ANALYSIS OF SNIFFING OF THE ALBINO RAT [1])

by

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(With 9 Figures)
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INTRODUCTION

The exploring albino rat exhibits a cluster of movement sequences usually referred to as "sniffing". The snout is probed along and around surfaces by a series of discrete head movements. The tip of the snout and the mystacial vibrissae are also in brisk motion during such excursions and all of these acts are accompanied by rapid respiratory movements. These sequential actions are prominent characteristics of rat exploratory behavior and their spatiotemporal organization suggests that the animal is sampling olfactory and tactile stimuli.

It was the purpose of the experiments reported below to describe and analyze some of the details of these action sequences; to identify the time of their first appearance, and to trace their subsequent ontogenetic development; to test certain hypotheses regarding their functional significance; and to determine some of the neural circuits involved in their operation.

MATERIALS AND METHODS

Subjects.

Sprague-Dawley albino rats of both sexes and several ages were used as subjects.

PROCEDURE

The subjects were divided into five experimental groups in order to examine several aspects of sniffing behavior.

Group I. Effects of olfactory bulb ablations. Eighteen female rats were obtained at 21 days of age. In nine of these (Subgroup A) the olfactory bulbs were aspirated bilaterally between 22-25 days of age. At the same age, the nine control animals (Subgroup B) were subjected to sham operations consisting of opening and closing of the scalp. The naso-

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2) Kenny Foundation Scholar, 1957-1961.

ciliary nerves, which are known to be activated by certain "olfactory" stimuli, were not sectioned in the operated animals of this group. All animals were housed in individual cages and were tested between 120-180 days postoperatively. Tests consisted of: (a) movie records of sniffing actions, (b) measures of latency of response (Latency), contact frequency (Touch), and frequency of occurrence of sniffing (Sniff) of two standard test objects (xylol-soaked cotton ball, or dry cotton ball), and (c) measures of duration of sniffing contacts with a cement block smeared with various edible substances.

In the cotton ball tests, each rat was given one trial per day with both xylol-soaked and dry cotton balls. The dry ball tests were separated from the xylol ball tests by about six hours, the temporal order of testing with these two stimuli being altered from one test day to the next. In each test, the cage door was opened and the cotton ball was placed on the floor just inside the cage door. At the moment that the cotton ball was presented, a timing clock was started by a silent push-button switch. The latency measure consisted of the time (in hundredths of a minute) elapsed from initial presentation of the cotton ball to the first oriented sniffing response directed at the stimulus. Each animal's average response latency was computed for the five tests with both xylol and dry cotton balls. A sniff score was obtained by tallying the frequency of occurrence of oriented sniffing responses toward the object by each animal on all five trials for each type of stimulus. A positive score was counted if the animal pointed its snout and sniffed at the stimulus from a distance of 1-2 inches before it either turned away or advanced and made physical contact. A touch response was tallied if a physical contact of tip of snout and/or vibrissae was made with the cotton ball.

Measures of duration of sniffing contact were made in a separate series of tests. All animals were given one test in their home cage during the evening on each of five successive days. The test object consisted of a small one-inch cube concrete block smeared with various non-aversive edible substances. For all animals the block was smeared with fresh pear on the first day and with peach, rat faeces, vegetable oil, and fig pulp on the subsequent four days respectively. The timing clock was started when the animal made its first physical contact by snout and vibrissae with the block. The clock was stopped when the animal either turned away, stopped sniffing, or grasped, bit, or started licking the block. The experimenter observed these actions from a distance of 1-1½ feet from the front of the cage.

A thermal masking noise was present 24 hours a day in the room in which Group I animals were caged and tested. This prevented the animals from

being distracted by slight noises made by the experimenter during his observations of the animals in their cages.

Group II. A. Effects of peripheral nerve section. The twelve animals in this group were divided into four subgroups in an attempt to determine the role of visual, auditory, and somatic-snout afferents in the initiation and patterning of sniffing behaviors. In all groups the olfactory bulbs were aspirated, and the nasociliary nerves were sectioned, bilaterally. Additional bilateral interference with sensory input was effected as follows: (a) Subgroup A. Somatic sensory sparing. This group consisted of three adult male animals. Somatic-snout (maxillary) afferents were left intact, but tympanic membranes were punctured and optic nerves were severed. (b) Subgroup B. Auditory sparing. This group consisted of six adult male animals. Ear drums were not damaged, but somatic-snout afferents and optic nerves were sectioned. At autopsy snout afferents were found to have regenerated. (c) Subgroup C. Visual sparing. This group consisted of six adult male animals. Optic nerves were left intact, but tympanic membranes were punctured and somatic-snout afferents were severed. At autopsy the snout afferents had regenerated. (d) Subgroup D. This group consisted of the three animals of Subgroup A in which the optic nerves and ear drums had been destroyed six months earlier. Since the severed somatic-snout afferents in the animals of Subgroups B and C had regenerated when film records of sniffing movements were taken, the three animals in this subgroup were tested within two days after section of the snout afferent nerves. In every case, behavioral tests and subsequent autopsy confirmed the adequacy of the nerve section.

All animals in Group II were housed in individual cages and, except for Subgroup D, were tested from one to four months postoperatively. The tests consisted of (a) movie records of the four sniffing actions, (b) observational records of inhibition of sniffing by xylol-soaked cotton balls, and (c) observational records of arousal to sniffing by visual, somatic sensory, or auditory stimuli.

B. Effects of excitant and depressant drugs. Three weeks after the first film records of sniffing rates were taken, the sniffing reactions of the twelve rats in Subgroups B and C were again photographed following intraperitoneal injection of either a neural excitant (Ritalin: methylphenidate hydrochloride; 5 mg./kg.) or depressant (Nembutal: sodium pentobarbital; 32 mg./kg.). The dosage of nembutal was sufficient in each case to inhibit locomotion, but insufficient to cause complete cessation of

sniffing movements. Movies of sniffing behavior were taken between 25-37 minutes after injection of these drugs. Three animals from each of the two subgroups were assigned to the Nembutal group, and the other three from each subgroup were assigned to the Ritalin group.

Group III. Effects of forebrain ablations. Four adult female rats (Subgroup A) were subjected to bilateral aspiration of olfactory bulbs and all cerebral neocortex. Subgroup B consisted of four adult female rats with bilateral ablations of the olfactory bulbs, anterior olfactory nuclei, and anterior portions of the tuberculum olfactorium. Two to nine months postoperatively, the sniffing actions of these animals were photographed.

Groups IV and V. Ontogenetic development. These groups consisted of the infant rats of two litters (N = 11 each). Group V was intended as a replication of Group IV. Movie records were taken of sniffing actions of Group IV infants daily for the first six postnatal days, and on alternate days thereafter until the 20th postnatal day. In addition, daily observational notes were taken of various movement complexes and reactions to stimuli. The observational records were repeated during the first 18 days postnatal for Group V infants, but movie records of the sniffing sequences of this group were taken only on the first six postnatal days. The items included in the developmental schedule (Fig. 9) were chosen on the basis of daily pilot observations of a third litter of rats. The preliminary data obtained from this litter were not included in the results reported below.

On each observation day, the entire litter was removed from the mother and placed one at a time upon a pillow. Observations were made of sensory status (eye, or ear canal opening), responses to mechanical, auditory, visual, and olfactory stimuli, resting respiration rate, time required (in seconds) to become quiescent after handling, tendency to "freeze" when placed on a strange surface, presence or absence of various locomotor and postural acts (head lifting and turning, forward body stretching, rearing, righting when dropped from an inverted position 10" above the pillow), locomotor stability or absence of body tremor during standing and walking, and time of onset of gnawing or biting of solids.

In order to photograph sniffing rates, each infant was placed within the field of the camera. Sniffing was induced in the youngest rats by strong mechanical (body pressure) or olfactory (perfume) stimulation. Older rats sniffed readily without such stimulation. The test for patency of the ear canals consisted of a sudden burst of high frequency hissing noise produced

by a rapid intake of air through the experimenter's pursed lips. Such a stimulus produced a pronounced total body startle in those rats in which the external auditory meatus appeared completely open. The observational periods lasted from 15-30 minutes for each litter and the infants were then returned to their mother one at a time in order to observe the adequacy of her retrieving.

CINEMATOGRAPHIC RECORDING PROCEDURES

The actions which comprise sniffing were recorded on Tri-X negative 16 mm. motion picture film. While recording behavior of Group I subjects the film was driven at either 32 or 64 frames per second by the camera's internal spring drive assembly. Slight speed variations were found to exist under these conditions when the film speed was callibrated by photographing a stop watch. Consequently, the data from Group I subjects are probably subject to some degree of error due to such variations. The behavior of the subjects in all other groups was recorded at 30 fps, the camera being driven by a synchronous motor drive assembly. Illumination required for photography consisted of four 75W or 100W bulbs placed one meter above and to the front of the open door of the animal's cage. All adult animals were photographed or otherwise tested in their home cages. During photography, the cage containing the rat was placed one meter from the battery of lights and the cage door removed. Typically, the animal came forward and explored the front edges of the cage or the objects placed just inside the door on the floor. These actions were photographed through a glass window from an adjoining room. Infant rats were placed on a towel spread upon the floor and the movie camera was mounted about one meter above them on a tripod.

Analysis of filmed data

The film records were analyzed by means of a single-frame time-motion analyzing projector. The film analysis provided data regarding the rate and temporal patterning of four prominent action sequences: (a) protraction and retraction of the mystacial vibrissae, (b) protraction and retraction of the nose or tip of the snout, (c) head approach and withdrawal (or extension and retraction), and (d) rapid expiration and inspiration (polypnea), as indicated by movements of the thoracic and abdominal walls.

SURGICAL PROCEDURES

Bilateral ablations of olfactory bulbs, anterior olfactory nuclei, tuberculum olfactorium, and cerebral neocortex were performed with the aspiration method under sterile operating conditions. In appropriate experimental groups

the optic, maxillary, and nasociliary nerves were sectioned bilaterally, also under sterile conditions. Auditory input was impaired by destroying the ear drums and, in one case, by destroying both cochlea with a dental scraper.

Histological analyses.

After the behavioral data had been obtained for each operated animal its brain was perfused with 10% formalin, removed from the skull, embedded, sectioned, stained with cell body and fiber stains, and mounted for subsequent microscopic determination of the extent of the lesions. Upon reconstruction of the ablations, it was typically found that the lesions were more extensive than had been intended. The approximate extent of the lesions within each of several major neural regions is indicated for each hemisphere of each animal in Fig. 7.

Statistical analyses.

The statistical significance of differences was determined in all cases by means of the test for unpaired replicates (WILCOXON, 1949). The two-tailed test was used in every case.

RESULTS

CHARACTERISTICS OF SNIFFING BEHAVIOR

Observation and cinematographic analysis of sniffing in normal albino rats (Subgroup IB) indicates that mildly novel visual, auditory, tactile, or olfactory stimuli are capable of arousing the animal to exhibit a characteristic pattern of behavior. This sniffing pattern consists of repetitive cycles of four major behavioral sequences: (a) polypnea, (b) protraction and retraction of mystacial vibrissae, (c) head movements and fixations and, (d) protraction and retraction of the tip of the nose. Each of these actions occurred at rates of between 5-11/sec. in bursts of varying duration of from less than one, to as long as ten, seconds. Polypnea and nose movements together may occur independently of vibrissae and head movements, but rapid vibrissae or head movements were never observed to occur independently of polypnea and nose movements. Successive head movements are most distinct and easily observed when the rat is sniffing an object from a stationary position. When the animal is actively moving about, however, successive individual head movements are often less clearly perceptible. The vibrissae on both sides of the head move together and in similar fashion unless those on one side are blocked by a solid surface. Fig. 1 (Plate XXIII) shows an albino rat as viewed from the position of an object being sniffed, and indicates several anatomical details of snout and vibrissae.

When they occur together, the polypnea, vibrissae, nose, and head move-

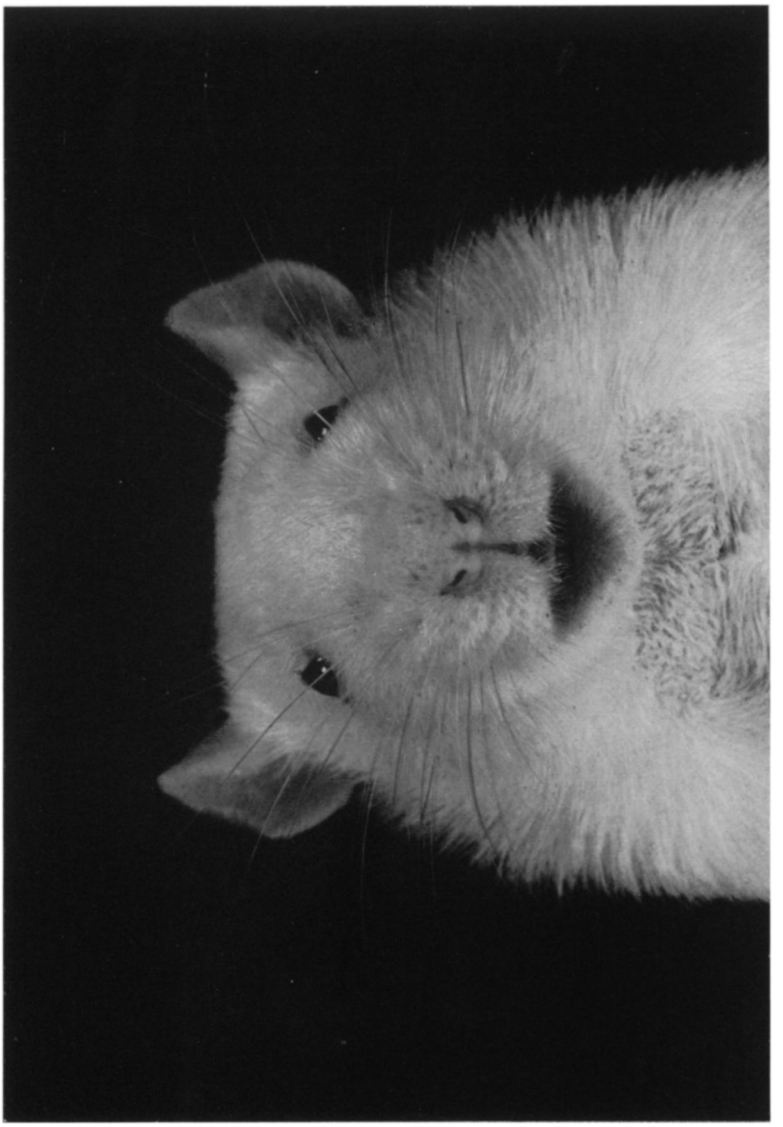

Fig. 1. Photograph of head of albino rat, rostral view.

ments take place at the same rate. Moreover, they exhibit a fixed temporal relationship to one another. Analysis of movie records in instances when two or more of the four act sequences were easily seen on the same series of frames has disclosed further details of these integrated movements. Beginning the analysis at a point in the sniffing cycle when the nose has just made contact with a surface, the film records reveal that the vibrissae are at maximal protraction, and are therefore also in contact with the surface. At this point in time the tip of the nose has fully retracted, and a point of maximal inhalation has been reached, thereby marking the termination of the approach phase of the cycle. The withdrawal phase now quickly follows. Thus, the head withdraws from its fixation point, the vibrissae retract, the tip of the snout protracts, or relaxes to its resting position, and the animal exhales. Upon conclusion of the withdrawal phase, the approach phase begins anew. The nose is again placed at a fixation point, usually spatially separated from the preceding one, the vibrissae sweep forward, the nose again retracts, and the animal inhales. This action complex is repeated over and over as the

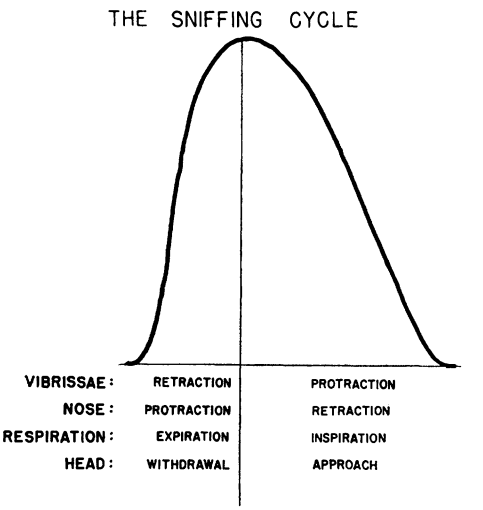

Fig. 2. Schematic representation of temporal organization of four sniffing actions during one complete cycle.

rat continues to explore the environment. It was not possible to determine from the film records whether there was a particular position for each of the component movements from which the first cycle of a sniffing burst began. During locomotion, the nose and vibrissae do not always make contact with objects or surfaces, and in such instances the cyclic movements are executed in mid air. The temporal coincidence of the four actions involved in sniffing

behavior is diagrammatically depicted in Fig. 2. This diagram is drawn to illustrate one complete sniffing cycle and to indicate that the withdrawal phase is more rapid (about twice at fast) than the approach phase.

The observed spatiotemporal patterning of these behaviors appears to be well suited for the most efficient and probable reception of sensory information from olfactory and somatic-snout receptors. Thus, the simultaneous head fixation and vibrissae protraction upon the surface of an object places somatic snout and vibrissae receptors into direct contact with surface stimuli. Concurrently, both nose retraction (which exposes flaring nostrils closely to the surface) and inhalation make olfactory stimulation more probable.

The results described in this section were found in each normal animal as well as in certain of the experimental rats, as described in subsequent sections.

EFFECTS OF OLFACTORY BULB ABLATIONS (GROUP I)

On the basis of the results summarized in the previous section, it would be reasonable to assume that olfaction is one primary function of sniffing behavior. It might be expected, therefore, that in rats deprived of olfactory bulbs, such sniffing acts would either disappear or be altered in some way. Analysis of the film records show, however, that there is no difference ($P \cong .50$) between the operated and normal animals of Group I in either average rate of sniffing (Fig. 3), or in the nicely timed integration of the four component movements. Each dot in Fig. 3 is an average score of several (2-6) rate measures for one animal. Repeated observations of the precise temporal integration of the four movements on successive occasions in any one animal (normal or operated) has revealed the stability of these phase relationships despite variations in rate. It is important to note that these sniffing actions appeared to be normal 100-160 days following olfactory bulb ablation.

Both inter- and intra-animal variations in movement rate were common (Figs 3 and 6). Careful examination of some of the film records disclosed occasional short abrupt pauses irregularly scattered within a particular sniffing sequence. It seems likely that variations in frequency of these pauses may account for some of the variability in the rates that were observed (see Discussion).

Fig. 4 depicts certain reactions of the normal and anosmic rats of Group I to xylol-soaked and dry cotton balls. Each animal was presented with each stimulus object on five separate occasions. Group scores for each measure (sniff, touch, latency) were obtained by averaging the mean scores of each

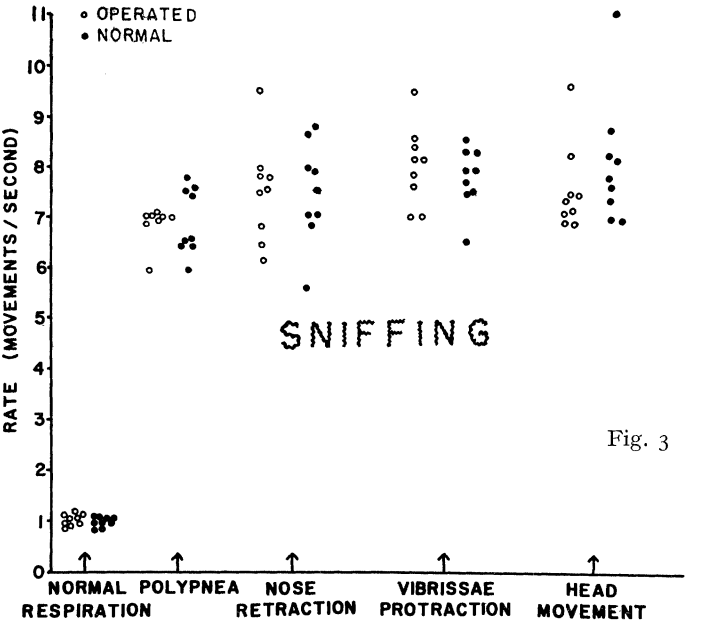

Fig. 3

Fig. 3. Movement rates of four sniffing actions of normal and anosmic rats (Subgroups 1A and 1B).

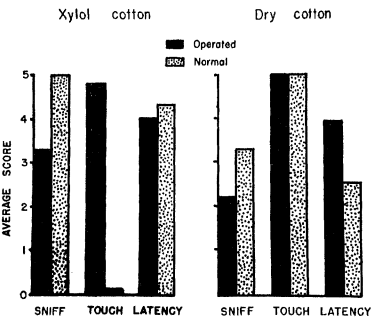

Fig. 4

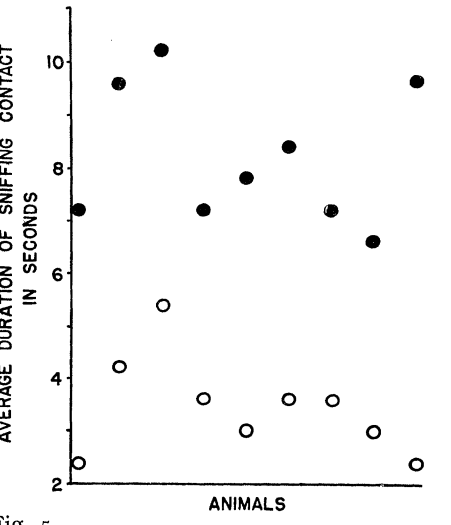

Fig. 5

Fig. 4. Sniffing (Sniff), contact (Touch), and contact latency (Latency) behavior of normal and anosmic rats (Subgroups 1A and 1B) to xylol-soaked and dry cotton balls.
Fig. 5. Average duration of sniffing contacts by normal and anosmic rats (Subgroups 1A and 1B) with edible substances.

animal of a group. All normal animals approached the xylol object hesitantly each time it was presented. This was accomplished by an outstretched posture, and they sniffed the pellet from a distance of an inch or two. All operated animals, on the other hand, approached the xylol object quickly and directly, making physical contact with it by both nose and vibrissae. Several operated animals carried the xylol cotton in their mouths to the rear of the cage. Xylol thus appears to be an aversive stimulus to the normal rats. The normal rats made significantly fewer contacts (T o u c h) with the xylol pellet than

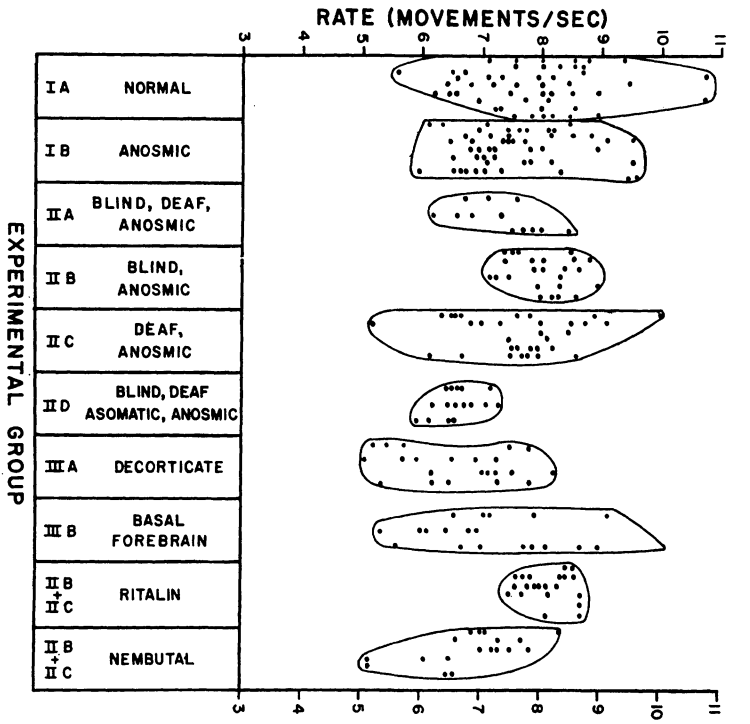

Fig. 6. Sniffing rates of individual animals in control and experimental groups. Each dot represents a rate of one or another of the four movements. The several scores recorded for each animal are arranged in horizontal rows.

did the operated animals ($P<.01$). Moreover, normal rats made fewer contacts with the xylol pellet than with the dry pellet ($P<.01$). All anosmic rats showed no aversion to the xylol pellets. Although the average frequency of occurrence of sniffing approaches (S n i f f) was greater for normal rats to both types of stimuli (Fig. 4), average S n i f f scores of normals were significantly greater only to the xylol pellet ($P<.01$). Fig. 4 also shows

that the latency to onset of sniffing after object presentation was not significantly different for the two types of stimuli between groups or between objects type ($P \cong .50$).

In order to observe sniffing reactions to a more preferred stimulus, a small concrete block was smeared with various edible substances and placed at the front of each rat's cage for a two minute period on each of five successive days. The duration of sniffing contact with the block was measured by a stop watch. Fig. 5 shows that, on the average, all operated animals spent less time sniffing the objects before turning to other stimuli than did any of the normal rats ($P<.01$). None of the animals in either group were hesitant to approach and contact the object.

All these data indicate that loss of input to the olfactory bulbs did not alter the rate, or disrupt the cyclical timing, of short-term sniffing actions. However, such loss does influence the t y p e of olfactory stimulus approached or avoided as well as the d u r a t i o n of such contacts. Olfactory input thus influences the character of the more prolonged exploratory sniffing sequences.

EFFECTS OF PERIPHERAL NERVE SECTION AND CNS ABLATION

Fig. 6 summarizes the sniffing rate data obtained from animals of all groups except IV and V. Each dot in this Figure represents one observation of a movement rate. Several rate measures were obtained for each animal and are plotted in vertical columns. It was not practicable to identify the type of movement represented by every dot in this figure. Consequently, all four movement types are plotted in Fig. 6.

It is clear from this figure that the rate scores for each group of animals fell within the range of sniffing rates indicated for the normal control group. Comparison of the dot arrays in horizontal rows in this figure discloses the fact that there is considerable inter- and intra-animal variability in movement rates even within a particular group. The number of animals in groups II A, D and III B were not sufficiently large to permit use of the Wilcoxon test for unpaired replicates. Nevertheless, the sniffing rates of animals in these groups fall within the range of normal rates (Fig. 6).

Rats deprived of somatic snout afferents, although exhibiting brisk vibrissae movements, were unable to locate food pellets efficiently. They made exaggerated ventrally-directed head movements and appeared to locate the pellets by contact with forepaws or the ventral aspect of the lower jaw. The sniffing rates of these animals were grouped at the lower end of the rate range exhibited by normal animals. In anosmic rats that were also deprived of cerebral neocortex bilaterally, clear discrete sniffing movements were

seldom observed. When they were seen on the film records, movements rates were below or within the lower range of that of normals. Moreover, the duration of the sniffing bursts in these operated rats was quite brief, and the component movements of the sniffing cycle were of reduced amplitude. It was not possible, therefore, to observe the temporal organization of the four component sniffing actions in these animals.

Film speeds were not fast enough to determine whether the slightly slower rates of somatic deafferented (II D), decorticate (III A), or narcotized (II B + C) animals were due to a greater number of pauses during a sniffing sequence or to slower movement during each phase of the sniffing cycle.

When the sniffing of the blinded animals of Subgroups II A and B had been observed, it became apparent that illumination required for photography was sufficiently strong to inhibit the duration of sniffing bursts in normal rats. Thus, visually intact rats were often hesitant about coming to the illuminated fronts of their cages. They came to the front of the cage infrequently and when there, sniffed about the door edges for only relatively short periods. Blinded rats, on the other hand, sniffed almost continuously and without hesitancy at the open doors to their cages. These data confirm the results of previous studies regarding the aversive and inhibiting effects of relatively strong levels of illumination upon exploratory behavior (WELKER, 1959).

An attempt was made in Group II animals to selectively eliminate visual, auditory, and somatic snout afferent activity in order to assess the roles of stimulation within a single modality upon the initiation of sniffing activity. Sleeping or resting rats in the appropriate groups were stimulated by either a light flash, hissing noise, or mechanical contact with body hairs and vibrissae. It was found that any of these stimuli were adequate to arouse a rat to a burst of sniffing actions. None of these rats was aroused to sniff when a xylol-soaked cotton ball was held close to the snout.

EFFECTS OF DRUG ADMINISTRATION

Statistical comparison of average sniffing rates was made between the animals in groups II B and II C under both drugged and nondrugged conditions. The difference in average rates between these two groups did not approach the .10 level of significance under the nondrugged condition, but was significant at less than the .01 level for the drugged condition. When compared with six randomly chosen normal animals, the Ritalin (excited) group showed a slightly higher average sniffing rate ($.10 > P > .05$), and the Nembutal (depressed) group a slightly lower rate ($P = .10$). Neverthe-

less, as Fig. 6 indicates, individual sniffing rates in these two groups fell within the range of rates exhibited by normal animals.

ANALYSIS OF EXTENT OF LESIONS

The proportional length of each solid bar in Fig. 7 indicates the estimated proportion of neural tissue that was removed from a particular neural group. The approximate extent of the lesions was determined for each hemisphere of each experimental animal by microscopic examination of stained sections. In almost all cases more neural tissue was removed than was intended. Thus, portions of frontal neocortex, basal ganglia, pyriform cortex, and even thalamus were lost in varying degrees in the several cases. None of the operated animals showed statistically significant differences in rate or pat-

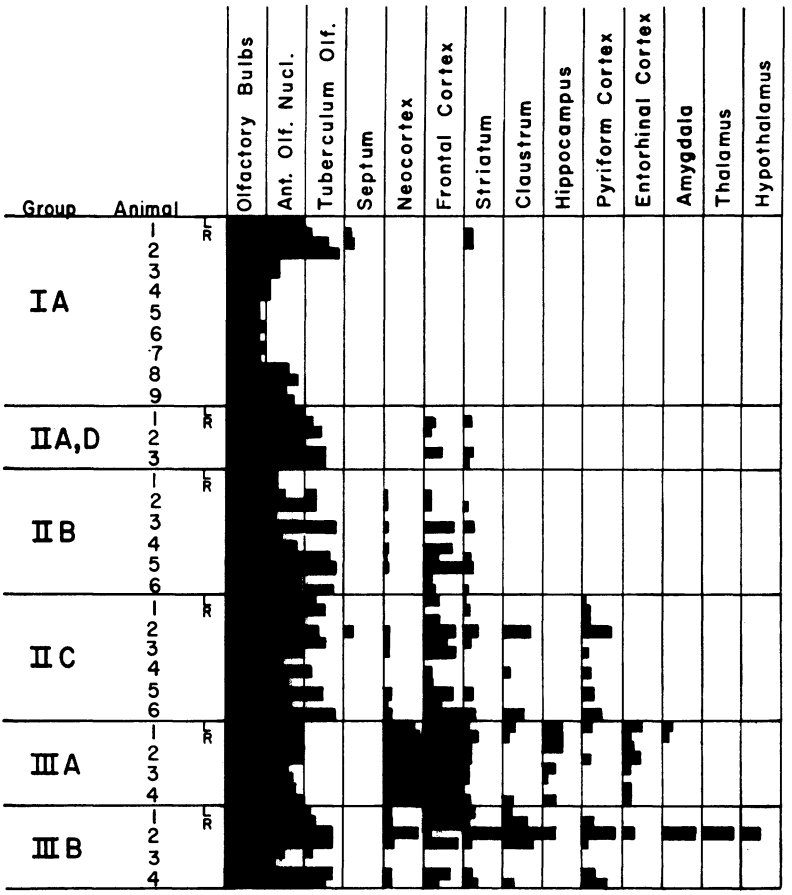

Fig. 7. Relative extent of lesions in various nuclear groups of right (R) and left (L) hemispheres in animals of several experimental groups.

terning of the sniffing actions from that of normal animals. Moreover, there were no significant rate differences between animals with the most and least extensive ablations.

ONTOGENETIC DEVELOPMENT OF SNIFFING BEHAVIORS

The sniffing reactions of the infant rats of Groups IV and V were measured during the first two and one half postnatal weeks. These animals were induced to sniff for the camera by the general body stimulation associated with handling, or by placing a perfume-soaked cotton pellet near their nostrils. During the first five days after birth, polypnea was the only sniffing movement clearly seen, recorded, and measured. Later, when

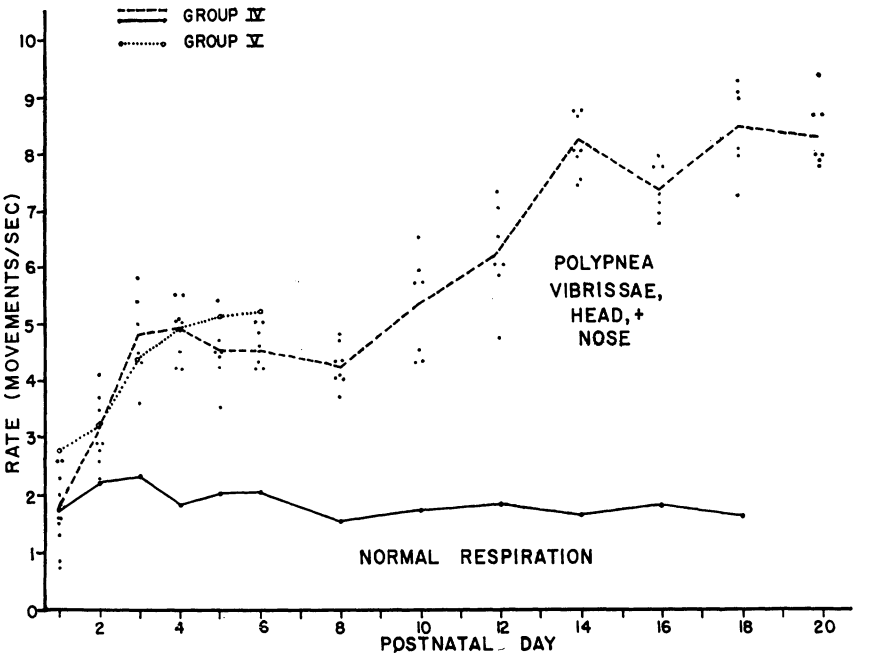

Fig. 8. Ontogenetic development of sniffing rates in infant rats of Groups IV and V.

Vibrissae, nose, and head movements became visibly prominent, they were also measured. Fig. 8 shows that polypnea frequencies were, on the average, only slightly higher than the normal resting respiration rates on the day of birth. There was a rapid increase in sniffing rate, however, during the next two days, reaching and holding a plateau for several days thereafter, and then gradually increasing again from the tenth to the eighteenth postnatal

day. By the 18th day, the average sniffing rate of all the young rats was within the normal adult range.

Each dot around the dashed line in Fig. 8 represents a sniffing rate observation for one animal in Group IV. Since at any given postnatal age it was found that all sniffing movements occurred at the same rate, only one or two movement types are represented in each animal's score in Fig. 8. Since at one time or another each of the four movements was measured, the data in this figure consist of composite scores of all four sniffing movements. The dashed line in Fig. 8 is drawn through the average sniffing rate of all Group IV animals on each day represented. The average normal resting respiration rate for these same animals is indicated by the solid line and shows little change in rate during the first three postnatal weeks. The dotted line in this figure indicates the average rate of polypnea for the eleven animals of group V on the first six postnatal days. As was found for Group IV animals, there was an initial rise, followed by a leveling off, in the rate.

The duration of sniffing bursts was very brief in newborn rats, consisting of only a few cycles. Longer bursts were clearly apparent by the fourth postnatal day, and longer still when active exploratory "searching" became pronounced between the 8th and 10th postnatal days. Individual variations in the amplitude of vibrissae movements were apparent among the several animals of each litter. Although all four movement types were not recorded on film for each animal on any particular postnatal day, rate data were obtained for each of these movements for each animal over the period of the study.

It is of interest to view the ontogenetic development of the sniffing movements within the context of other items in the expanding behavioral repertory. The data pertaining to several developmental items are illustrated schematically in Fig. 9 which indicates the times at which particular items first appeared and reached full expression. Although the developmental schedule illustrated in this figure was prepared primarily on the basis of data obtained from animals of Group V, the data obtained from Group IV animals showed similar trends with respect to all items. There were individual variations of course, both within and between litters, in the postnatal day on which particular items (*e.g.* eye, or ear canal opening) occurred. These variations were restricted to a range of about 1½ days.

Of the four sniffing movements, polypnea alone was clearly evident on the day of birth. The tiny vibrissae began to move synchronously with polypnea on the fourth day, but then primarily in the retraction phase. Distinct vibrissae protraction not only appeared later than retraction (day 7),

but also did not achieve its maximum amplitude until a day or so after that of retraction movements. Repetitive movements of the nose and nostrils during sniffing began to appear clearly on the seventh day, and discrete head movements associated with sniffing were first clearly visible on the eighth postnatal day. Analysis of the movie records disclosed that although these sniffing movements matured at different times in ontogeny, when each did appear, it developed at the same rate as those actions already present, and in the same temporal sequence as that described above for the adult.

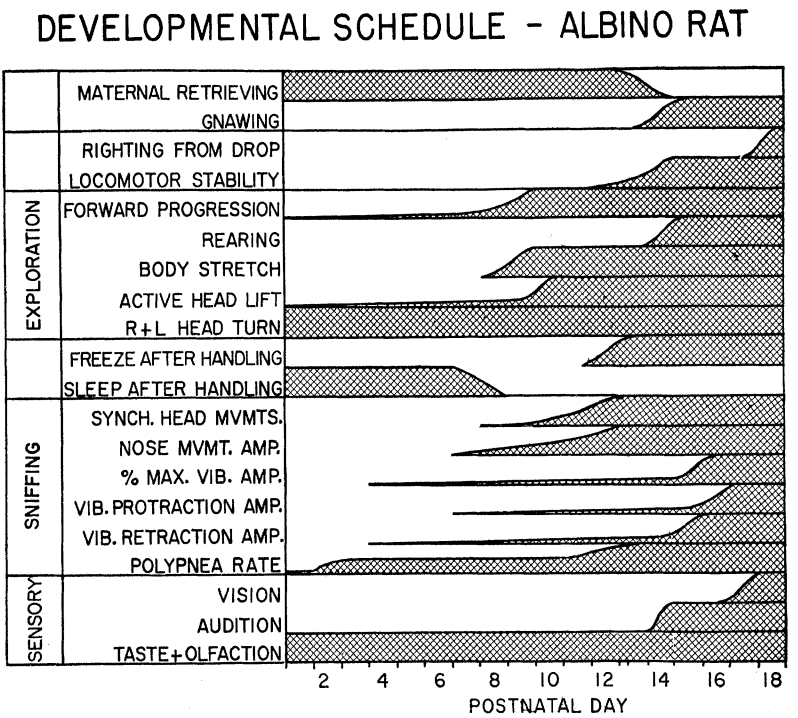

Fig. 9. Ontogenetic development of several items in behavioral repertory of infant rats in Groups IV and V.

Thus, by the eighth day after birth, the four sniffing movements were clearly present although they had not reached their maximal amplitude or prominence. As a group, these actions next became associated with the three postural components of exploratory behavior which became prominent between the eigth and tenth postnatal days. These actions: forward progression, active head lifting and turning, and the forward body stretch, are all prominent characteristics of adult rat exploratory behavior and appear to direct the sniffing actions toward specific portions of the environment as

well as to bring them into contact with a greater range and variety of sensory stimuli. During the forward extension phase of the body stretch sequence, the vibrissae appeared to exhibit an enhanced protraction-retraction amplitude, whereas the amplitude of these vibrissae actions became minimal during the withdrawal from the extended body position. Between the eighth and tenth postnatal days, these exploratory locomotor-postural actions become increasingly prevalent. All rats of both litters exhibited this spurt in behavioral development at about this time. This rapid development of the behavioral repertory of exploration occurred at about the same age as it did in the albino rat infants studied by SMALL (1899) and TILNEY (1933). Earlier than the eighth day the infants typically became quiescent shortly after being removed from the nest and placed alone on the observation pillow. After the eighth day, however, the time to quiescence after handling rapidly increased, and the subsequent three day period marked the rather sudden maturation of the rat's characteristic full pattern of activation and persistent exploratory responses to novel external stimuli. These developments all took place before eyelids had separated or ear canals had opened.

The data represented in Fig. 9 indicate that the 18day old albino rat has a fully developed exploratory and sniffing repertory. It becomes immobile ("freezes") when disturbed by strong stimulation. Its eyes and ears are open. It has well developed righting reactions as well as adequate postural and locomotor stability. It has begun to gnaw and ingest solid foods. At about this time, and possibly induced in part by the persistent and impulsive exploratory activities of her young, the mother begins to sleep apart from them. During the third and fourth postnatal weeks, she retrieves her pups with lessened vigor, and, unless suckling them, attempts to escape from their persistent climbing, nibbling, and sniffing contacts with her body.

DISCUSSION

The four sniffing movements examined in this study compose an integrated behavioral assembly which appears to promote efficient and organized olfactory and somatic sensory perception. Indeed, an animal, in which olfactory bulbs and afferent vibrissae nerves have been destroyed, has difficulty in finding food and does not quickly or efficiently discriminate between noxious and innocuous substances until they are actually held in the mouth. Despite these sensory defects, the motor expression of sniffing actions was not significantly altered in such animals when compared to normal control rats.

The experiments reported above show that either auditory, visual, olfactory, or somatic sensory as well as olfactory, stimuli may induce a rat to sniff as

long as they are of moderate degree of intensity or novelty. Strong stimulation in any of these modalities tends to inhibit sniffing, and usually results in locomotor immobilization (*Cf.* BINDRA & SPINNER, 1958). Although sensory stimulation may initiate, terminate, or regulate the duration of sniffing actions, the temporal organization of the component movements is unaffected by various patterns of afferent input, or feedback, from the major sensory receptors. For example, the data have shown that, in any individual rat, the rate as well as the temporal patterning of the sniffing movement were similar regardless of whether the vibrissae made physical contact with surfaces or only swept freely in mid air. Moreover, it was noted that head movement rate was unaltered by variable patterns of afferent feedback from vestibular and somatic sensory neck afferents which would necessarily accompany repetitive head movements of varying degrees of acceleration, speed, and direction. Thus, forward head thrusts, head swings to the left, right, up, or down, and head movements of short or long linear extent, were all inserted, in variable order, into a sniffing sequence of c o n s t a n t rate and temporal pattern.

Although interruption of somatic snout afferents, decortication, and partial anesthesia did result in slightly slower average sniffing rates, it was not clear in these cases whether the basic sniffing rates had been interrupted by more frequent pauses than normally occur, or whether the rate itself was reduced. Faster film speeds must be used to answer this question. Bilaterally decorticated rats seldom sniffed under the conditions of this study. Even when they did sniff, the amplitude of the responses was minimal and difficult to observe. The significance of this finding is not clear. It is possible that the effect of neocortical ablation is not due directly to a loss of cortical facilitation of spinal and medullar centers. Rather, the effect may be more indirect. For example, decorticate rats may experience the test situation as strongly novel due to the loss of detailed perception of familiar stimuli which results from removal of the main neocortical sensory areas. In this connection BINDRA and SPINNER (1958) have shown that stronger degrees of novelty decrease the incidence of sniffing in rats. The existence of such a possibility suggests a need for caution in interpretation of the role of cerebral structures in such behavior. Further studies involving more detailed selective cerebral ablations and more careful parametric control of the stimulus situations will be required to clarify these issues.

Nevertheless, the behavioral data suggest several general hypotheses regarding the location and character of the neural circuits involved in sniffing actions. Thus, at least four neural groups can be identified which constitute the final common pathways for activation of the four muscle groups involved.

Vibrissae and nose movements are probably activated by two separate neural groups within the 7th (facial) cranial motor nucleus in the medulla (GREENE, 1935). Muscles of the head and neck which produce head movements are innervated by fibers with cells of origin in the ventral horns of the cervical spinal cord. Respiratory movements produced by thoracic and diaphragmatic muscles are activated by ventral horn cells within the thoracic segments of the spinal cord. The motor cell groups which directly activate the four sniffing movement sequences are thus presumably located at specific, anatomically distinct portions of the spinal cord and medulla. There are at least two possible means by which the integrated and fixed temporal organization of the four sniffing actions could be achieved by the four motor cell groups hypothetically involved. The observed temporal organization could be imposed either: (1) from w i t h i n the four motor neuron groups by a system of fiber interconnections that would produce the necessary timing relationships, or (2) from another distinct nuclear group located o u t s i d e the immediate territory of the four motor cell groups, but which sends efferent fibers to each of them, activating them in the proper temporal pattern on the basis of its own internal organization of interconnections. Such general hypotheses require further refinement, elaboration, and experimental verification by means of recording, stimulation, and ablation-degeneration techniques. Electromyographic recording methods could be used to identify more accurately those muscle groups which actually are involved in the four sniffing actions. Such data would also lend greater precision to inferences regarding the location of the motor cell groups involved. In any event, the present study supports the notion that behavioral data, if obtained in sufficient detail, may be valuable aids to the formulation of testable hypotheses regarding the spatiotemporal action patterns of known neural groups within the central nervous system.

Neither of the two general hypotheses proposed above would account for the observed rates of movement of the four muscle groups. It is possible that sniffing rate is programmed by the same neural centers as is licking rate in albino rats. Licking rates of 6-7/sec. (STELLAR & HILL, 1952), 5-6/sec. (DAVIS & KEEHN, 1959), 6-8/sec. (KEEHN & ARNOLD, 1960), and of 5.3-11.4/sec. (SCHAEFFER & PREMACK, 1961) have been reported for albino rats. These values are within the sniffing rate ranges reported above. The locus of neural circuits that might determine either sniffing or licking rates is unknown. Several studies have shown that licking rates decrease during licking bursts lasting several seconds (KEEHN & ARNOLD, 1960; SCHAEFFER & PREMACK, 1961). Moreover, DAVIS and KEEHN (1959) and KEEHN and ARNOLD (1960) reported consistent individual differences in licking rates. Al-

though such intra- and inter-animal differences in sniffing rates were observed in the present study, systematic attempts were not made to determine their causes, nor to assess their reliability or validity. It is important to note that licking rates, as well as sniffing rates, have been found to be relatively independent of various afferent stimulus and experience factors (HILL & STELLAR, 1951; DAVIS & KEEHN, 1959; KEEHN & ARNOLD, 1960; SCHAEFFER & PREMACK, 1961).

The ontogenetic development of sniffing rate as well as the differential development of the four component sniffing movements suggest that certain changes are taking place in the anatomical, physiological, and biochemical characteristics of the nerve cell groups involved. It would be of particular interest to study maturation changes in cytoarchitecture, cell size and density, and in dendritic growth (*Cf.* EAYRS & GOODHEAD, 1959) in the four nuclear groups discussed above. SAMSON, *et al.* (1960) have charted the ontogenetic changes in mitochondrial activity in the albino rat and have found increases during the first three postnatal weeks that resemble those for sniffing rate described above. A number of such interdisciplinary studies could make important contributions to an understanding of neural bases of sniffing behavior.

All these lines of evidence indicate that sniffing is a relatively fixed and stable response pattern. The complete sniffing pattern appears early in ontogeny before the infant rat has entered its first period of intense exploratory activity. The rate and temporal organization of the component sniffing movements seem to be unaffected by general experience since they are essentially identical in 18 day old and adult albino rats of either sex. Moreover, they persist unaltered in infants or adults for at least six months after olfactory sensory reception has been obliterated.

That sniffing behavior is a rather common phenomena among certain groups of rodents is suggested by the author's observations of strong, brisk, repetitive mystacial vibrissae movements in individuals of several other rodent genera. These movements appeared to be similar to those described above for the albino rat. Thus, rapid vibrissae movements were seen in hooded rats (*Rattus*), several types of wild and albino mice (*Mus*), flying squirrels (*Glaucomys*), gerbils (*Meriones*), chinchillas (*Chinchilla*), and in hamsters (*Mesocricetus*). Vibrissae movements during sniffing were not observed in rodents such as guinea pig (*Cavia*), Capybara (*Hydrochoerus*), or pocket gopher (*Geomys*). Such movements were also not seen in any of the carnivores examined such as the domestic cat (*Felis*) and dog (*Canis*), raccoon (*Procyon*), coatimundi (*Nasua*), ring tailed cat (*Bassariscus*), lesser panda (*Ailurus*), or seals (*Callorhinus ursinus,* and *Phoca vitulina*), all of which

have prominent mystacial vibrissae. Neither the rate of the rapid vibrissae movements, nor that of any of the other sniffing movements were measured in these other animal types. A systematic comparative study of species differences and taxonomic distribution of these activities would shed light upon the evolution of sniffing behavior as well as of the neural centers which control it.

SUMMARY

Sniffing behavior of albino rats was photographed on movie film at 30, 32, or 64 frames per sec. The ontogenetic development of sniffing was studied and the component movements were described. The effects of olfactory, somatic, visual, and auditory stimuli, and of certain drugs, nerve sections, and cerebral ablations upon sniffing were examined. The following results were obtained: "Sniffing" by rats consists of an integrated and precisely timed movement sequence in which four distinct synergistic muscle groups participate to produce: (a) bursts of polypnea, (b) recurrent protraction and retraction of mystacial vibrissae, (c) repetitive retraction and protraction of the tip of the snout, and (d) a rapid series of discrete head movements and fixations. These four component movements occur at rates between 5-11/sec. in bursts of varying duration (1-10 sec.). Some of these movements may occur independently of the others, but when they all occur together, which is more common, they blend at the same rate and in a fixed temporal pattern with respect to one another. The sniffing bursts are brought into play when the animal is aroused to explore by olfactory, visual, auditory, or tactile stimulation. Sniffing actions are not dependent on olfactory or tactile vibrissae input for their initiation or synchronous patterning despite the fact that the main adaptive values of these actions would seem to be those of olfactory and somatic sensory perception. In normal rats, olfactory input affects sniffing activities by regulating the degree of their persistence; the duration being affected by the character of the olfactory stimuli encountered. Thus, aversive stimuli are contacted only briefly, whereas, preferred stimuli elicit prolonged bursts of sniffing. Each of the four component movements of the sniffing sequence emerge at different times during ontogeny. This suggests that separate neural centers driving them also mature at different times. As each type of movement appears, it swings into synchrony with those actions already present. Interruption of olfactory input does not alter the normal rate of sniffing. Interruption of somatic sensory input from vibrissae may reduce the overall sniffing rate slightly. Bilateral ablation of neocortex drastically interferes with the sniffing sequences. In the decorticate rat these actions were elicited only with difficulty; the sharpness of the individual movements was blurred, and their magnitude and rate were diminished. The possibility that these effects are not directly related to cortical control of sniffing patterns is suggested. The various lines of evidence indicate that sniffing is a fixed and stable response pattern that is relatively independent of age and of long or short term experiential factors.

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ZUSAMMENFASSUNG

Schnüffelnde Albinoratten wurden mit 30, 32 oder 64 Bildern/Sek. gefilmt. Untersucht wurde die ontogenetische Entwicklung des Schnüffelns; die es zusammensetzenden Bewegungen wurden beschrieben und die Wirkungen von Geruchs-, Berührungs-, Gesichts- und Hörreizen, einiger Drogen, Nervdurchschneidungen und Abtragungen von Gehirnteilen auf das Schnüffeln untersucht. Wie es sich ergab, ist das Schnüffeln der Ratte eine wohlgeordnete und zeitlich genau festgelegte Bewegungsfolge, an der sich vier verschiedene zusammenarbeitende Muskelgruppen beteiligen: a) Ausbrüche beschleunigter Atmung, b) abwechselndes Aufrichten und Senken der Schnurrhaare, c) wiederholtes Vorschieben und Wiederzurücknehmen der Schnauzenspitze, d) ganz bestimmte Kopfbewegungen und -stellungen in sehr rascher Folge. Diese vier Bewegungsanteile treten je 5-11 Sek. 1-10 Sek. lang auf. Manchmal lauft eine unabhängig von den anderen ab, aber wenn sie alle, was häufiger ist, zusammentreffen, verschmelzen sie bei gleichem Rhythmus zu einem zeitlich festgelegten Muster. Die Ratte schnüffelt beim Erkunden auf Geruchs-, Gesichts-, Gehörsreize und Berührungen hin. Das Schnüffeln muss nicht durch Düfte oder Schnurrhaarberührung ausgelöst werden, wenn es auch dazu dient, diese Reize wahrzunehmen. Bei normalen Ratten regeln Duftreize die Ausdauer des Schnüffelns: abstossende Gerüche lösen nur kurze, bevorzugte lösen langanhaltende Schnüffelfolgen aus. Die Tatsache, dass jeder der vier Bewegungsanteile zu einem anderen Zeitpunkt der Entwicklung auftritt, lässt auf ebensoviele Zentren schliessen, die zu verschiedenen Zeiten reifen. Jede neuauftretende Bewegungsweise schliesst sich dem Rhythmus der schon vorhandenen an. Bei Unterbrechung der Duftzufuhr wird im gleichen Tempo weitergeschnüffelt. Bei Wegfall der Schnurrhaarreizungen verlangsamt es sich manchmal, nach beidseitigem Wegfall der Grosshirnrinde ist das Schnüffeln nur mit Mühe auslösbar; die einzelnen Bewegungen sind undeutlich, klein, seltener und langsamer, was nicht unmittelbar auf eine Rindenlenkung des Schnüffelns deuten muss. Alle bisherigen Befunde legen nahe, dass das Schnüffeln ein ziemlich starres, vom Alter und von lang- bzw. kurzfristigen Erfahrungen einigermassen unabhängiges Verhalten ist.