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BRIEF COMMUNICATION

Acute Anosmia in the Rat: A Behavioral Test of a Peripherally-induced Olfactory Deficit[1]

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ALBERTS, J. R. AND B. G. GALEF, Jr. *Acute anosmia in the rat: a behavioral test of a peripherally-induced olfactory deficit.* PHYSIOL. BEHAV. 6 (5) 619–621, 1971.—Hooded rats trained to use olfactory cues to locate scented food pellets buried in an open field were unable to do so following bathing of the olfactory mucosa with a 5% zinc sulphate solution. An identical intranasal injection of physiological saline had no effect on performance of the task. This peripherally-induced anosmia lasted from 2 to at least 14 days in different subjects. The advantages of the present technique over surgical ablation of the olfactory bulbs as a means of producing anosmia are discussed.

Anosmia Nasal mucosa Olfactory bulbs Zinc Sulphate

IN RECENT years psychological, physiological and ethological research has indicated that olfactory inputs can excite, inhibit, and direct many vital behaviors and organismic states in a variety of mammalian species [1–7, 11]. Olfactory stimuli are difficult to measure and manipulate directly and many studies concerned with the effects of olfactory stimuli on behavior have resorted to examination of the consequences of total deprivation of olfactory input, surgical ablation of the olfactory bulbs being the accepted technique for producing anosmia in laboratory animals.

Whitten [10] has emphasized the difficulty of interpreting data from bulbectomized animals, in that destructive intervention into the central nervous system may produce unwanted secondary effects in addition to the desired anosmia. It is always possible that effects observed following bulbectomy do not result from anosmia per se but rather from destruction of structures in the olfactory bulbs. It would clearly be desirable to produce anosmia by destruction of peripheral sensory structures so that the effects of anosmia on behavior could be evaluated more directly.

Smith [8] has reported that olfactory, sustentacular and basal cells in the sensory epithelium of the nasal cavity of the rat are destroyed when bathed briefly by a 1% solution of zinc sulphate in 0.5% saline solution. Damage was variable across the epithelium, ranging from complete degeneration to possible destruction of dendritic processes that later regenerated. Smith's report contains no behavioral test or suggestion of anosmia in his subjects. Clinical evidence [9] includes reports of chronic and acute anosmia in humans following use of nasal sprays containing 1% zinc sulphate.

The following procedure was developed to investigate the effects of zinc sulphate on the rat's olfactory sensitivity.

METHOD

Animals

Animals were 6 male hooded rats, 250–300 g, obtained from the Quebec Breeding Farms.

Procedure

Training and testing. Rats reduced to 75 per cent of ad lib body weight were trained to locate, dig up and eat 0.5–1.0 g pieces of Purina rat chow pellets each scented with a drop of oil of lavender. Testing was conducted in a 2 × 4 × 2 ft open field with 2 in. of shavings covering the floor of the enclosure.

Each rat was first placed in the open field where a number of pellets were scattered on the surface and buried under the shavings. After each animal was accustomed to finding and eating food hidden in the enclosure, individual pellets were randomly buried beneath the shavings and discrete trials were run with the rat returned to its home cage between trials. Each animal received 5 trials/day until its baseline latency reached a criterion of 5 consecutive trials averaging < 30 sec/trial. Latency was defined as the time required for a rat to locate, dig up, and grasp a pellet with either teeth or forepaws following placement in the field. The next day sham treatments were administered. Twenty-four hr following sham treatment rats were again run for 5 trials in the field with one

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scented pellet available per trial. Zinc sulphate treatment was administered the following day and testing was again resumed 24 hr later.

Following zinc sulphate treatment, each subject was given 2 discrete 5 min trials/day, until it succeeded in locating one pellet. After retrieval of the first pellet, 5 consecutive trials were again run daily, allowing a maximum of 5 min/trial for the rat to locate the scented pellet.

A sufficient quantity of unscented rat chow was given to each rat in its individual home cage to maintain 75 per cent body weight.

Treatment procedure. An etherized rat was placed on its back, its tongue held out and pulled to one side, and a hooked catheter adapted from a standard 3.5 in. 20 ga syringe needle inserted into its mouth. The needle had been blunted, heated until maleable, and bent 180° to form a hook measuring 3 × 7 mm (See Fig. 1). The bent tip of the catheter was run back along the hard palate, while held in the orientation shown in Fig. 1, until the rounded apex (a) was felt to enter the esophagus at the caudal end of the palate. The catheter was then retracted rostrally so that the tip (b) entered the nasal cavity via the posterior choanae located behind and above the palate. The solution (physiological saline or zinc sulphate) was injected via a 1 cm^3 syringe until 8 drops drained out the external nares; 0.5–0.8 cm^3 of solution was required for the treatment of each rat. The rat's mouth was intermittently aspirated to remove saliva and excess solution and following treatment, the rat was held head down until the animal recovered from anesthesia to facilitate drainage from the nares.

Five % zinc sulphate (wt/vol) was used for experimental treatment and physiological saline was injected in sham treatment to control for the effects of anesthesia followed by introduction of a salt solution into the nasal cavity. 7.65% zinc sulphate is isotonic with body fluids.

Histology. Four additional animals were treated with a 5% zinc sulphate solution and one week later perfused with isotonic saline and a solution of 10% formalin. The brains were removed and sectioned at 80μ. Sections were stained with cresyl violet and examined microscopically for evidence of damage as a result of experimental treatment.

RESULTS

The main results of the experiment are presented in Fig. 2. It is clear from examination of the figure that untreated rats can learn to locate buried pellets with considerable speed (Day PRE.). It is also evident that sham treatment (Day SAL.) does not disrupt performance. By contrast, treatment with zinc sulphate solution rendered all subjects tempora incapable of finding the scented pellets. Five of the 6 tre rats recovered olfactory function to the extent required this task within 3–10 days of treatment, while one ani remained anosmic for 14 days, at which time the experin was terminated.

All 6 animals rapidly devoured Purina pellets prese to them in their home cages following daily test trials an showed apparent searching activity (sniffing, exploration random digging) while in the test situation.

Histological examination revealed no damage to olfac bulb structures as a result of treatment of the olfactory mu with zinc sulphate solution.

DISCUSSION

The data strongly suggest that the effects of zinc sulp on the olfactory epithelium render rats temporarily anos The alternative explanation, that the failure of the rat find pellets was caused by motivational deficits follo treatment, is contradicted by the willingness of the rat eat pellets presented to them following treatment, and their apparent searching for food in the test situation.

The generality of the anosmia produced by the pre technique remains open to question. It is possible that sulphate treatment simply renders rats incapable of reac to the scent of oil of lavender. However, pilot experim have demonstrated that rats are capable of finding unsce Purina pellets buried in the test enclosure. Oil of lavender added to the pellets in the present study to increase avail olfactory cues. Furthermore, data to be reported elsew (Alberts and Galef, in preparation) indicates that Norway rats treated with a 10% zinc sulphate solution no longer distinguish familiar from unfamiliar intruders i their territory and no longer attack unfamiliar intruder do saline treated controls. Treatment with zinc sulp would, thus, appear to cause a general loss of olfactory sitivity.

It is not likely that animals who had regained their f finding ability following zinc sulphate treatment utilized o information, such as tactile cues, in food location. F animals recovered from zinc sulphate treatment did not up small wooden blocks, cut to the size of pellets that v also buried in the field.

Unfortunately, it is not clear whether the recovered anir in the present study had, in fact, recovered their full olfac capabilities or had merely regained a sufficient degre olfactory sensitivity to perform the relatively simple disc ination required here.

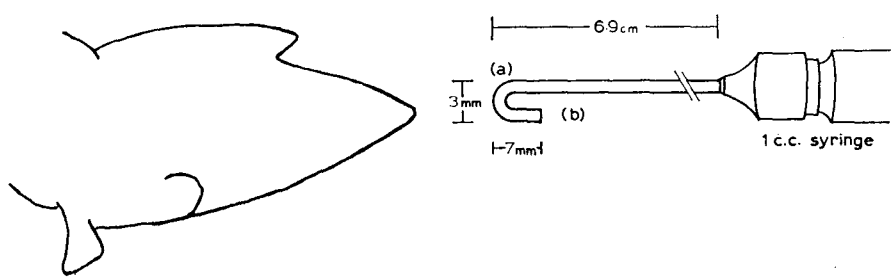

FIG. 1. Hooked catheter for treatment procedure showing rounded apex of hook (a) and tip (b) that enters the posterior nasal cavity via the choanae.

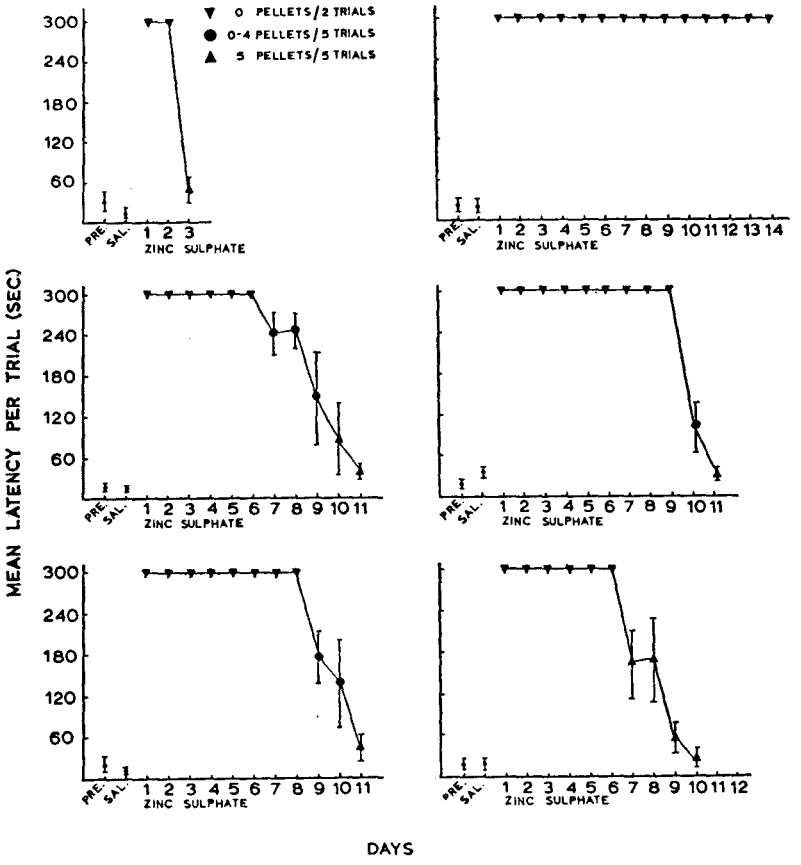

FIG. 2. Individual mean latencies per trial and the standard error of these means. (PRE.) pre-treatment baseline latency; (SAL.) mean latency 24 hr after saline sham treatment; Day (1, 2,...*n*) daily performance beginning 24 hr after treatment with a 5% zinc sulphate solution.

The present technique would appear to have a number of advantages over surgical ablation of the olfactory bulbs as a means of producing anosmia. First, it is possible to differentiate the effects of anosmia from those of removal of portions of the CNS and from the effects of disruption of intrinsic olfactory bulb activity. Second, the effect is at least partially reversible permitting subsequent study not possible after bulbectomy.

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