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THE RELATION OF SCENT-MARKING, OLFACTORY INVESTIGATION, AND SPECIFIC POSTURES IN THE ISOLATION-INDUCED FIGHTING OF RATS

by

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(With 3 Figures)
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INTRODUCTION

The purpose of this study was to record and analyze the sequences of acts and postures of rats during tests for isolation-induced fighting with a particular emphasis on scent-marking and olfactory investigation. From these data it has been possible to construct a model for the sequences of behavior which lead to and maintain isolation-induced fighting in the rat.

Fighting in rats, as in most rodents, is closely related to olfaction. Wild rats mark their territories with glandular secretions, and they apparently discriminate between the odors of male and female rats, strange and familiar rats, and strange and familiar scent-marks on objects within their territory (CALHOUN, 1962; BARNETT, 1963). Olfaction is critical for the isolation-induced fighting of an isolated male rat against a strange male intruder, the behavior which appears to be the laboratory analogue of the territorial aggression of wild rats. Isolation-induced fighting is abolished by olfactory blockade, and attack only occurs if the olfactory stimuli from the opponent are unfamiliar and those of an adult male (ALBERTS & GALEF, 1973). In a contest between two rats the one on its own home ground usually wins (BARFIELD *et al.*, 1972) which may be based, at least in part, on the discrimination of the olfactory cues of the home cage, especially those from previous scent-marking.

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The glandular sources of odorous substances on the body surface and scent-marks on objects in the environment have not been investigated in the rat, but comparable data from the mouse have implicated secretions from the preputial glands (MUGFORD & NOWELL, 1971; BRONSON & CAROOM, 1971), coagulating glands (JONES & NOWELL, 1973) and sebaceous glands of the dorsal body surface (STRAUS & EBLING, 1970). The ventral sebaceous glands have been shown to play a critical role in the aggressive behavior of the gerbil (THIESSEN, 1973). One previous mention of scent-marking behavior in the laboratory rat may be found in the work of GRANT & MACKINTOSH (1963) who described two behavioral acts apparently related to scent-marking: crawl-over-object and rub.

In light of these previous data, the present study places particular emphasis on scent-marking and olfactory investigation. Scent-marking was measured during the weeks prior to testing by weighing the weekly accumulation of odorous material deposited in drops of urine on a Petri dish in the home cage of the isolated male rat. It was independently measured in terms of the crawl-over-dish and rub behaviors observed during the isolation-induced fighting test. Acts of olfactory investigation were carefully noted along with other stereotyped behavior patterns during the tests.

MATERIAL AND METHODS

The subjects were five strains of highly inbred rats chosen for use in another study as yet unpublished, on strain differences in aggressive behavior. Data of home rats were pooled from 37 males, 10 each of the DA, Irish, and Lewis strains, and 7 of the Fischer strain. Data of intruder rats were pooled from 37 males, 8 each of Fischer and DA strains and 7 each of Irish, Lewis, and WAG-Rij strains. Data from WAG-Rij home rats were not included here because they did not show isolation-induced fighting; otherwise, there were no significant strain differences in the behaviors of home or intruder rats which could be determined to affect the analysis employed here.

All animals were weaned at 25 days of age and placed into 64 × 10 in. pens with their siblings. At 90 days of age the home rats were isolated in 32 × 10 in. cages along with a single glass Petri dish on the floor of the cage. The other males, the intruders, were housed together as groups of five (same strain) in 14 × 24 in. cages.

To record scent-marks, the glass Petri dish was weighed, washed thoroughly, and replaced in the cage at one week intervals. In most cases there was an appreciable accumulation of an oily odorous substance apparently deposited with urine on the dish. The substance could often be seen in the form of a small stream or droplet on the side of the Petri dish corresponding to the form of the urine droplets left by a rat during crawl-over-dish. On the day of testing the Petri dish was weighed prior to testing, replaced in the cage without washing it, and then weighed again one day later in order to record the accumulation of material during and after the test.

Tests were conducted when the home rat had been isolated for five weeks. Preliminary experiments had shown that more fighting was obtained after this isolation interval than after only one or three weeks of isolation. An intruder rat, chosen according to a counterbalanced design so that each strain of home rat was given two tests with each strain of intruder, was introduced into the home cage of an isolated rat during

the night cycle and under red light illumination. Each home rat was tested once, there being 37 tests in all.

During the test, which was 20 minutes long, each act and posture of each rat was continuously recorded on a moving paper tape. A scoring system of abbreviations was adapted from the classification of acts and postures of rats by GRANT & MACKINTOSH (1963) including the following: sniff-anogenital-region of other rat; sniff-Petri-dish; exploring the cage; offensive sideways posture; full attack posture, attack; defensive upright posture (one rat); boxing (both rats); submissive posture; rub-against-cage; crawl-over-dish; mounting; aggressive groom; and groom-self. Attack was further characterized as a bite-and-kick attack, following the detailed cinematographic analysis by BANKS (1962) of attack in the mouse and a cinematographic analysis of attack in the rat kindly supplied to the author by Klaus MICZEK of Carnegie-Mellon University. The first ten tests were recorded by two independent observers and their data later compared. A reasonable degree of inter-observer reliability was found, and further testing was carried out by a single observer.

Distinctions between interrupted behavior and separate incidences of the same behavior were often difficult to make; therefore data from paper tapes were transcribed into tabular form in terms of occurrence or non-occurrence of each act and posture during each minute of the twenty minute test regardless of the number of times they seemed to occur during that minute. In addition to the quantitative minute-by-minute tabulation, sample behavioral sequences were analyzed on a second-by-second basis.

RESULTS

The time course of the major behavioral acts and postures of home and intruder rats is presented in Fig. 1. It may be seen that during the course of a typical test, the home rat first investigated the intruder with the behavior termed "sniff-anogenital-region" and then investigated its own cage including the Petri dish. Bite-and-kick attack, offensive sideways posture, and crawl-over-dish or rub-against-cage behavior by the home rat usually did not begin until several minutes into the test. Similarly, the intruder usually began with olfactory investigation, although it usually investigated the cage first and the home animal later. The percentage of home rats showing sniff-anogenital-region and aggressive-groom within the first five minutes was significantly higher in home rats than in intruders, reflecting the different sequences of behavior in those animals. Scent-marking of the Petri dish by the intruder (crawl-over-dish) began immediately, in contrast to the home rat, while rub-against-cage was not shown by intruders. Defensive behaviors (upright posture or submissive posture) by the intruders usually did not begin for several minutes and apparently corresponded to the onset of offensive behaviors by the home rats.

A detailed qualitative examination of the ethogram records, looking at sequences of behaviors on a second-by-second basis, supplemented the preceding quantitative data. The intruder, when first put into the cage, usually explored the cage, sniffing the floor and sides and often pausing to sniff at the Petri dish which contained the scent-marks of the home rat. Frequently

the intruder would scent-mark the dish after sniffing it (crawl-over-dish). The home rat followed the intruder around, sniffing its anogenital region and "grooming" the intruder's head or back, behaviors which appeared to function as olfactory investigation of the sebum from the dorsal surface of the animal and the specialized sebaceous glands in the anogenital region.

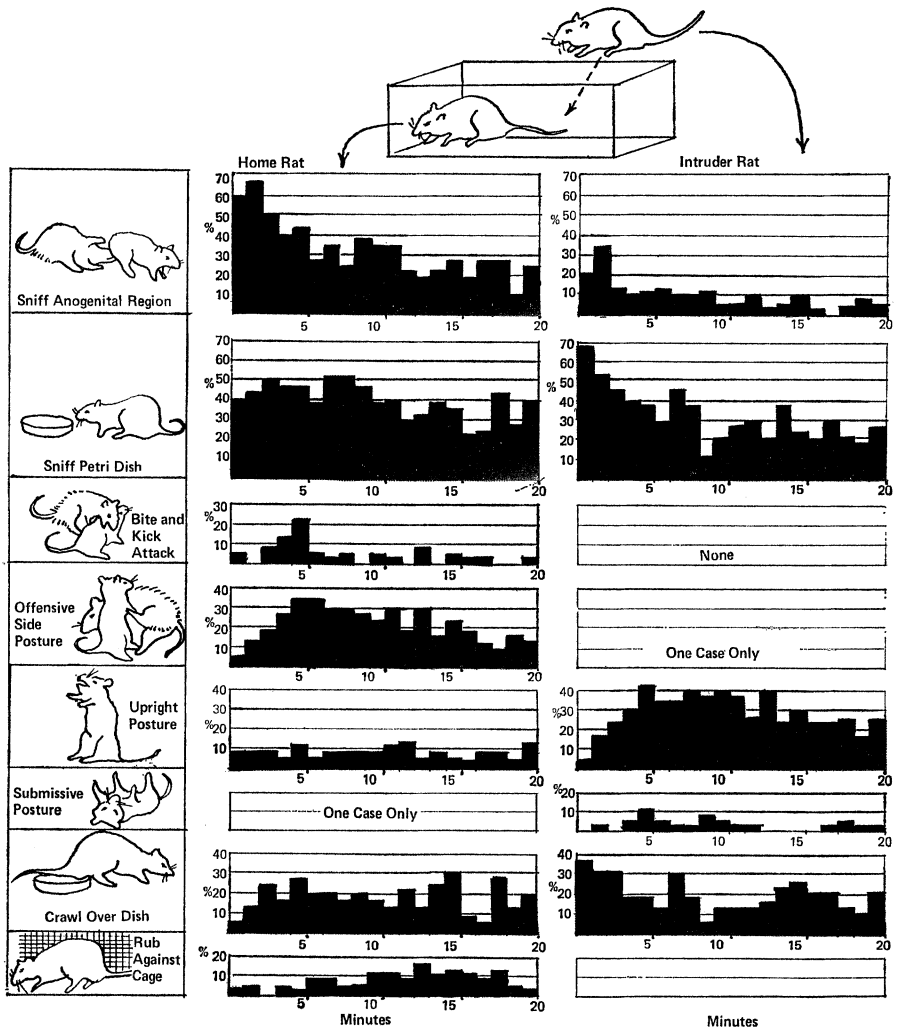

Fig. 1. Percentage of animals showing acts and postures on a minute-by-minute basis in isolation-induced fighting tests. Data from home rats shown on left and from intruder rats shown on right. Upright posture, for purposes of this analysis, calculated exclusive of boxing. Drawings in this and succeeding figures derived in part from GRANT & MACKINTOSH (1963).

Often, after the intruder had scent-marked the Petri dish, the home rat sniffed the dish in turn and scent-marked on top of the intruder's secretions. At times, the home rat jumped back and forth, sniffing first the intruder and then the dish, giving the observer the impression that a comparison of the odors was being made. Occasionally at first, and more frequently as the test progressed, the two rats exchanged roles, the intruder sniffing the home rat, and the home rat exploring the cage. In most cases, attack and defense did not occur until after several minutes of olfactory investigation.

Bite-and-kick attack, offensive sideways posture, and rub-against cage were shown exclusively by home rats and will be termed "offensive" acts and postures. The bite-and-kick attack was shown by 19 rats; it usually occurred once about the seventh minute and often occurred once again two or three minutes later following an apparent refractory period. Bite-and-kick attack was almost always accompanied by offensive sideways posture: 28 of the 34 instances were accompanied within two seconds by offensive sideways posture and 18 of the 19 rats with bite-and-kick also showed offensive sideways posture during the test. Whereas the bite-and-kick attack occurred only once or twice during a test, the offensive sideways posture continued to occur throughout the test following the initial attack (Fig. 2). Rubbing behavior was also correlated with attack: home rats with bite-and-kick attack showed rub-against-cage behavior an average of 1.8 minutes as compared to only 0.6 minutes for home rats without bite-and-kick attack ($p < .05$ by analysis of variance). Rub-against-cage behavior did not usually begin until four or five minutes after the attack.

Upright posture (exclusive of boxing which involved both rats) and submissive posture were shown primarily by intruders in response to attack and will be termed "defensive" postures. Although the upright posture also occurred at low levels in both home rats and intruder rats which were not attacked, it occurred most frequently in intruder rats which were attacked. Attacked intruders showed upright posture an average of 9.3 minutes of the 20 minute test session, compared to 4.0 minutes for threatened intruders, and 1.5 for unthreatened and unattacked intruders (threatened refers to intruders which were subjected to offensive sideways posture but no bite-and-kick attack by the home rat). These differences were significant at $p < .01$ by analysis of variance. In the minutes following an attack the percentage of submissive posture returned to low levels, but the percentage of upright posture remained high, in parallel to the high levels of offensive sideways posture shown by the home rat.

Scent-marking behavior was consistent from week to week and across two independent measures (Petri-dish accumulation and crawl-over-dish be-

havior). The strains which were highest in scent-mark accumulation during the early weeks prior to testing (Lewis, Fischer, and Irish) were also highest in crawl-over-dish behavior during the test session. If strain differences were excluded there was also a significant correlation of scent-marking measures across individual rats. From week to week individual rats of the same strain retained the same rank order of scent-mark accumulation: correlation of .76 from week one to week two ($p < .01$) and .37 from week one to week five ($p < .05$). Also if strain differences were excluded there was a significant correlation between the rank order of rats in accumulation of scent-marking substance during week five and the number of minutes with crawl-over-dish behavior during the test session (correlation of .37, $p < .05$). Although the

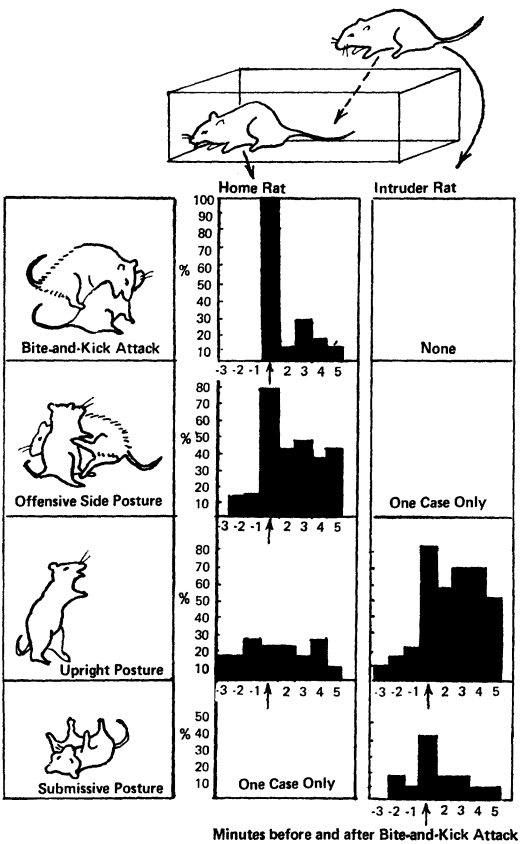

Fig. 2. Percentage of animals showing various behaviors on a minute-by-minute basis in isolation-induced fighting tests with the time scale based on minutes before or after the initial bite-and-kick attack by the home rat. Data from home rats shown on left and from intruder rats shown on right.

data from all strains were pooled to test for significance, the direction of the correlation was positive for every strain calculated separately.

The amount of scent-marking by individual rats did not correlate with probability of attack. It should be noted, however, that most rats which showed attack had *some* scent-marking substance in their Petri dish and sniffed it at some time during the test prior to attack. Two rats were exceptions, however: one Lewis rat leapt upon the intruder and attacked without any preliminary investigation, and one DA rat attacked the intruder despite never having shown any accumulation of scent-marks or crawl-over-dish behavior.

A negative relationship was found between scent-marking by intruders and their having been attacked. In the three minutes prior to the first bite-and-kick attack, 22% of these intruders showed crawl-over-dish behavior per minute, while in the four minutes after the attack crawl-over-dish behavior dropped to only 4% per minute ($p < .05$ by X^2 test). Not-bitten intruders, which were the appropriate controls, remained relatively constant in crawl-over-dish rate, with 15% per minute in minutes 4-6 and 13% per minute in minutes 8-11 (these minutes were chosen for comparison because the average initial bite-and-kick attack took place in the seventh minute). The reduction in scent-marking by bitten intruders was part of a more general behavioral syndrome which followed subjection to attack and which was characterized by diminished investigatory behavior. Aggressive groom dropped from a mean of 28% of rats showing the behavior per minute in the three minutes prior to initial attack to a mean of only 1% in the four minutes after attack. Sniff-anogenital-region dropped from 18% to 4%; exploration of the cage dropped from 80% to 14% and sniff-Petri-dish dropped from 46% to 21%. All of these changes were significant when compared to behavioral rates of non-bitten intruders, by the use of the X^2 statistic.

The lower rates of scent-marking and investigation by intruders after they were bitten was not paralleled by any change in the scent-marking and general investigatory activity of the home rats which administered the attack. In the three minutes prior to the first attack, 18% of the home rats showed crawl-over-dish behavior, per minute, and in the four minutes after the attack crawl-over-dish behavior continued at 20% per minute. Similarly exploration of the cage remained high: (34% per minute prior to attack and 32% after attack) as did sniff-Petri-dish (36% per minute prior to attack and 36% after attack). Investigation of the intruder did diminish after attack, however. Aggressive groom fell from 36% per minute to 14% per minute, and sniff-anogenital-region dropped from 46% to 24%. There

was no change in rate of either of these latter two behaviors in the appropriate control minutes for home rats which did not attack.

DISCUSSION

A hypothetical model of isolation-induced fighting has been presented in Fig. 3 as derived from the results of previous studies and the sequences of behaviors observed in the present study. The sequence begins with olfactory investigation by the two animals triggered by the presence of strange odors. The home rat begins with investigation of the intruder, while the intruder begins with investigation of the cage especially the heavily marked Petri dish. Following initial investigation, each rat sniffs both the other rat

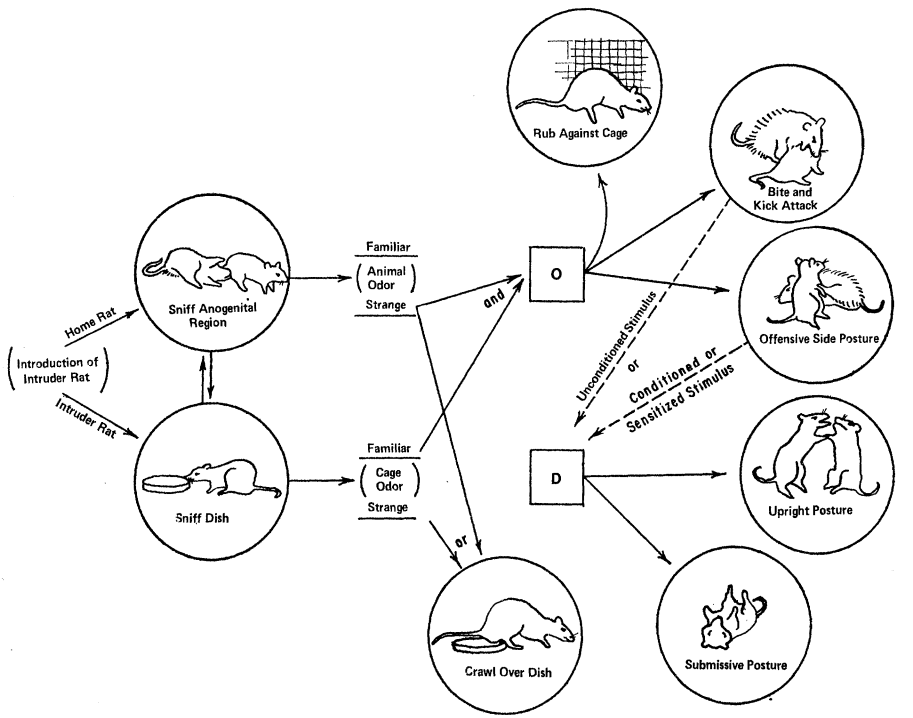

Fig. 3. Hypothetical model of isolation-induced fighting in the rat. The behaviors shown by home and intruder rats are presented as a sequence initiated by olfactory investigation and mediated by hypothetical neural mechanisms for offense (O) and defense (D). The offensive mechanism is triggered by a comparison between strange rat odor and familiar home cage odor. The defensive mechanism is triggered by the pain of a bite-and-kick attack. It is hypothesized that the defensive behaviors shown by an intruder rat to offensive sideways posture are due to sensitization of those responses following attack.

and the cage, and each rat shows scent-marking behavior, especially on the Petri dish. The olfactory comparison of cage odor and other rat odor becomes critical for succeeding events. If the other rat is strange, but the cage odor is familiar, a hypothetical neural mechanism of "offense" (O) is triggered in the home rat, leading to bite-and-kick attack and offensive sideways posture. The pain inflicted by the bite of the home rat triggers in turn, a hypothetical neural mechanism of "defense" (D) in the intruder which produces upright posture or submissive posture. The exact brain mechanisms of offensive and defensive behavior in the rat are not well known, although ADAMS (1971) has shown that they may be distinguished by lesions of the lateral and medial hypothalamus, and EDWARDS & ADAMS (1974) have implicated the midbrain central gray in defensive upright behavior.

The emphasis given here to olfactory stimuli in the elicitation of attack is not meant to deny that other sensory systems and internal states contribute to the behavior. Olfaction should probably be interpreted broadly to include sensations in the vomeronasal organ as well as the olfactory mucosa, since the behaviors described here as olfactory investigation may often involve extension of the tongue and licking as well as sniffing. Visual, tactile, and auditory stimuli may also be involved in elicitation and maintenance of the behavior. Internal states related to the presence of male sex hormones and changes induced by isolation are no doubt involved as well. Data from CALHOUN (1962) and TELLE (1966) regarding territorial behavior of wild rats might be explained in terms of an isolation effect on aggression. Only 3 of the 61 adult males were truly territorial in CALHOUN's colony, and the most dominant male (# 49) grew to maturity under the protection of a dominant mother that excluded other adult males and kept her male infant in relative isolation. TELLE's finding that territorial defense was shown by wild rats in small and medium sized groups, but not by rats in large groups might have been due to the greater inter-male isolation in small groups.

The functional role of bite-and-kick attack appears to be simply the infliction of pain and the unconditioned elicitation of defensive behaviors in the intruder (upright posture or submissive posture in the present tests and presumably flight in more free-ranging animals). The functional role of offensive sideways posture may be more complex, however. Since offensive sideways posture is usually shown by the home rat within a few seconds before or after a bite-and-kick attack, it is possible that it becomes a conditioned stimulus eliciting defensive behaviors in the intruder because of its close temporal pairing with the previous painful attack. But not all defensive

behaviors are conditioned responses to pain; some defensive upright posture is exhibited by home rats and by intruders prior to attack, and in other experiments we have observed defensive upright posture exhibited prior to attack by intruders which have been raised since weaning in total isolation from other rats. It seems more likely, therefore, that the defensive upright behavior of the intruder is sensitized by attack, and that the approach of the home rat in offensive sideways posture elicits it as a "sensitized" response. In this way, the offensive sideways posture may be understood as a truly functional threat behavior. It would explain how the high rates of offensive sideways posture by the home rat throughout the present test sessions continued to trigger high rates of defensive upright posture by the intruder although there were no further bite-and-kick attacks.

What are the functional roles of the defensive postures? The key to this question may lie in the nature of the bite-and-kick attack. In every case we have observed, the bite is delivered by the home rat on the opposite flank of the intruder following assumption of the aggressive posture (the home rat extending from one side and at right angles across the back of the intruder) or following a leap onto the back of the other animal. When the intruder is in defensive posture or submissive posture, however, the back of the intruder is shielded from the home rat, the home rat cannot assume an aggressive posture, and attack may be inhibited.

Scent-marking has been considered as an integral part of the repertoire of behaviors involved in aggressive behavior in the present paper as in many previous studies (see JOHNSON, 1973). It would appear that the presence of scent-marking odors increases the confidence of home animals and decreases the confidence of intruders. If so, it may play an important role in the elicitation of attack or defense, although definite proof is still lacking (JOHNSON, 1973) and at least one animal showed isolation-induced attack in the present study without ever showing scent-marking behavior.

Previous studies on the gerbil have shown that dominant animals scent-mark more than subordinates and that newly defeated animals decrease in scent-marking dramatically (*e.g.* THIESSEN, 1973). In the present study, no relationship could be found between the amount of scent-marking and attack if strain differences were excluded. However, most of the rats which showed attack also showed some scent-marking previous to the territorial test, and hence there were deposits of scent-marking material in the Petri dish when the intruder was placed into the home rat's cage. The second finding of Thiessen was paralleled in the present study; intruders showed less scent-marking after they were attacked than before.

The decreased rate of scent-marking by intruders after they were attacked

was clearly related to the attack, since non-bitten intruders showed no such decrease. It could not be explained by assuming that the rats were so busily engaged in the aggressive interactions that they had no time to scent-mark; the attacking home rats, which were also taking part in the interactions, continued their pre-attack rates of scent-marking. Instead, the decreased scent-marking appeared to be part of a general depression of behaviors, including investigatory behaviors of all kinds, which was produced by the attack of the other rat. The present test sessions were too short to make possible a full comparison of this effect to the long term, apparently conditioned depression of scent-marking in defeated gerbils as reported by THIESSEN.

SUMMARY

The temporal sequences of acts and postures of rats during tests for isolation-induced fighting were recorded and analyzed. Scent-marking and olfactory investigation, which have been related to fighting by previous studies, were particularly emphasized. From the data a model was constructed for the sequence of behaviors which lead to and maintain isolation-induced fighting.

The typical sequence begins with olfactory investigation and scent-marking; the home rat initially investigates the intruder, and the intruder initially investigates the cage. The combination of olfactory perception of a strange male and a familiar environment, it was suggested, serves to trigger an offensive mechanism in the home rat which leads to bite-and-kick attack and offensive sideways posture. The pain of the attack then triggers defensive mechanism in the intruder rat which leads to defensive upright posture and submissive posture.

Whereas the functional role of the bite-and-kick attack appears to be simply the infliction of pain and elicitation of defense in the intruder, the function of offensive sideways posture as a threat behavior may be more complex. It is possible that it becomes a conditioned pain stimulus due to its close temporal pairing with bite-and-kick attack, but it is more likely that it produces defense by a process of sensitization. In any case, following the initial attack, the offensive sideways posture continues to elicit defensive behavior by the intruder even when there are no further attacks.

The functional roles of the defensive postures were interpreted as positioning the intruder in such a way that the home rat cannot assume the aggressive posture from which attack is launched.

Scent-marking behavior was consistent within strains, within individuals, and across different types of measures (accumulation of scent-marking marking material and performance of the stereotyped scent-marking act, crawl-over-dish). Amount of scent-marking was not correlated with attack, however, and its role in isolation-induced fighting remains unclear. In parallel to findings in other rodents, it was observed that scent-marking was diminished in animals after they had been subjected to attack.

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RÉSUMÉ

La séquence des comportements montrés par le Rat „résident" et par le Rat intrus au cours de tests de combat résultant d'isolements a été analysée en détail, et un modèle hypothétique a été proposé pour rendre compte des données obtenues. Le séquence typique commence par la recherche olfactive; le Rat résident a examiné l'intrus, et celui-ci explore la cage. La perception olfactive d'un mâle étranger dans son domicile déclenche chez le Rat résident une réaction offensive, tandis que la douleur d'attaque a déclenché de la part de l'intrus une riposte défensive.

On a donné une définition opérationnelle à la posture nommée offensive-bordée comme un comportement menaçant. En raison de la quasi simultanéité de l'offensive et de la morsure exécutées par le rat résident au début du test, la posture offensive est devenue le stimulus de défense conditionnée ou sensibilisée, et a déclenché un comportement défensif chez l'intrus.

Le comportement de marquage par l'urine était du même ordre dans chaque souche comme chez les individus (de semaine à semaine) et cela quels que soient les tests utilisés. Ces tests n'ont montré aucune corrélation entre la tendance à marquer et la probabilité de l'attaque. Cependant, la tendance à marquer a baissé chez les animaux ayant été attaqués.