reinforcement cup using a progressive 1-trial fixed ratio (FR) schedule (from FR 1 to FR 20). After this 20-trial progression, a variable ratio (VR) 20 schedule was in effect for an additional 20 reinforcements. The animals were then shaped to touch the response bar for a minimum duration of 300 ms, first under an FR 1 schedule and later under a VR 3 schedule. After learning these responses, training sessions were given in which an initial bar press resulted in the presentation of a high concentration of odorant (10⁻².⁹) and a subsequent bar press resulted in the delivery of water reinforcement. A lick at the water dispenser terminated the delivery of the odorant. After this sequence of behaviors was performed correctly on 20 successive trials, a photo beam break was required to initiate the odorant presentation. This resulted in the proper orientation of the rat’s nose for monitoring the incoming airstream. After the rat attained 80% correct performance on this task in a given session of at least 100 trials, blank (S⁻) non-reinforced trials were interspersed among the S+ trials in which the final trial parameters, described in the next paragraph, were in effect. Following achievement of 80% correct or better performance on these S+ and S⁻ trials (the correct responses being touching and not touching the bar, respectively), the odorant concentration was decreased to a lower level (10⁻³.⁵). After 80% performance was achieved at this level, the animals were successively trained at 10⁻³.⁷ and 10⁻⁴.³ concentrations until 80% performance was similarly attained.

The ascending/descending final trial parameters were then introduced. As above, the rat positioned its snout at the neck of the chamber to initiate a trial. The photo beam break resulted in a 1 s diversion (to exhaust) of the airstream from the chamber and simultaneously activated either the odor or blank air delivery valve, thereby directing the appropriate 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 bar touch response by the rat during this period aborted the trial. After this diversion, the odor (S⁺) or blank air (S⁻) stream was delivered into the chamber for 5 s. A bar touch under the S⁺ condition resulted in the immediate termination of the trial and delivery of a 0.02 ml water reward under an FR 1 reinforcement schedule. In the absence of the odor stimulus (S⁻), a paw contact terminated the trial. A paw response made during the initial 2 s of the 5-s stimulus period was not reinforced, although it was recorded to establish the response latency measure. Thus, the reinforcement contingencies were designed to delay the response long enough to insure that an adequate period of time was available for sampling the stimulus. If no responses were made during the subsequent 3 s of stimulus delivery, the trial was automatically terminated and a 4-s intertrial interval intervened before another photo beam break could initiate a trial.

A daily test session consisted of a total of 260 trials per subject, the first ten of which consisted of five S+ (10⁻³.² concentration) and five S⁻ warm-up trials not used 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., 10⁻³.², 10⁻³.⁷, 10⁻⁴.³, 10⁻⁴.⁸, and 10⁻⁵.⁴ relative to saturation), with the order of presentation of the five S+ and five S⁻ trials at a given concentration being random with the restriction that no more than three of a kind occurred in succession. After this descending series of 50 trials, two analogous ascending and two analogous 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. The proportion of hits (i.e., bar contacts under the S+ condition) and false alarms (FA; bar contacts under the S⁻ condition) were used to calculate the nonparametric sensitivity index SI and the responsivity index RI using the formulae of Frey and Colliver (1973):

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SI = \frac{P(HIT) - P(FA)}{2[P(HIT) + P(FA)] - [P(HIT) + P(FA)]^2}
\]

\[
RI = \frac{P(HIT) + P(FA) - 1}{1 - [P(HIT) - P(FA)]^2}
\]

In addition to not requiring the parametric assumptions of homogeneity of variance and distribution normality, these two measures can be calculated when the hit rate is 1.00 or the false alarm rate is 0.00, unlike the parametric signal detection indices of d' and beta (Frey and Colliver 1973). SI can range theoretically from 0 (no detection) to 1.00 (perfect detection), whereas RI can range from -1.00 (very conservative response criterion) to +1.00 (very liberal response criterion).

Since the stimulus concentrations and number of trials were constant across the various drug conditions, both SI and RI were first calculated for each animal’s performance.

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**Fig. 2. Rat testing chamber.** Two of these chambers were used, each housed within temperature-controlled and sound-attenuated boxes, as illustrated in Fig. 1. See text for details.
for all 250 trials combined (i.e., for the entire test session involving all ethyl acetate odor concentrations). 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 the stimulus intensity.

To establish whether motor responses were being either facilitated or disrupted in a manner that might influence the odor detection performance measure, we also monitored the latency of each subject to touch the bar under the S+ condition following the initiation of the test trial. Since the S− condition was automatically terminated at 5 s and since the lack of a response was the correct operant under this condition, S− latencies were not similarly used.

**Experimental design and injection protocol.** Four concentrations of d-amphetamine sulfate (0.2 mg/kg, 0.4 mg/kg, 0.8 mg/kg, and 1.6 mg/kg; Sigma A-5880) were used in addition to the saline vehicle control. On a treatment day, the selected injectant was administered subcutaneously 5 min before the start of the 260 trial test sessions. Although each animal received daily test sessions, the treatment days were separated from one another by 2 saline-injection days to minimize or eliminate effects of prior injections. The order of the five treatment conditions was derived for 15 of the 16 animals from three 5×5 Latin squares, two of which were isomeric (Zimney 1961). The order of the treatment conditions for the 16th animal was randomly determined.

**Results**

The medians and interquartile ranges of the SI values based on all of the daily test trials are presented in Fig. 3. A Friedman 2-way nonparametric analysis of variance (ANOVA; Siegel 1956) revealed that amphetamine treatment was associated with a significant change in the sensitivity (SI) measure (Chi Square = 23.2, df = 4, P < 0.001). Wilcoxon matched-pairs signed-ranks test comparisons between each of the amphetamine treatment conditions and the saline control revealed a significant increase under the 0.2 mg/kg condition (t = 23, P < 0.02) and a significant decrease under the 1.6 mg/kg condition (t = 4, P < 0.001).

Friedman ANOVAAs performed on the sensitivity data for each of the ethyl acetate odorant concentration levels revealed significant alterations at the 10−4.8, 10−4.3, 10−3.7, and 10−3.2 levels (respective Chi Square values = 20.3, 20.6, 25.6 and 25.7, all Ps < 0.001). Individual comparisons using the Wilcoxon test revealed a significant increase in SI (relative to the saline treatment) at the 10−4.3 odor concentration for the 0.2 mg/kg amphetamine dosage (t = 21.5, P = 0.016; 15% median increase over saline). A tendency towards enhancement was present also at the 10−4.8 concentration at this dose level (t = 31.5, P = 0.06; 5% median increase over saline). Significant decrements in sensitivity were present at the 1.6 mg/kg dose level for the four highest odorant concentrations (respective t values = 11 (P = 0.004), 4 (P = 0.002), 11.5 (P = 0.004), and 15 (P = 0.004); respective percentage decreases in median SI values = 25%, 28%, 24%, and 54%).

In general, there was an inverse relationship between an animal's drug-related change in performance and his baseline performance under the saline condition. Under the 0.2 mg/kg dosage, animals with lower initial SI scores performed, on the average, better than those with higher initial SI scores. Under the 1.6 mg/kg dosage, animals with higher initial SI scores tended to evidence greater decrements in performance. This phenomenon is reflected by strong correlations between (a) the SI value under the saline condition and (b) the change in sensitivity performance after the administration of the drug. For example, using the 250 trials under the 0.2 mg/kg condition, the Spearman correlation coefficient between these two measures equals −0.65 (P < 0.005). The analogous r for the 1.6 mg/kg condition is −0.51, (P < 0.05). The median of the 20 coefficients computed among all drug dosages and odorant concentrations is −0.63 (P < 0.005). The 25th and 75th percentile values for these 20 correlations are −0.54 and −0.70, respectively.

To assess whether the enhancement following the 0.2 mg/kg amphetamine injection reflected an increase in performance during later phases of the test sessions (when fatigue might be expected), we compared the performance of the rats during the first 100 and last 100 trials of the test session under both the saline and drug conditions. No evidence of significant decrements in performance across the test session under either the saline condition or the drug condition was observed [median SI values for control and drug subjects for the first 100 trials = 0.59 and 0.70, respectively (Wilcoxon t = 44, P = 0.22); analogous medians for the second 100 trials = 0.65 and 0.67, respectively (t = 65, P = 0.88)].

Although an overall Friedman ANOVA performed on the RI data calculated from all five odorant concentrations combined was not statistically significant (Chi square = 8.19, df = 4, P > 0.10), analysis of the data for each concentration revealed significant effects at the 10−5.4 and 10−4.8 levels [respective Chi squares = 23.4 (P < 0.001) and 10.4 (P < 0.05)]. At the 10−5.4 level, individual comparisons revealed significant decreases in RI at the 0.8 mg/kg and the 1.60 mg/kg dosages relative to the saline condition: for 0.8 mg/kg, the RI value decreased from the saline value of 0.83 to 0.45, and for 1.6 mg/kg, the RI value decreased from the same saline value to 0.00. At the 10−4.8 concentration, such comparisons revealed a significant decrease in RI at the 1.6 mg/kg dose (from a saline value of 0.61 to 0.06). This decrease in RI reflected fewer total bar touches emitted by the subjects.
In general, the latencies for touching the response bar under the S+ condition increased as a function of amphetamine dosage (median latencies from saline to 1.6 mg/kg, respectively: 1.43, 1.48, 1.72, 1.75 and 2.29 s; Friedman ANOVA Chi Square = 20.80, df = 4, P < 0.001). However, a significant drug-related increase relative to saline was present only for the 1.6 mg/kg drug condition, in which 14 of the 16 animals evidenced increased latencies (Wilcoxon t = 15, P < 0.001). Interestingly, there was a near-significant tendency under the 0.2 mg/kg dosage towards shorter latencies (11 of the 16 animals evidenced decreased latencies; Wilcoxon t = 32, 0.05 < P < 0.10). This lack of significance was probably due to an atypically short latency under the saline condition for one of the subjects.

When the S+ latencies were analyzed for each of the odorant concentrations separately, significant decreases were noted for the 0.2 mg/kg amphetamine dosage condition at the 10^-3.2 and 10^-4.3 odorant concentration levels (respective t values = 29.5 and 26, Ps < 0.025). Increased latencies were observed under the 0.8 mg/kg dosage for the 10^-5.4 odorant concentration (t = 20, P < 0.005), as well as under the 1.6 mg/kg dosage for all odorant concentrations (10^-3.2 t = 22, P < 0.01; respective t values for 10^-3.7 to 10^-5.4 = 14, 6, 3.5 and 12, Ps < 0.005).

**Discussion**

The present data suggest that low doses of amphetamine enhance, and moderate doses depress, the odor detection performances of male Long-Evans rats for ethyl acetate. The enhancement appears to be independent of alterations in the subjects’ response criteria. To our knowledge, this is the first well-controlled empirical demonstration (in any modality) of an amphetamine-related quantitative enhancement of sensory detection performance in a non-human animal, although (a) amphetamine has been shown to increase the performance of rats in a two-lever operant task at the lower amphetamine doses used in this study, it was significantly decreased at the 1.6 mg/kg dosage level, reflecting a tendency for the animals to emit fewer bar touch responses. Significantly increased S+ response latencies were also present at this dosage. Although these changes may be associated with the decrement in sensitivity, it is likely they reflect additional drug-related factors, including alterations in motor function, attention, cognitive processing, or some combination of such variables. Unfortunately, it is not possible from the present data to establish which, if any, of these factors are responsible for these behavioral alterations.

It is of interest to note that significant increases in amphetamine-related sniffing behavior by rats occur in a dose-related manner from concentrations as low as 0.3 mg/kg (Fray et al. 1980; Ervin et al. 1981), with so-called stereotypic sniffing being clearly present at the 1.0 mg/kg dosage (Porrino et al. 1984). Since increased sniffing occurs in normal rats as stimulus concentration is decreased (Youngentob 1984), it is conceivable that such sniffing may reflect, in part, compensation for alterations in smell function. Additional research is needed to determine if this intriguing notion has merit.

The mechanism by which amphetamine alters odor detection ability is unknown. Amphetamine releases catecholamines, and blocks both their reuptake and degradation, in a number of brain regions directly associated with olfactory processing (e.g., anterior olfactory nucleus, olfactory tubercle, amygdala, and entorhinal cortex; cf. Horn et al. 1974; Louilot et al. 1985), as well as in brain regions involved in general arousal (e.g., the reticular activating system; cf. Bradley and Key 1958). The locus coeruleus is a prime candidate as a region for such involvement, since it is the primary, if not sole, source of noradrenergic fibers to the olfactory bulb, and is responsible for most of the norepinephrine content of more central olfactory related structures (Fallon et al. 1978; Fallon and Moore 1978; Shipley et al. 1985). This nucleus is probably the origin of the noradrenergic centrifugal fibers near or within the lateral olfactory tract which, when stimulated electrophysiologically, inhibit (via granule cells) mitral cell activity (Felix and McLennan 1971). Amphetamine-related releases of norepinephrine by such cells might be expected to decrease the firing of mitral and tufted cells and thereby decrease the perceived intensity of an olfactory stimulus. On the other hand, Jahr and Nicoll (1982) found that iontophoretic application of norepinephrine can lead to less firing of the inhibitory granule cells, which would be expected to produce an increase in the perceived intensity of an incoming stimulus. Theoretically, both of these phenomena could be present, depending upon the regions and amounts of catecholamines which are discharged (see Louilot et al. 1985). Since we have recently demonstrated that 6-hydroxydopamine depletion of norepinephrine within the olfactory bulb proper has little or no influence on the rats' detection of ethyl acetate (unpublished data), it is probable that amphetamine-related alterations in odor detection performance to this compound depend upon more central olfactory or arousal pathways.

In light of the data of the present study, it is conceivable that a number of behavioral alterations reported in the literature following manipulation of catecholaminergic systems could be the result of alterations in olfactory function. For example, Landauer and Balster (1982) found that doses of 1.0 and 3.0 mg/kg d-amphetamine administered to both sexually naive and sexually experienced male mice resulted in
less time spent investigating a compartment housing a female anestrous conspecific—a result that would be expected if such doses decreased olfactory sensitivity or altered the olfactory percept in some manner. Interestingly, aversions conditioned to a novel food using lithium chloride are decreased following 6-hydroxydopamine lesioning of the olfactory bulb, another finding that would be expected if smell perception is selectively altered or lessened by catecholaminergic manipulation (Royet et al. 1983). The possibility that such ablation produces subtle alterations in smell hedonics or identification ability is suggested by a recent study of sheep (Pissonnier et al. 1985). In that work, the lesioning of the centrifugal noradrenergic projections to the olfactory bulbs of maternal ewes was found to eliminate their ability to form a selective bond with their own lambs. However, the ewes still avoided food contaminated by infants’ amniotic fluid, implying that total anosmia was not present.

Whatever the basis for the alterations in odor detection performance noted in this study, it is clear that the effects of amphetamine are dose related and reasonably robust. Additional research is needed to determine if such effects are odorant dependent, vary with organismal factors (e.g., hunger, hormonal state, sexual arousal), and explain some of the other alterations noted in rodent behaviors following amphetamine administration (e.g., increased sniffing at low to moderate dosage levels).

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