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Enhancement of the Locomotor Response to d-Amphetamine by Olfactory Bulb Damage in Rats

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GADDY, J. R. AND D. B. NEILL. *Enhancement of the locomotor response to d-amphetamine by olfactory bulb damage in rats.* PHARMAC. BIOCHEM. BEHAV. 5(2) 189–194, 1976. – Bilateral removal of approximately 60% of the olfactory bulbs of ovariectomized female rats enhanced the locomotor response to d-amphetamine. The bulb damage did not reliably alter amphetamine-induced stereotypy or anorexia. The results were interpreted as being consistent with the idea that the olfactory bulbs may exert an inhibitory influence over some aspects of behavior in rats.

Locomotor response d-Amphetamine Bulbectomy Ovariectomy Olfactory bulbs Anorexia Stereotyped behavior

BILATERAL removal of the olfactory bulbs in the rat produces behavioral effects which are often difficult to interpret as due solely to a loss of sensory facility [2,15]. Consequently, it has been proposed that the diverse behavioral changes following bilateral bulbectomy indicate a general "modulating" function of the olfactory bulbs on behavior which may be independent of sensory function [6].

In trying to parsimoniously account for the spectrum of changes in behavior produced by bulbectomy, some investigators have suggested that the bulbs may exert an inhibitory influence on many behaviors. Thus, rats with bulb damage may be somewhat hyperactive in tests of spontaneous activity [24,33], acquire 2-way avoidance responding faster than normal [33,36], barpress at higher than control rates for food [25], and show subnormal levels of spontaneous alteration [14,24].

Many of the above behavioral alterations are also characteristic of lesions in a number of brain regions in rats, such as the septum [9,17], hippocampus [13], and midbrain raphe nuclei [30]. In addition, lesions of these latter areas enhance the locomotor response to systemically administered amphetamine [8, 10, 27], which is consistent with the idea that these areas exert an inhibitory influence over many behaviors.

The following experiments were conducted to examine the hypothesis that the olfactory bulbs, like the above brain regions, may play an inhibitory role in some aspects of behavior. We examined the effect of bulbectomy on 3 separate behavioral effects of amphetamine: increased locomotor activity, decreased food intake, and stereotyped behavior. Three measures were used to evaluate the specificity of the olfactory influence on the behavioral effects of amphetamine.

METHOD

Animals

Twenty three 90–120 day old female Long-Evans rats obtained from Marland Farms (NJ) were ovariectomized soon after their arrival in the laboratory in order to eliminate confounding influences from cyclicly varying levels of ovarian hormones. Thirteen of the animals underwent olfactory bulb ablation by the method of Edwards and Warner [16]; the remaining 10 controls were sham-operated by opening the scalp and removing some of the frontal bone. The wound was then sutured shut following the local application of an antibiotic. A period of 2–6 weeks after surgery was allowed before the first behavioral tests. The animals were housed in groups of 3–4 for all experiments except the tests of food intake. One bulbectomized animal died as a result of respiratory infection before the food-intake experiment. The experiment was conducted as an original study and a replication. Since the data were consistent between the 2 studies, they are presented as a single experiment.

Procedure

Amphetamine-induced stereotypy. While low doses of

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amphetamine usually increase locomotor activity in the rat, higher doses result in lowered scores [34]. This decrease in locomotor activity is due to the emergence of repetitive stationary sniffing or gnawing behaviors which are incompatible with high levels of locomotor activity. These behaviors are classed as "stereotypy." Although stereotypy may sometimes include repetitively-patterned locomotor activity in individual rats, most animals settle into a pattern of stationary behaviors. Therefore, an amphetamine-induced locomotor activity dose-response curve typically shows an inverted U pattern. Since the fall-off of locomotor activity at higher doses is due to competing stereotypic behaviors, the absence of locomotor activity at high doses of amphetamine is indicative of the presence of stereotypy.

Stereotypy was measured in eight 29.0 x 18.0 x 17.5 cm stabilimeters constructed of black Plexiglas with wire mesh floors on a central fulcrum. The lid of the apparatus was clear Plexiglas, allowing direct observation of the animal during tests. Crossings of the rat from one end of the box to the other tilted the floor slightly and closed a microswitch on one end. These devices were designed to detect gross locomotor activity and not fine movements such as sniffing. The stabilimeters were located in a sound-attenuating, darkened room. Stabilimeter crossings were automatically recorded by an Esterline-Angus event recorder and impulse counters.

Each rat was placed in a stabilimeter for a 30 min adaptation period and then injected intraperitoneally (IP) with 4.0 mg/kg of d-amphetamine sulfate (Smith, Kline, and French) in an isotonic saline vehicle. The duration of stereotypy was measured by observing the rise in stabilimeter crossings which follows amphetamine administration, the sudden cessation of crossings attendant to stereotypy, and finally, the reemergence of crossings. Since pilot work had indicated that habituation effects from repeated testing tended to lower crossing scores during the emergence from stereotypy and thus obscure the determination of emergence from stereotypy, the stereotypy tests were conducted before testing the locomotor response to amphetamine. The fact that the animals were in stereotypy was verified by direct observation.

The amount of time each animal spent in stereotypy was determined from the event records by the following method. The first cessation of responding which occurred within 1 hr of drug administration was designated as the onset of stereotypy. "Cessation of responding" was defined as a crossing rate of less than 2 in any 5 min period for a minimum interval of 15 min. Emergence from stereotypy was considered to have taken place when at least 3 consecutive 5 min intervals occurred in which the response rate was greater than the criterion value for "cessation of responding." Total time spent in stereotypy was computed for each animal by summing the amount of time classified as "cessation of responding" between onset of and emergence from stereotypy. Locomotor activity was measured concurrently by the method described below.

Amphetamine-induced locomotor activity. Locomotor activity (crossings) was measured in the same stabilimeters used for the stereotypy test. The animals were placed in the chambers for a 30 min adaptation period, after which they were given an IP injection of amphetamine or isotonic saline, and crossings were recorded every ½ hr for 3 hr. This testing began 2 days after the stereotypy test and continued with 2-day intervals between tests. Saline was given on the first test, 1.0 mg/kg of d-amphetamine on the second, 2.0 mg/kg on the third, 0.5 mg/kg on the fourth, and saline again on the fifth test. All injections were 1.0 ml/kg of vehicle or vehicle plus drug. Periodic observation of each animal was performed to determine if severe stereotypy was occurring during locomotor activity tests.

Amphetamine-induced suppression of food intake. Upon completion of locomotor testing, animals were singly housed and body weight under an ad lib feeding schedule measured for each animal. A food deprivation regimen was then initiated that maintained each animal at 85% of its ad lib body weight for at least 1 week before drug testing began.

At the beginning of a test each animal was given a preweighed 15 20 g portion of dry food pellets (Purina Lab Chow) in its home cage. The remaining pellets were removed ½ hr later and weighed to the nearest 0.1 g to determine the amount eaten. Food bits beneath the cages of both groups were measured to determine if drug treatment affected spillage. The practice was discontinued in the replication, however, since inspection of the data from the original study showed no differences in spillage between groups. Food intake was measured after injections in the following order: saline, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg, and saline. Tests were conducted daily, with drug or saline injections given every third day. This procedure assured that food intake had returned to baseline before subsequent saline or drug was given, thus precluding cumulative drug effects. Drugs were injected intraperitoneally in a volume of 1 ml/kg vehicle or vehicle plus drug 20 min before testing.

Lesion Evaluation

Bulbectomized animals were sacrificed under deep barbiturate anesthesia by intracardial perfusion with normal saline followed by 10% Formalin. The brains were removed from the skull at the time of sacrifice and fixed in 10% Formalin. Following fixation, each brain was examined under a dissecting microscope. The external features of the olfactory bulb were used as landmarks in order to determine the amount of bulbar tissue missing. The olfactory bulb is connected to the remainder of the cerebral hemisphere by the olfactory peduncle which lies between the olfactory bulb and the olfactory tubercle. If the brain of a rat is viewed ventrally, a circular groove the circular fissure at the base of the olfactory is easily seen. This groove marks the boundary between the olfactory bulb and the olfactory peduncle. The region rostral to the circular fissure comprises the main and accessory olfactory bulbs and the anterior portion of the anterior olfactory nucleus; the region caudal to the circular fissure is the olfactory peduncle and contains with it the remaining cell structure of the anterior olfactory nucleus. The amount of tissue missing anterior to the circular fissure was drawn on previously prepared brain drawings. Each contained two drawings of a rat brain. One drawing depicted the brain as viewed dorsally; the other drawing depicted the brain as viewed ventrally. Damage was quantified by dividing the area of the drawing anterior to the circular fissure into quadrants. The amount of tissue missing in each quadrant was then estimated. For any given brain, the average of these 4 values (a dorsal and ventral view for each bulb) was taken as the estimate of the percentage of olfactory bulbs missing. Following these procedures, the brains were embedded in celloidin and every fifth 50 μ coronal section stained with cresyl violet for histological evaluation.

Data Evaluation

The stereotypy results were evaluated by a t test for independent samples [32]. Locomotor response and food intake suppression dose-response curves were evaluated by repeated-measures analyses of variance [37]. Since the 4.0 mg/kg dose resulted in severe stereotypic behaviors, incompatible with locomotor activity, locomotor activity scores from this dosage level were omitted from the analysis. Saline measures taken at the beginning of the dose series were compared with those taken at the end of the series to detect any cumulative drug effects.

RESULTS

Lesions

Bilateral olfactory bulb damage ranged from 37.5% to 66.8%. The average damage was 59.1%. Diagrammatic representation of this damage is shown in Fig. 1. Dissection-microscope examination revealed no visible damage to the frontal cortex or any other portion of the brain other than the olfactory bulbs. Examination of 50 μ cresyl violet-stained sections showed glomerular degeneration in the lateral aspect of the remaining portions of the bulbs. Glomeruli on the medial aspect of the remaining bulbs were often left quite intact. Eight of the 13 brains exhibited at least some gliosis or loss of tissue in the accessory olfactory bulb. Gliosis was found in the anterior olfactory nuclei of only 2 brains. In 6 of the brains some frontal cortex gliosis was noted. Because frontal cortex ablation potentiates the locomotor response to amphetamine [18, 19, 23], we paid close attention to any frontal gliosis. This damage, however, was so slight that it was detectable only in a few stained sections and was limited to the outer lamellae of the cortex. There was no apparent correlation between frontal gliosis and any behavioral response to d-amphetamine. A section through the remaining portion of one of the most severely damaged bulbs taken at the level of the accessory olfactory bulb using the brain orientation of Pellegrino and Cushman [28] is shown in Fig. 2.

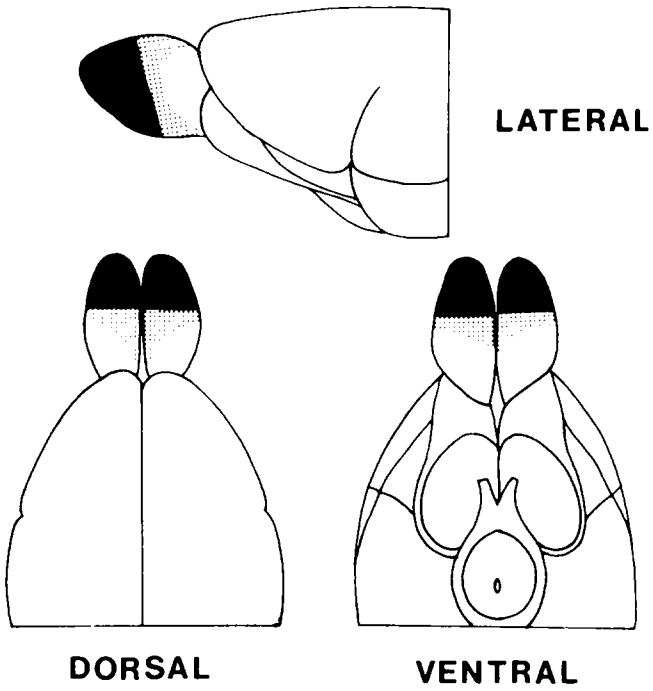

FIG. 1. Diagrammatic representation of olfactory bulb damage. Black area represents minimum damage; black plus shaded area represents maximal damage to brains fixed in 10% formol-saline.

Stereotypy

The bulbectomized group spent an average of 111.6 min in stereotypy while the sham-operated group spent an average of 131.6 minutes in stereotypy. These means were not statistically different ($t(22) = 0.43, p > 0.05$).

Locomotor Response

The dose-response curves for the locomotor responses of both groups after amphetamine administration are presented in Fig. 3. The non-monotonic nature of these dose-response curves was expected since, as noted in the introduction, stereotypy occurring at high doses of amphetamine is usually incompatible with locomotion. The control group's comparatively high locomotor score at 4.0 mg/kg is due to a single animal whose stereotypy pattern consisted of sitting on the axis of the tilt floor and successively shifting her weight from one side to the other. If this animal's score is omitted from the data, the mean value of locomotor activity for the control group is reduced to 174 crossings. An analysis of variance for repeated measures over all but the highest dose of amphetamine indicated that bulbectomized animals showed higher locomotor responses to amphetamine than did the control animals ($F(1,21) = 6.25$, $p < 0.01$) and that there was a significant interaction between olfactory bulb ablation and amphetamine dose ($F(3,63) = 3.63$, $p < 0.025$). Both groups exhibited significant dose-dependent increases in locomotor activity ($F(3,63) = 13.1$, $p < 0.01$). Comparison of first and last saline test scores revealed no significant differences between groups ($F(1,21) = 0.39$, $p > 0.05$) or test ($F(1,21) = 1.37$, $p > 0.05$), and no interaction effect ($F(1,21) = 1.26$, $p > 0.05$), providing evidence against cumulative drug effects.

Food Intake Suppression

As shown in Fig. 4, both groups of animals showed comparable amphetamine-induced suppressions of food intake in a dose-dependent fashion ($F(3,60) = 164.4$, $p < 0.001$). However, there was no difference between groups ($F(1,20) = 0.7$, $p > 0.05$) nor was there a significant interaction between olfactory bulb ablation and amphetamine dose ($F(3,60) = 0.1$, $p > 0.05$).

DISCUSSION

These experiments demonstrated that while bilateral olfactory bulb ablation in the rat enhanced amphetamine-induced locomotor activity, it did not reliably affect our particular measure of amphetamine-induced stereotypy or anorexia. These results are consistent with the idea proposed on the basis of other experiments (see Introduction) that one aspect of the bulbs' influence on behavior is inhibitory in nature.

Our finding that bulbectomy selectively altered the locomotor response to amphetamine is also in agreement with work showing that the effects of amphetamine on locomotor activity, stereotypy, and food intake are probab-

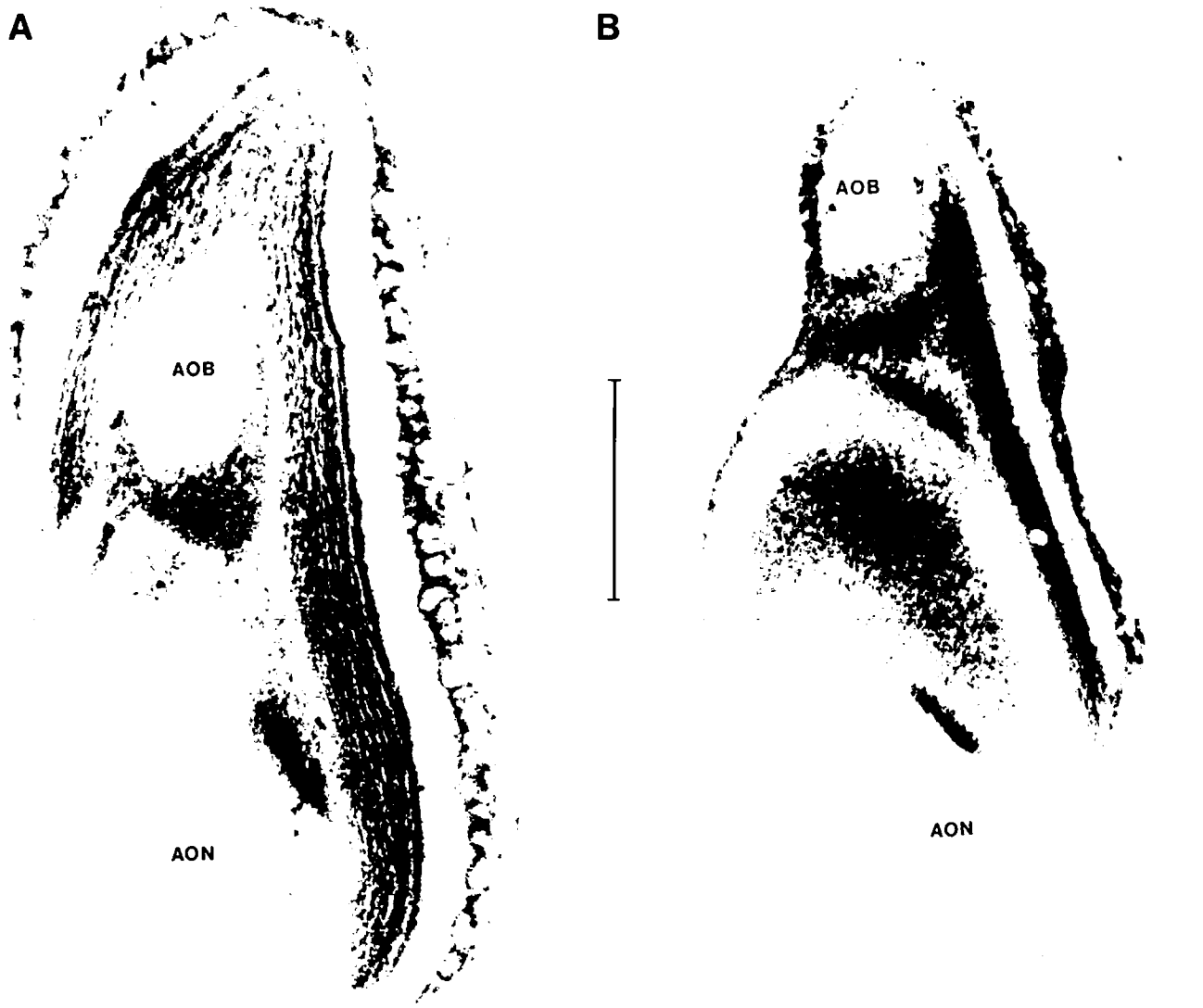

FIG. 2. A. Coronal section through normal olfactory bulb. B. Coronal section through olfactory bulb with severe tissue loss anterior to plane of section. 36X for both sections. Line = 1 mm. AOB, Accessory Olfactory Bulb; AON, Anterior Olfactory Nucleus. Section orientation that of Pellegrino and Cushman [28].

ly due to the drug's actions on functionally separate neural systems. Experimental results published to date indicate that amphetamine may increase locomotor activity by increasing brain dopaminergic transmission [22,31], perhaps in the mesolimbic dopamine system [29], but probably produces stereotypy by increasing dopaminergic transmission in the corpus striatum [4,26]. It is possible that our automated measure of stereotypy did not detect changes in some of the components of stereotypy brought about by the bulbectomy. Such selective changes, e.g., in sniffing but not gnawing, have been shown by Costall and Naylor [11]. Nonetheless, we could not detect any deficit in the cessation of locomotor activity which is usually attendant to severe stereotypy [34] produced by amphetamine. The anorexic effect of the drug appears due to an action on components of the ventral noradrenergic bundle [1].

Since the food intake series was always performed after the locomotor series, it is possible that the lack of differences between lesioned and control groups' food intake was due to recovery from lesion-induced hypersensitivity to amphetamine. Glick has noted such phenomena following frontal lesions [18,19]. We believe that this is an unlikely explanation for the different results. Such time-dependent differences are usually found comparing tests performed within a few days of lesioning with those

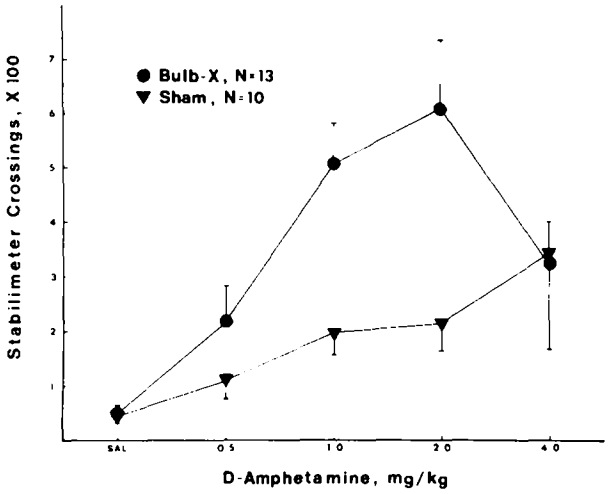

FIG. 3. Locomotor response in stabilimeter devices to different doses of d-amphetamine for rats with olfactory bulbectomy (bulb-x) or sham operations.

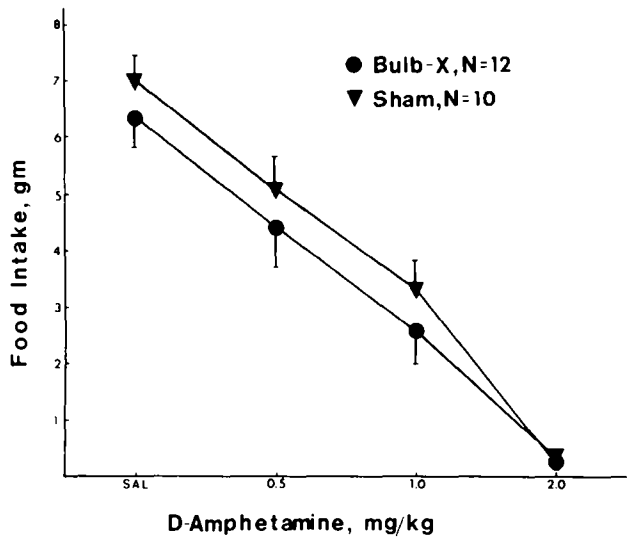

FIG. 4. Intake of dry food pellets in a 30 min test under different doses of d-amphetamine for rats with olfactory bulbectomy (bulb-x) or sham operations.

tests performed several weeks after lesioning [18]. We allowed a minimum of 3 weeks for recovery from surgery before testing was initiated. Thus, these tests are probably not as susceptible to the large differences seen in comparing immediate postlesion measurements with later measurements. In the initial performance of this experiment, locomotor activity tests were conducted approximately 6 weeks postsurgery. In the replication, locomotor tests were conducted approximately 3 weeks postsurgery. Regardless of the differences in recovery time, data from the 2 replications were indistinguishable.

Combining the results of the present experiments with the above, it is possible that the olfactory bulbs exert an inhibitory influence over neural activity in the mesolimbic dopaminergic system. This system has been speculatively linked with the olfactory system on the basis of comparative neuroanatomy and behavioral observations [12]. Although olfactory bulbectomy has been reported to lower forebrain levels of norepinephrine [7,30], this effect is only seen if the lesions invade the olfactory peduncle (D. Edwards, personal communication). Since our lesions did not encroach upon the peduncle, it is not likely that a change in forebrain norepinephrine levels was involved in the drug-lesion interaction.

Some of the effects of olfactory bulb damage on species-specific behaviors in the rat may be related to an alteration of the animals' responsiveness to arousing stimuli. For instance, bulbectomy has been shown to enhance sexual responsivity in the female rat [16]. Amphetamine, when injected into female rats bearing lesions of the anterior hypothalamus, reverses the deficit in sexual receptivity which follows the lesions [20]. It may be that the olfactory bulbs are functionally inhibitory over a catecholaminergic mechanism which is facilitatory on receptivity. Finally, the present findings with the rat may be species-specific, because bulbectomy increases general activity in mice but does not alter their response to amphetamine [5].

It must be noted that, as suggested some time ago by Herrick [21], the olfactory bulbs also appear to exert excitatory influences on arousal processes [3,6]. The separation of the excitatory and inhibitory actions of the olfactory bulbs on behavior is a task for future studies.

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