Influence of Adrenalectomy on the Odor Detection Performance of Rats

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DOTY, R. L., J. M. RISSE AND G. M. BROSVIC. Influence of adrenalectomy on the odor detection performance of rats. PHYSIOL BEHAV 49(6) 1273-1277, 1991.—The influence of adrenalectomy (ADX) on the odor detection performance of male Long-Evans rats was assessed using high-precision olfactometry and a go/no-go operant signal detection task. Nonparametric signal detection measures of sensitivity and responsivity, as well as measures of $S^+$ response latency, the number of aborted trials, and session time, were obtained in daily 250-trial test sessions prior to and after adrenalectomy. Four ADX animals were tested using the odorant pyridine, three using the odorant eugenol, and two using the odorant ethyl acetate. Nine other rats served as sham-operated controls. Neither odor detection nor related nonsensory performance measures were influenced by adrenalectomy or sham-operation procedures. These results imply that adrenalectomy has little or no influence on the odor detection performance of the rat.

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A marked increase in olfactory sensitivity has been reported in humans with adrenal cortical insufficiency (ACI). For example, Henkin and Barter (8) reported that patients with ACI could detect, by olfactory cues alone, differences between water and aqueous solutions of sodium chloride, potassium chloride, NaHCO₃, sucrose, urea, and hydrochloric acid at concentrations 1/100,000 that required by normal subjects. Such heightened sensitivity was also noted for the odorants pyridine, thiophene, and nitrobenzene. Interestingly, daily treatment with the mineralcorticoid deoxycorticosterone acetate failed to decrease the heightened sensitivity, whereas such treatment with the corticosteroid prednisolone returned sensitivity to normal.

In the sole study examining the influences of adrenalectomy (ADX) on the olfactory sensitivity of rats, Sakellari (12) reported that a) two ADX rats had slightly lower olfactory thresholds than two sham-operates ($<0.5$ log dilution unit) and b) another rat, the only one tested pre- and postoperatively, had a lower threshold following the operation. Unfortunately, no controls were tested to ascertain the influences of repeated testing on the test measure [see (4)].

Given the reportedly large influence of ACI on human olfactory thresholds and both the theoretical and practical value of discovering ways to enhance the olfactory sensitivity of animals (5,9), the question of whether ADX enhances the odor detection performance of rats needs to be addressed in a thorough manner. Thus, the present study used high-precision olfactometry and computer-controlled operant procedures to evaluate the influences of this operation on the odor detection performance of rats to pyridine, ethyl acetate, and eugenol. A comparatively large number of animals were tested daily in 250-trial test sessions which incorporated not only pre- and postoperative tests in the same animals, but a control group receiving an intervening sham operation. Signal detection methodology was chosen because of its ability to provide a separate measure of sensitivity and response criterion, as well as its sensitivity to subtle changes in detection performance (1, 3, 4, 7).

EXPERIMENT 1: INFLUENCES OF ADRENALECTOMY ON PYRIDINE ODOR DETECTION PERFORMANCE

Subjects

Twelve adult male Long-Evans rats, obtained from Charles River Breeding Laboratories, served as subjects. The animals were approximately three months of age at the time of initial testing. Six animals received ADX and six received sham (SHAM) operations following the odor detection training and testing described below. Since two of the ADX animals did not survive for postoperative testing, the final study group consisted of four ADX and six SHAM subjects. All subjects were housed in pairs in $24 \times 21.5 \times 45$ cm polystyrene laboratory cages in a humidity- and temperature-controlled vivarium and maintained on a 12:12 h light:dark cycle (L: 0500-1700) with Purina Lab Chow available ad lib.

Odorants

Chromatographic grade pyridine (Fisher Chemical), the odor-
ant used by Sakellaris (12), was used as the test stimulus at concentrations (relative to saturation) ranging from $1 \times 10^{-3.5}$ to $1 \times 10^{-6}$ in half-log dilution steps.

**Stimulus Generation Apparatus**

As described elsewhere (4, 5, 10, 11), an air-dilution olfactometer was used to generate various concentrations of the test stimuli. Specifically, filtered air was passed through two poly-carbonate filters into a refrigerant dryer. The airstream was then split; one segment flowed through an over-the-surface saturator filled with pyridine and the other through an empty saturator (3,4). Both saturators were immersed in a water bath maintained at 24°±1°C. The odorized and clean air lines were then passed through a 6-stage olfactometer which provided incremental odor dilutions. Each dilution could be delivered to two final mixing manifolds (one for each of two test chambers) via separate three-way Teflon™ solenoid valves. A stream of nonodorized air continuously flowed through the final manifolds and into the common port of a larger three-way Teflon solenoid valve (termed the “final valve”). At the onset of a trial, the final valve and the three-way valve controlling the flow of the selected stimulus [either air (S-) or a given odorant concentration (S+)] were activated. Activation of the final valve shunted the airstream away from the chamber and into an exhaust line to allow the selected stimulus to mix with the nonodorized air flowing through the manifold. The final valve was then deactivated and either air or a selected concentration of pyridine entered the chamber.

**Test Chamber**

A 9.5 cm dia., 22 cm long Plexiglas tube served as the test chamber. The airflow from the olfactometer passed through a vertical wind tunnel located at one end of the chamber. A 3 cm dia. sampling port in the side of the wind tunnel allowed the rat to sample the passing airstream. The chamber was housed in a thermostatically controlled enclosure maintained at 20°±1°C. A photocell and light were positioned across the sampling port to detect the nose of the animal and, when broken, initiated the trial sequence described below. A small stainless steel cup projecting through the floor of the chamber served as the response cup. Licks to the cup were monitored through a detection circuit (6). In addition to mediating the operant response, this cup served as the drinking spout from which the rat received water reinforcement.

**Testing Procedures**

Testing took place during the first half of the light phase of the light:dark cycle. The animals were placed on a 23.5-h water deprivation schedule two weeks before training and subsequent testing. The daily test sessions consisted of 250 trials per subject. Following five S+ (odor) and five S- (air) warm-up trials which were not included in the data analyses, six 20-trial blocks of S+ and S- trials were presented according to a descending method of limits, followed by an analogous ascending method of limits, resulting in a total of 40 trials per day for each of the six odorant concentrations. Within each 20-trial block, the S+ and S- trials were presented randomly, with the exception that a) no more than three trials of the same type occurred in succession and b) that an equal number of S+ and S- trials occurred in each 20-trial block.

To initiate a trial, the rat positioned its snout in the vertical wind tunnel, thereby breaking the photobeam, and simulta-

neously activating a stimulus valve and the final valve. One second later, the final valve was deactivated, shunting the odorized airstream into the wind tunnel. During the final valve period, any response immediately terminated the trial. A 2-s fixed interval (FI) period followed in which the rat was required to break the photobeam for at least 0.2 s to ensure that it was exposed to the stimulus. Responses during this 2-s period were not counted. If the animal did not sample the stimulus for at least 0.2 s, the trial was terminated after the FI. If sampling took place during the FI, a 3-s response period followed the 2-s FI. During this period, if the stimulus was an S+, a response resulted in 0.05 ml of water and termination of the trial. If the stimulus was an S-, such a response resulted in no reinforcement. Each trial was separated by a 2-s intertrial interval during which a photobeam break could not initiate a trial sequence.

**Performance Measures**

The proportion of hits (cup contacts under the S+ condition) and false alarms (cup contacts under the S- condition) was used to calculate the nonparametric sensitivity index (SI) and responsivity (RI) index described by Frey and Colliver (7). Addition-

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**FIG. 1.** Mean (±SEM) sensitivity index (SI), responsivity index (RI), and S+ response latency measures for 12 rats calculated across all pyridine odorant concentrations before and after adrenalectomy (ADX) and sham (SHAM) operations. Note lack of significant alterations across the pre- and posttest periods. See text for details.
ally, we measured the latency of each subject to respond following the initiation of the 3-s response period, as well as the total session time and the number of aborted S+ trials. Response latency was calculated only for S+ trials, since the operant for S− trials was not to lick the water reservoir and successful S− trials terminated automatically at the end of the fixed period.

**Surgical Procedures**

Each rat was adrenalectomized under Ketamine anesthesia as described previously (1). A salt lick was provided in each cage postoperatively, and all animals were given daily access to a 1% saline solution for 1 h after testing.

**Experimental Design**

The subjects received daily 250-trial test sessions for six weeks (5 days/week), the last two of which provided the data for assessing preoperative baseline performance. The animals were then placed on an ad lib food and water regimen for two days and operated upon. Following surgery, they were given, in addition to their salt lick, ad lib access to food and a 1% saline solution for seven days and subsequently placed on a 23-h water deprivation schedule for an additional seven days. Testing was then resumed for 5 days per week for the following 3 weeks.

**Statistical Procedures and Data Analysis**

Analyses of covariance (ANCOVA) and Scheffe comparisons were used to examine the potential influences of adrenalectomy and sham-operation procedures on each dependent measure: SI, RI, S+ response latency, total session time, and the number of aborted S+ trials. Preoperative dependent measures were used as covariates in these analyses to assess and control for potential between-group baseline differences in the dependent measures.

**RESULTS**

**Odor Detection Performance Measure**

Mean overall SI values are presented in Fig. 1A. Results of analysis of covariance indicated that mean SI values did not differ between the ADX and SHAM groups [covariate, F(1,101) = 0.5, p>0.5; surgery, F(1,101) = 1.23, p>0.5; postoperative week, F(2,101) = 0.67, p>0.5]. A significant main effect of odorant concentration for mean SI values was observed, F(5,101) = 6.81, p<0.001, with Scheffe comparisons indicating that SI’s for the two strongest concentrations (1 x 10^{-3.5} to 1 x 10^{-4}) were significantly higher than those of the two weakest concentrations (1 x 10^{-5.5} and 1 x 10^{-6}). The interaction terms did not reach statistical significance (all Fs<1, all ps>0.5), indicating no significant changes in overall olfactory sensitivity at any point during the study.

**Nonsensory Performance Measures**

Mean overall RI values are presented in Fig. 1B. Results of an analysis of covariance on mean RI values did not differ between the ADX and SHAM groups [covariate, F(1,101) = 0.98, p>0.5; surgery, F(1,101) = 0.43, p>0.5; postoperative week, F(2,101) = 1.49, p>0.5]. A significant main effect of odorant concentration for mean RI values was observed, F(5,101) = 4.98, p<0.001, with Scheffe comparisons indicating that RI’s for the two weakest concentrations (1 x 10^{-5.5} and 1 x 10^{-6}) were significantly higher than those of the two strongest concentrations (1 x 10^{-3.5} and 1 x 10^{-4}). As seen in Fig. 1C, mean S+ response latencies did not differ between the ADX and SHAM groups [covariate, F(1,101) = 1.01, p>0.5; surgery, F(1,101) = 0.06, p>0.5; postoperative week, F(2,101) = 1.13, p>0.5; odorant concentration, F(5,101) = 0.81, p>0.05]. Finally, no between-group differences for mean total session time [40 minutes (SEM = 8)] and the mean number of aborted S+ trials [ADX, 7 (SEM = 2.5) SHAM, 3 (SEM = 1.5)] were observed (all Fs<1, all ps>0.5). The interaction terms for these dependent measures did not reach statistical significance (all Fs<1, all ps>0.5), indicating no significant changes in overall nonsensory behavior at any point during the study.

**Relationship of Test Measures With Corticosterone Levels**

Two to four months after the completion of behavioral testing serum corticosterone levels were measured in 9 of the 12 rats using radioimmunoassay procedures described previously (1). Although the delay in obtaining these measures may have resulted in some regeneration of adrenal gland remnants (13), mean serum corticosterone levels were significantly lower in the ADX rats (13.3 μg/dl, SEM = 2.93) than in sham-operates (23.2 μg/dl, SEM = 2.93), t(8) = 3.1, p<0.02. As seen in Fig. 2, the distributions had only one point of overlap. None of the dependent measures were significantly correlated with the corticosterone levels (median Spearman r = .06, all ps>0.8).

**EXPERIMENT 2: INFLUENCES OF ADRENALECTOMY ON ODOR DETECTION PERFORMANCE FOR ETHYL ACETATE AND EUGENOL**

**METHOD**

Although adrenalectomy had no influence on the rat’s odor detection performance to the odorant pyridine, it is possible that this operation might alter such performance for other odorants. Therefore, Experiment 2 explored the influences of adrenalectomy on the detection performance for ethyl acetate and eugenol.

**Subjects, Odorants, and Procedures**

Eight adult male Long-Evans rats similar to those described in Experiment 1 served as subjects. Four animals were tested...
FIG. 3. Mean (±SEM) sensitivity index (SI), responsivity index (RI), and S+ response latency measures for 8 rats calculated across all pyridine odorant concentrations before and after adrenalectomy (ADX) and sham (SHAM) operations. Four rats were tested with the odorant ethyl acetate (two ADX and two SHAM) and four with the odorant eugenol (three ADX and one SHAM). Note lack of significant alterations across the pre- and posttest periods. See text for details.

FIG. 4. Serum corticosteroid levels of five adrenalectomized (ADX) rats and two sham-operated (SHAM) rats from Experiment 2. See text for details.

with ethyl acetate (two ADX, two SHAM). Three ADX and one SHAM were tested with eugenol. The chromatographic grade odorants (Fisher Chemical) were used at concentrations (relative to saturation) ranging from $1 \times 10^{-3.5}$ to $1 \times 10^{-6}$ in half-log dilution steps. The test procedures were the same as those described in Experiment 1.

RESULTS

Analysis of variance indicated that SI, RI, S+ latency, session time, and the number of aborted S+ trials did not differ between animals trained with ethyl acetate and those trained with eugenol (all Fs<1.5, all ps>0.5). Therefore, the data were combined across odorants, and potential between-group differences were analyzed using the procedures described in Experiment 1.

Odor Detection Performance Measure

Mean overall SI values are presented in Fig. 3A. Results of an analysis of covariance indicated that mean SI values did not differ between the ADX and SHAM groups [covariate, F(1,96) = 1.25, p>0.5; surgery, F(1,96) = 1.82, p>0.5; postoperative week, F(2,96) = 1.03 p>0.5]. A significant main effect of odorant concentration for mean SI values was observed, F(1,96) = 7.89, p<0.001, with Scheffe comparisons indicating that SI’s for the strongest three odorant concentrations ($1 \times 10^{-3.5}$ to $1 \times 10^{-4.5}$) were significantly higher than those of the weakest two concentrations ($1 \times 10^{-5.5}$ to $1 \times 10^{-6}$). The interaction terms did not reach statistical significance (all Fs<1.56, all ps>0.5).

Nonsensory Performance Measures

Mean overall RI values are presented in Fig. 3B. Results of an analysis of covariance indicated that mean RI values did not differ between the ADX and SHAM groups [covariate, F(1,96) = 1.76, p>0.5; surgery, F(1,96) = 1.27, p>0.5; postoperative week, F(2,96) = 1.03, p>0.5]. A significant main effect of odorant concentration for mean RI values was observed, F(5,96) = 0.23, p>0.5, with Scheffe comparisons indicating that RI’s for the weakest two concentrations ($1 \times 10^{-5.5}$ to $1 \times 10^{-6}$) were significantly higher than those of the strongest two concentrations ($1 \times 10^{-3.5}$ to $1 \times 10^{-4}$). As seen in Fig. 3C, mean S+ response latencies did not differ between the ADX and SHAM groups at any point during the study [covariate, F(1,96) = 1.76, p>0.5; surgery, F(1,96) = 0.62, p>0.5; postoperative week, F(2,96) = 0.3, p>0.5; odorant concentration, F(5,96) = 0.18, p>0.5]. Finally, no between group differences were observed for mean total session time (45 minutes SEM = 6) and the mean number of aborted S+ trials [3 (SEM = 1)] (all Fs<1.5, all ps>0.05). As for SI, the interaction terms for these dependent measures did not reach statistical significance (all Fs<1.44, all ps>0.5), indicating no significant changes in non-sensory behavior at any point during the study.

Corticosterone Level Measurements

As in Experiment 1, mean serum corticosteroid levels were significantly lower in the ADX rats (8.7 µg/dl, SEM = 2.5) than in sham-operates (23.5 µg/dl, SEM = 0.35), t(5) = 4.9, p<0.01 (Fig. 4). None of the dependent measures correlated significantly with corticosteroid levels (median Spearman r = .10, all ps>0.5).

DISCUSSION

The present data, which are based upon a comparatively large number of subjects, three odorants, pre- and postoperative test-
ing, and signal detection methodology, lend no support to the hypothesis that adrenalectomy and the resultant corticosteroid deficiency appreciably alters the olfactory sensitivity of rats. These findings stand in stark contrast to those of Sakellaris (12). Although the reasons for these differences are unknown, the changes in olfactory thresholds for the single ADX rat tested pre- and postoperatively by Sakellaris may have been the result of sampling error, since only a few test trials were used in his study. On the other hand, procedural differences (e.g., Sakellaris used a conditioned suppression paradigm) may also have contributed to this effect.

We have recently shown that adrenalectomy has no significant influence on signal detection measures of the rat’s taste sensitivity and responsivity to sucrose and sodium chloride (1). Although, to our knowledge, there are no studies of the effects of adrenalectomy on behavioral thresholds to stimuli in sensory modalities other than gustation and olfaction, studies have examined the effects of adrenalectomy on electrophysiological responses within the rat’s auditory pathway. Thus Conn and Mast (2) compared the cochlear microphonic response (CM), the auditory nerve action potential (AP), and the auditory evoked response (AEP) of eight adrenalectomized rats to those of eight sham-operated animals and found no statistically significant adrenalectomy-related effects on the CM and AP measures. However, adrenalectomized rats exhibited smaller AEP amplitudes than controls at the higher stimulus intensities tested (i.e., 40 and 50 dB), implying that adrenalectomy may have decreased sensitivity at these intensities. More recently, Weigel, Prazma and Pillsbury (14) made pre- and postoperative determinations of a) the threshold intensity of a sound needed to induce cochlear microphonic action potentials in rats at 4 and 16 kHz input frequencies, b) the latency and amplitude of responses at these same frequencies, c) latency and amplitude measures for stimuli at 20 dB and 40 dB above threshold, and d) measures of the auditory brain stem threshold and various conductance latencies (14). Adrenalectomy had no influence on the threshold measures, although impaired neural transmission was reported for the first and second order neurons.

In addition to their work on rats, Conn and Mast (2) evaluated auditory-induced CM, AP, and AEP measures in chinchillas who had been either treated or not treated with metyrapone, an adrenal suppressant which inhibits the 11-beta hydroxylation in the biosynthesis of cortisol, corticosterone, and aldosterone. Although no differences were observed between the treated and the untreated animals in CM and AP responses, the metyrapone-treated subjects evidenced, relative to controls, smaller increases in AEP amplitudes at high stimulus levels (95 and 100 dB) and larger decrements in amplitude at low stimulus levels (40 and 50 dB).

In summary, the experiments described in the present paper suggest that adrenalectomy and the resultant corticosterone deficiency have no meaningful influences on the odor detection performance of rats to the odors of pyridine, ethyl acetate, and eugenol. These findings imply that the observation of markedly enhanced odor detection performance in patients with adrenal cortical insufficiency (8), if true, is unlikely present in the rat. Our findings, along with the lack of the influences of ADX on taste sensitivity observed by a number of authors [e.g., (1)], suggest that the original findings by Henkin and Bartter (8) of heightened taste and smell function in humans as a result of adrenocorticotrophic insufficiency need to be replicated.

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REFERENCES