OLFACTORY AND BEHAVIOURAL REGULATION OF TERRITORIAL AGGRESSION IN FERAL RATS

OLFACTORY AND BEHAVIOURAL REGULATION OF TERRITORIAL AGGRESSION IN FERAL RATS (*Rattus norwegicus*)

by

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SCOPE AND CONTENTS:

This thesis is primarily concerned with the problem of recognition among feral Norway rats. Wild rats are known to behave amicably in the presence of colony members and act in a stereotyped aggressive manner towards unfamiliar conspecifics in a territorial context. Previous reports suggest that identity is basically olfactory and is organized around colony-specific odors residing in the nesting material or the metabolic excretions of the male members of a colony.

The first experiments in this study were attempts to replicate various reports concerning the maleability of clan odors, thereby giving a wild rat the olfactory cahracteristics of an alien group and, conversely, to alter a resident's odor such that he was no longer identifiable as a member of his original clan. Further experiments examined identifier and regulator functions of behavioural cues on social interaction subsequent to recognition. The interactions of unfamiliar rats on a mutually common terrain was studied, and compared with that of familiar rats paired on an uncommon territory. The final experiment investigated the functional nature of olfactory stimulation in these situations and compared the reactions of anosmic residents towards familiar and unfamiliar conspecifics.

These experiments indicate that recognition is indeed an olfactory process but that identity is highly resistent to alteration. The identifier substance does not appear to be contained in either the nesting matter nor in a colony's urine or feces. Bond of familiarity are not specific to a common terrain and in most cases behavioural cues appear to control the onset and termination of fight behaviour following the olfactory identification. Studies of anosmic residents indicate that novel odors elicit aggressive tendencies while familiar odors are not inhibitors of aggression. The results suggest the presence of a specific male pheromone that is essential for the arousal of aggression while the topography of interaction is regulated largely by the reciprocal behaviours of the animals.

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General Introduction

Within an established pack or colony of feral Norway rats (Rattus norvegicus) overt fighting is rarely, if ever, observed (Barnett, 1963; Steiniger, 1950). Lorenz (1966) has described the wild rat as a "model of social virtue" with respect to the peaceful relations seen within the clan where food, burrows, territorial defense and rearing obligations are freely shared. The adult members of a colony do not merely refrain from engaging in overt aggression but perform a variety of stereotyped "amicable" behaviours (Barnett, 1963). Many of these acts, huddling, crawling under, walking over, and mutual grooming (allogrooming), involve cutaneous contact.

It has been observed, however, in a variety of laboratory situations (Barnett, 1958, 1960; Barnett & Spencer, 1951; Galef, 1970) and in large, relatively unconfined outdoor environments (Calhoun, 1962; Steiniger, 1950) that feral rats will attack unfamiliar conspecifics that intrude into their territory. The actual fighting behaviour of the feral rat can be characterized by the postures and acts described for the laboratory rat (Grant, 1963; Grant & Mackintosh, 1963) but these aggressive behaviours are performed with greater vigor and in many more situations by wild strains (Barnett, 1958, 1960, 1968). The specific cues that mediate conspecific identification have not been subjected to experimental scrutiny although several authors have asserted that recognition is under olfactory control (Eibl-Eibesfeldt, 1961, 1970; Lorenz, 1966; Steiniger, 1950).

The present study aims at empirical consideration of (1) the stimuli that facilitate peaceful cohesiveness between potentially aggressive conspecifics, permitting them to live in functionally coordinated societies and (2) the functional nature of the specific cues that serve to identify individual rats as either familiar or novel.

Experiment 1

Eibl-Eibesfeldt (1970, p. 351) has characterized the feral rat colony as a "closed anonymous group" in which members and interlopers are recognized by the group odors they carry, and not as individuals. It is has been reported (Eibl-Eibesfeldt, 1970), but again without substantial experimental or observational citation, that male rats mark other group members of both sexes with their urine. Observational data suggest that rats utilize olfactory cues for recognition of familiar and novel rats in that sniffing investigations always precede agonistic behaviours caused by intruding conspecifics (Barnett, 1963; Steiniger, 1950).

Such group smells are reportedly manipulable. Steiniger (1950) mentions, in anecdotal fashion, that a wild rat removed from its colony and exposed to other rats for "some days" was treated as an intruder upon re-introduction to his former clan. Lorenz (1966) substantiates this and adds evidence attributed to Eibl-Eibesfeldt indicating that odorants contained in the nesting material serve to identify rats as familiar or unfamiliar to specific groups.

Experiment 1 was designed to empirically examine the social consequences of manipulating such colony odors as those described above. In the present experiment territorial residents were tested for differential reactions to intruders that were (1) exposed to odors specific to the resident colony, or (2) similarly exposed to the odors of some other, foreign, colony.

METHODS

Subjects

Sixteen second and third generation laboratory bred, feral, male rats selected from the breeding and experimental populations of the Psychology Department of McMaster University were used as subjects.

Procedure

Residents: Eight pairs of feral male siblings were weighed, marked with spot shavings and introduced simultaneously into large homecages constructed of 3/4 in. plywood, 3 x 3 x 3 ft. Two in. of wood shavings covered the galvanized metal floors of these homecages. In order to maximize and maintain specific colony odors the shavings were never changed completely but once every four wk. half the material was replaced. A single slot-roofed nest box (12 x 3 x 5 in.) with open ends was shared by both occupants of each homecage.

These rats, referred to below as residents, were given 4 - 6 wk. to establish themselves in these homecage territories. During this period they were placed on a 3 hr./day feeding schedule eating powdered Purina rat chow presented in a bowl. The overhead fluorescent lights were on for the duration of the feeding period each day (1:30 - 4:30 p.m.), providing regular periods suitable for observation. Each pair of residents was observed for at least 12 hr. prior to initiation of experimentation to determine its normal activity patterns and establish a baseline level of inter-resident aggression during the observation period.

Intruders: Each intruder was anesthetized with Equi-Thesin (i.p. injection .22 cc/ 100 gm body weight) and scrubbed thoroughly with an emulsifying detergent soap solution (Phisohex), rinsed with clean water, and his fur smoothed down with a gauze pad soaked in rubbing alcohol. Washed rats were placed in plastic holding cages (12 x 17 x 8 1/2 in.) containing the urine and feces impregnated shavings of a specific established resident colony; they continued to be maintained on Purina chow and water. Twenty-four hr. prior to testing, the fur of each intruder was wet with the urine (about 15 ml.) of the resident pair in whose shavings he was being kept and the residents' fresh feces were deposited in the holding cage. The resident urine and feces were collected by storing both residents in a metabolism cage overnight and returning them to their homecage territory for the next feeding period.

Wild rats treated in this manner presumably carried odors representative of a specific territory and its resident pair. A "matched intruder" was a treated rat introduced into the colony that had contributed the variety of odors imposed on him. "Unmatched intruders" were the same treated rats placed in the homecage of any other colony.

Testing

Resident reaction to intruding rats was tested by introducing a single animal into a homecage territory for 1/2 hr. during the 3 hr. feeding period. Observations were made via closed circuit television monitored in a separate room. Experimental sessions were often video taped for repeated scrutiny and slow motion analysis. The experimenter recorded the activities, movements and interactions of the tested animals. Of the 48 postures and acts described by Grant (1963) the three most salient ones associated with aggression were selected for quantification. They were the (a) lateral approach or "threat posture", (b) chase, and (c) attack.

Resident rats in homecages 1 - 4 were tested individually for differential reactions to matched and unmatched intruders presented in counter-balanced order across subjects. When residents were tested individually the partner of the animal to be tested was removed from the homecage 3 hr. prior to the feeding period. After approximately 15 min. of feeding the intruder was introduced for a period of 30 min. and then removed. The second resident was returned to his territory and observation continued for an additional 30 min.

Resident rats in homecages 5 - 8 were tested as intact colonies and were presented with unmatched intruders only. Resident animals tested in pairs were also observed for 30 min. after departure of the intruder.

RESULTS

Residents tested individually (N = 8) directed a mean of 4.0 (sd = 4.0) attacks against unmatched intruders while the same animals attacked matched intruders an average of 1.8 times (sd = 3.2) in one 30 min. session. When resident pairs were tested as intact colonies (N = 4) unmatched intruders were attacked an average of 4.5 times (sd = 3.2) and matched intruders received a mean of 2.0 (sd = 1.0) attacks/ test session. Figure 1 gives a more complete illustration of the interactions occurring under the various conditions. Included in Figure 1 are a variety of taxon-specific acts that are generally accepted as behavioural indices of amicability, disruption, or agonism.

Twelve hr. of observations during the feeding period revealed no aggression within resident pairs. Feeding normally began within 5 min. of food presentation and both residents fed freely, usually spending most of the first hour at the food bowl. Huddling, allogrooming, crawling under, and walking over were observed in all resident pairs.

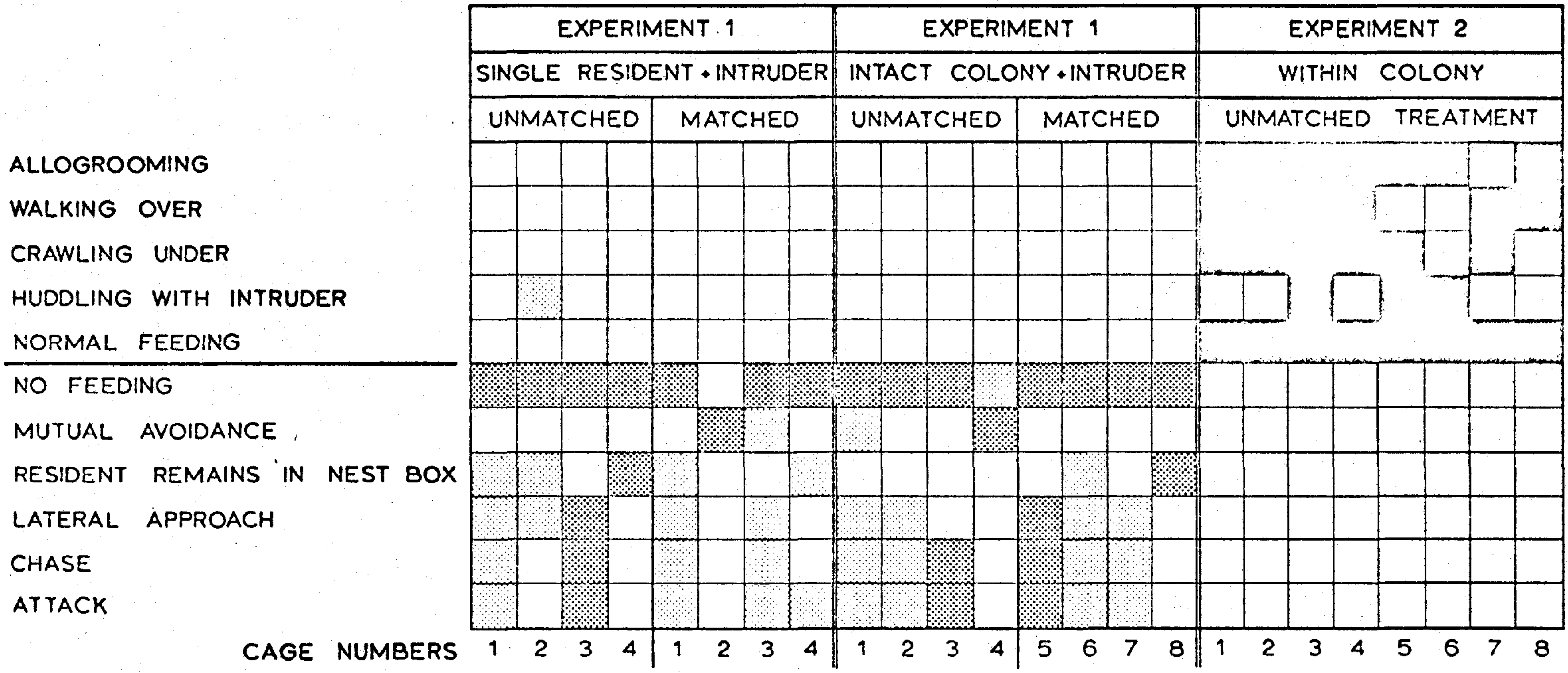
Figure 1 about here

As can be seen in Figure 1, even in the absence of overt aggression, behaviours distinguishable as amicable were not exhibited between residents and intruders, regardless of pretest intruder-treatment. Furthermore only one resident fed for more than 5 min. in the presence of an interloper during any of the 20 confrontations. When the second resident was returned after the 30 min. intruder presentation, normal feeding was consistently resumed and a full range of amicable behaviours exhibited.

DISCUSSION

The results of Experiment 1 indicate that territorial residents tested individually and in established pairs correctly recognized both "matched" and "unmatched" intruders as being unfamiliar. Resident reaction to the presence of an interloper ranged from displays of persistent overt aggression to less quantifiable disruptions of normal activity such as cessation of feeding and the lack of stereotyped amicable behaviour. The different numbers of attacks directed toward matched and unmatched intruders suggests that matched intruders received lower levels of aggression than unmatched intruders.

FIGURE 1. Resident Reaction to Familiar and Unfamiliar Conspecifics Given Various Odorant Treatments. Behavioural events associated with amicability, disruption, or aggressive arousal are plotted with respect to occurrence under different experimental conditions. Light hatching indicates that only one resident of a pair was scored for a particular response; medium hatching indicates both residents responded as scored; dark hatching (Experiment 2) is individual data for untreated resident confronting a familiar conspecific treated as an "unmatched intruder".



However, to rely upon quantification of "aggression" under the present experimental conditions is not apt to provide a complete or necessarily valid description of the results. Individual residents appeared to have characteristic styles of fighting such that some animals extended single attacks into continuous bouts lasting as long as 75 seconds. Other animals attacked intruders with numerous series of discrete but shorter encounters. Since there is no clear way of measuring the relative "intensity" or "level" of aggression under such circumstances, the data does not differentiate between them. Furthermore, "behaviours" like the lateral approach, which are easily distinguishable when fully performed, do not have a constant intensity and cannot be assigned a precise duration since onset and termination of the behaviour is gradual rather than discrete. For this reason the data is expressed most accurately in the form shown in Figure 1 where resident reaction is measured not in "units" of behaviour but in the form of the response.

Despite the apparent simplicity of the experimental conditions in this experiment (small colony size, single site for refuge) many factors were nevertheless present which seemed to contribute to the variability in results across and within trials. The nest boxes produced much of this inconsistency. Intruders often secured and defended the nest box after the first attack or even before any overt aggression had occurred. Residents with a clear fighting advantage over their opponents were successfully impaired from further contact with the intruder once the nest box was secured.

However, the major source of variability seemed to be related to the differential behaviours exhibited by intruders when introduced into a novel environment. This aspect of the experiment could not be directly controlled but a number of issues were raised that will be examined in subsequent experiments in this paper. Intruders were attacked only when in motion; thus, those intruders assuming "freeze", "crouch", or "submissive" postures (Grant, 1963) were not attacked, although they were clearly recognized by residents whose feeding behaviour and general activity was disrupted by their presence.

Other experimenters who have quantified intruder-directed aggression in wild rats (Barnett, 1958; Galef, 1970) recorded a greater percentage of aggressive encounters in such pairings than was observed here. This discrepancy can be attributed to methodological differences. In the case of Barnett (1958), nest box entrances were blocked prior to intruder introduction. The present author has observed that rendering the habitual cover or retreat of wild rats inaccessible can induce fighting even among clan members. Galef's (1970) results were collected from fights in enclosures of much smaller dimensions (12 x 18 x 9 in.) and he too blocked normally accessible passage-ways. Fighting in rats has been shown to be inversely related to enclosure size (Ulrich and Azrin, 1962).

Experiment 2

Lorenz (1966, p. 138) refers to an uncited experiment by Eibl-Eibesfeldt in which wild rats, familiar to a group of conspecifics, were removed from their colony and maintained under two conditions. According to Lorenz, rats removed from their colony and stored for "only a few days" in the nesting materials of an alien group become, in fact, alien to the original clan. Rats removed and stored with materials containing the odorants of the home colony successfully maintain the clan identity and are accepted peacefully upon re-introduction, even after "several weeks" of absence. Experiment 2 attempts to replicate these findings or demonstrate that, on the basis of manipulated olfactory characteristics, a rat once familiar to a conspecific will be attacked or otherwise treated as a stranger.

METHODS

Subjects

The eight established pairs of wild rats from Experiment 1, above, served as subjects for this experiment.

Procedure

From each pair of residents the subordinate or "less aggressive" rat was selected. Subordinate rank was assigned on the basis of previous fighting performance or, when this was not possible, on the basis of lighter body weight. The selected subordinate rat was removed from the homecage and subjected to the Intruder Procedure described in Experiment 1. Residents were returned to their homecages after 7 days of separation during the first 15 min. of the regular feeding period, and the initial 30 min. of reinstatement served as the test session. The experimenter recorded the events of the test session as in Experiment 1.

RESULTS and DISCUSSION

Inspection of the results illustrated in Figure 1 reveals that after being absent for 7 days and returned to the homecage as an "unmatched intruder", treated residents failed to evoke any aggressive or agonistic behaviours from the non-displaced residents. Normal feeding patterns remained undisturbed and, in addition to the lack of overt aggression, all 8 reinstated pairs demonstrated stereotyped patterns of amicable behaviour.

Thus, the notion that exposure to the odors of a foreign group of conspecifics is sufficient to render a wild rat alien to his home territory (Lorenz, 1966; Steineger, 1950) was not supported under the conditions of the present experiment. Despite the vigorous regime of changing the colony-specific olfactory characteristics of a clan member employed here, the treated individual was clearly recognized as familiar to the remaining colony member on the home territory.

The dramatic contrast between resident behaviours displayed to the presence of a real intruder, regardless of odorant exposure, and to the presence of an 'intruding' resident treated with novel odorants is shown in Figure 1.

A number of possible explanations can account for the above findings. One is that the treatment used in these experiments did not alter the rat's odor despite the relative thoroughness of the procedure. A second possibility is that identification of familiar rats depends on some non-olfactory cue such as behavioural identity. Intruders would thus be recognized as unfamiliar because of their behaviour (eg., finickiness in the novel cage) or residents recognized as familiar because of their behavioural characteristics and despite their odorous differences. The striking correlation of attack initiation with movement found in Experiment 1 is also suggestive of this relationship.

A major question remains as to the discrepancy between the present findings and those reported by Lorenz (1966) mentioned above. Unfortunately, personal communication with Eibl-Eibesfeldt has not elicited any careful description of his experimental procedures. However, it is important to note that the 8 pairs of residents used in the present experiment were littermates and there is some evidence suggestive of a familial olfactory attachment process in rodents (Marr & Gardner, 1965; Marr & Lilliston, 1969) similar to imprinting.

Tinbergen (1951, p. 179) reports a study which shows that the conspicuous coloration of the shell parakeets' cere serves as a sexual identifier and a releasing stimulus for either the sexual displays or aggressive defensive behaviour in established males. Both these behaviours will be exhibited towards the same unfamiliar bird with the appropriate manipulation of cere color. After formation of a pair bond, however, changing the female's cere to the male color will not produce a deficit in recognition.

It is possible, even likely, that Eibl-Eibesfeldt did not use littermates in his experiments and that for this reason he found much greater sensitivity to olfactory manipulation in his subjects.

Also, it is not known how many animals comprised his experimental colonies. Eibl-Eibesfeldt (1970) maintains that rat societies, in the wild, are "closed anonymous groups" in which individual members are not recognized idiosyncratically but merely as familiar within the clan. Thus, in the present experiment, the clan bond may have been strengthened or reinforced by the familial relations of the pairs of littermates comprising the 8 experimental colonies.

Experiment 3

The reports of Steineger (1950) and Lorenz (1966) mentioned above, indicate that the odorants contained in the nesting materials of rat colonies are the specific identifiers determining clan affiliation. If in fact these stimuli are the sole means of intruder identification in the feral rat, then two visually identical objects containing unfamiliar colony odors should evoke similar effects on the behaviour of wild rat colonies in which they are introduced.

In the present experiment established colonies of feral rats were presented with two identical objects, one containing the nesting material from a foreign colony of male rats and the second containing an anesthetized unfamiliar male rat. Observation of the colonies was undertaken to determine whether apparent discriminations were made between the two stimuli. Such discriminations would provide further evidence of the importance of cues other than those contained in nesting materials in the process of consecific identification.

METHODS

Subjects

Four of the established pairs of male wild rats were used as subjects and four adult male hooded rats obtained from the Quebec Breeding Farms were incorporated as stimulus objects.

Procedure

The hooded rats were deeply anesthetized with Equi-Thesin (i.p. injection, .25cc/ 100 gm. body weight). The anesthetized rats were inserted into a sleeve of seamless tubular gauze bandage (Tubegauz, size 56, Scholl Mfg. Co.) which was doubled over the limp animal and secured at his posterior end with an elastic band. At the same time impregnated shavings were removed from the homecages of wild adult male rats and a second sleeve of similar dimensions was prepared. In accordance with the standard testing procedure these bags were simultaneously placed into the homecage territory of an established pair of adult feral rats in the first 30 min. of the feeding period and observations made.

RESULTS and DISCUSSION

Both gauze 'socks' presented to each colony were investigated by at least one member of each resident pair. Residents of two colonies exhibited full biting attacks to the bag containing the anesthetized male rat, and ignored the shavings-filled "dummy" after investigative sniffing. These attacks tended to be inordinately prolonged because intense biting attacks were often continuations of intermittent pulling at the gauze tubing. In other colonies where attacks were not demonstrated, agonistic behaviours were observed. Piloerection and lateral or 'threat' postures were exhibited by residents when approaching or in the vicinity of the sock containing a live immobile rat, but not in the presence of the shavings-filled bag.

These results indicate that the odor of a rat is clearly discriminable from the odors of urine and feces contained in nesting materials. The differential reactions to the two gauze 'objects' not only demonstrate the discriminability of their contents but also suggests that the odor of a male rat will elicit and direct specific behaviours that accessory odors do not. Furthermore, it should be noted that the residents in this situation do attack motionless rats even when visually occluded by the gauze sock. This finding may imply that behaviour other than respiratory movements is not absolutely necessary to elicit attack. However, the attacks that were directed at the sock containing an immobile intruder differed in topography from stereotyped aggressive patterns normally displayed by feral rats. Attacks were often initiated after the resident had climbed upon the bag containing the anesthetized animal. Under more natural conditions such pre-attack behaviour would not occur. The same uncommon prelude to attack was also observed in pilot studies in which a single live, anesthetized male hooded intruder was presented to resident pairs; these intruders were also attacked reliably (in all four cases).

In both the present experiment and the above pilot study inter-resident aggression, an unlikely occurence (Steineger, 1950; Barnett, 1963), was observed. Fighting between familiar rats seen in conjunction with the presence of an immobile, unresponsive intruder was brief, quickly terminated when one resident adopted a submissive posture (Grant, 1963), and occurred when and only when both residents were in the immediate vicinity of an immobile intruder. It is noteworthy that the second resident was never sought for an attack and if he was at the time within the nest box or out of the intruder's vicinity, the second resident would not, in fact, be the target of aggression. In these cases, when the second resident was not spatially present, a species-typical form of digging behaviour (Pisano & Storer, 1948) was often displayed by the investigating, piloerected colony member.

The situation-specific aspects of these behaviour sequences suggest that the inter-resident conflicts may be likened to a pattern of behaviour shown by a variety of species termed "redirection activities" (Bastock, Morris, & Moynihan, 1953). These authors have described numerous situations where the releasing stimulus that normally activates a behaviour is present but the aroused behaviour is directed toward a stimulus other than the activating one. They cite a study that indicates "when two angry animals are prevented from attacking one another by an intervening fence, one or both will turn and attack other animals". Thus, the immobile intruder may arouse a resident to aggression but fail to provide the necessary behavioural cues that normally release aggression. A second mobile resident, when in the vicinity of the anesthetized intruder would provide the necessary releasing cues and thus elicit attacks. The following experiment examines this possibility more closely.

Experiment 4

In the present experiment the established pairs of wild rats were presented with two unfamiliar male intruders. One of these was a live, but motionless hooded rat. The other was a free-moving intruder (hooded rat) introduced simultaneously. If the inter-resident conflicts reported in the previous experiment were, in fact, behavioural aberrations caused by an arousing but unresponsive intruder then the residents in this experiment should not only fail to demonstrate aggression between themselves, but should also direct most of their agonistic responses towards the more suitable stimulus.

METHODS

Subjects

The 8 established pairs of feral rats and 8 mature male hooded rats obtained from the Quebec Breeding Farms were the subjects for this experiment.

Procedure

Two intruders were introduced into each homecage with both residents present. One intruder was immobilized with an intraperitoneal injection of Equi-Thesin 15 min. prior to introduction. The second intruder was taken directly from its holding cage and placed into the territory at the same time. Observations were made in the usual manner.

RESULTS and DISCUSSION

The results of this experiment were consistent across all groups. Given two intruding rats differing only in mobility, motionless intruders were investigated and never attacked while freely moving intruders were repeatedly subject to consistent aggressive attack ($\bar{x}$ = 8.1; sd = 4.2). Furthermore, given an immobile intruder and a "behaving" rat, no aggression between residents was observed in this situation.

These data indicate that the attacks against immobile intruders and against fellow residents in Experiment 3 was a form of aberrant behaviour induced by a highly unnatural stimulus situation. Similarly, the inter-resident conflicts reported earlier now appear to have been caused by the immediate external stimuli (a moving resident in the vicinity of an arousing, immobile intruder). In light of this, it would appear that the behaviour, most particuarly the movement, of the interlopers does in fact function as a releaser of attacks once the resident has been aroused to aggression.

Experiment 5

Maintainance of colonial and territorial integrity requires that accurate discriminations be made between conspecifics that share a territory from those that are alien to it. The experiments presented thus far clearly demonstrate the wild rats' ability to make such distinctions. Earlier experiments in this paper demonstrate empirically that this discrimination can resist gross manipulations of superficial olfactory cues. These experiments however, are limited to the extent they demonstrate only the rats' ability to accurately recognize familiar rats on a common territory and to distinguish them from conspecifics who are themselves unfamiliar within that area. Recognition, therefore, could be based on a territorial rather than clan or individual identity. It may be the case that rats who are collectively familiar to a common territory will be, on that basis, familiar to each other. Intruder recognition may rely on a comparison of individual odor with some background odor of the terrain. The present experiment compares the interactions between conspecifics that (a) are familiar to each other but are not mutually common to a territory, and (b) are common to a territory but unfamiliar to each other.

METHODS

Subjects

Six resident pairs of wild rats used for previous experiments were subjects in Experiment 5.

Procedure

Resident pairs of rats were simultaneously removed from their territories. The larger or more aggressive rat of each pair was placed in a vacated homecage of another colony and the second member of each pair was placed in an individual hanging cage. Rats were introduced into new territories only minutes after the former residents had been removed and the nesting material and soiled shavings of the former residents were left intact. Re-located residents were maintained on the standard feeding schedule and permitted three days to establish themselves. On the third day following the reversal of territories a second rat was introduced for 30 min. into the homecage held by a relocated resident in accordance with the standard testing procedure. For half these residents the first intruder into the new territory was the familiar littermate that shared the original homecage. The next day a second intruder was presented in the same manner and this intruder was the lower ranking former resident of the same territory. Thus, each relocated resident confronted two intruders on successive days. Both intruders were equivalently ranking animals with similar histories; one was unfamiliar to the territory but familiar with its present resident and the other was familiar to the territory but foreign to the current resident.

RESULTS and DISCUSSION

The relocated residents, confronted on the new territory with an unfamiliar rat associated with that homecage failed, in every case (N = 6) to accept the former resident. Rejection of the unfamiliar former resident was evidenced by overt and persistent aggression in 4 out of 6 pairings and by feeding disruptions and lack of amicable behaviours in all cases.

Introduction of a familiar rat to the new territory produced contrasting results. In all 6 of these pairings, stereotyped amicable behaviours were exhibited between the two rats during the 30 min. test. Some agonistic behaviour was observed following introduction of a 'familiar intruder' to the new territory but in the 2 instances aggression was observed it occurred in the first 10 min. of the session and was followed by amicable interactions.

All aggressive encounters observed in these situations were initiated by the resident rat holding the territory for the 72 hr. prior to intruder introduction.

The results demonstrate that familiar and alien conspecifics can be differentiated independent of the territorial context. Intruder recognition appears to function without the existence of some incongruity between the intruder and the territory. Experiment 2 indicates that absence from the homecage for as long as 7 days will not abolish a rat's territorial identity so that the observed rejection of a former resident in the present experiment cannot be attributed to the 72 hr. removal of the previous resident. It appears, however, that some characteristic of the individual rat is the crucial identifier and that identity is not specific to the territory with which animals are mutually familiar. The results also imply that the behaviour of a wild rat in a novel territory is not a sufficient stimulus to release aggression or identify an interloper since the attacked alien rats in this experiment were familiar to the homecage while the accepted 'intruders', though familiar to the territorial resident, were not themselves familiar with the territory.

Experiment 6

The experiments presented in this study demonstrate that there are olfactory identifier substances that are intimately involved in the recognition processes of feral rats which, to some extent, determine the nature of interactions subsequent to recognition. The evidence examined thus far, however, does not indicate whether the cues function (1) to inhibit aggression towards conspecifics possessing familiar odorant qualities or (2) to elicit aggression towards conspecifics with novel odors. If the identifier odor functions to inhibit aggression, then anosmic residents should treat familiar and unfamiliar rats similarly and behave agonistically towards both. On the other hand, if the olfactory stimulus is an elicitor of aggression then an anosmic resident should again react identically to novel and familiar rats but instead, attack neither. The present experiment tests this hypothesis.

The most common method of producing anosmia in rats is by surgical ablation of the olfactory bulbs. This procedure, however, has many undesirable aspects, particularly in that it involves massive destructive intervention in the central nervous system. Whitten (1956; 1966) has stressed that evidence from bulbectomized animals must be interpreted with caution, noting evidence provided by Sawyer (1955; 1957) which indicates that normal hormonal function, particularly in the gonads, is dependent on intrinsic activity of the olfactory bulbs. The olfactory bulbs also contain the accessory olfactory bulbs which synapse onto pathways to the hypothalamus via the median forebrain bundle, or the amygdala and the stria terminalis (Allison, 1953).

Ropartz (1968), who destroyed the olfactory bulbs in mice, asserts that the resultant effects may be associated not only with elimination of olfactory input, but with the removal of a "facilitation or arousal mechanism" of the CNS. A technique has recently been reported which, on the basis of a simple behavioural test, produces acute anosmia in hooded rats (Alberts & Galef, in preparation). Anosmia is produced by briefly bathing the olfactory mucosa with a solution of zinc sulphate, leaving the olfactory bulbs and the rest of the CNS entirely intact.

In this experiment one resident of each established pair was given the intranasal zinc sulphate treatment and tested in the homecage for differential reactions to familiar and unfamiliar conspecifics. Treated residents were tested 24 hr. after zinc sulphate treatment when, according to the data with hooded rats, anosmia is complete; these animals were tested similarly 10 days after treatment when the anosmic condition subsides or disappears in treated laboratory rats.

METHODS

Subjects

Six resident pairs of male feral rats and six adult hooded males were subjects for this experiment.

Procedure

Resident pairs were removed from their homecages and the smaller or subordinate member of each pair was placed in an individual hanging cage. While under ether anesthesia each dominant resident was treated with a 10 per cent solution of zinc sulphate (wt./vol.), injected into the nasal cavity via the posterior choanae (Alberts & Galef, in preparation). Treated animals were returned immediately to their homecages following recovery from anesthesia and experimentation began during the next feeding period, 24 hr. later.

Dominant residents were tested first with an adult male hooded intruder placed in the homecage for 15 min. while the standard observations and recordings were made. After the hooded intruder was removed the untreated resident was returned. Observations were again made for at least 15 min. Ten days following zinc sulphate treatment the same testing procedure was repeated; subordinate residents had been removed for 21 hr. prior to the 10 day test.

RESULTS and DISCUSSION

Feral rats normally display vigorous attacks toward unfamiliar adult male hooded rats. Figure 2 illustrates that when rendered anosmic by a manipulation of peripheral portions of the olfactory system, residents showed a variety of behaviours towards both unfamiliar hooded intruders and familiar feral rats.

The most striking consequence of the treatment was that 4 of the 6 treated residents displayed stereotyped amicable behaviours towards the hooded intruders; 3 of these residents fed normally during the 15 min. test. Two anosmic residents demonstrated an additional dramatic shift in response to the unfamiliar male intruder. After investigating the intruder these residents mounted and attempted copulation with the interloper. In one instance the hooded rat upon one of these anosmic residents who had made 3 copulatory attempts, and displayed an aggressive posture. The resident then ceased copulatory behaviours and participated in aggressive interactions with the intruder. In another instance a hooded rat also displayed some agonistic behaviours to which the anosmic resident behaved submissively, turning on its back and remaining motionless until the hooded rat withdrew.

Anosmic residents appeared to treat reintroduced colony members in the same fashion as the hooded intruders during the second phase of the 24 hr. test (see Figure 2). Amicable behaviours or normal feeding patterns were maintained in all cages although one anosmic resident (Cage 1) who had previously mounted the hooded intruder also attempted copulation with the second resident which produced momentary inter-resident conflict that subsided within 5 min.

Figure 2 about here

The results of the 10 day test were somewhat different than the findings on the day immediately following the zinc sulphate, treatment; most hooded rats treated with zinc sulphate show recovery of function, measured by the ability to locate buried food after this period of time. When presented with the unfamiliar male rat, 4 of the 6 residents attempted copulation and in the 3 cases where the hooded intruders responded aggressively the wild rat adopted the characteristic submissive postures. One resident appeared to have recovered olfactory capabilities to some extent in that this resident, after feeding normally for 11 min., approached the hooded

FIGURE 2. Response Patterns of Anosmic Residents Presented with Familiar and Unfamiliar Rats. Darkened cells in matrix indicate occurrence of behavioural responses categorized as amicable, disruptive or aggressive, and sexual.

	24hr. TEST												10 day TEST											
	INTRUDER						RESIDENT						INTRUDER						RESIDENT					
ALLOGROOMING						▨				▨					▨				▨	▨				
WALKING OVER			▨			▨												▨			▨			▨
CRAWLING UNDER					▨				▨										▨			▨		
HUDDLING WITH INTRUDER				▨			▨				▨	▨	▨	▨					▨			▨		▨
NORMAL FEEDING			▨	▨	▨			▨	▨	▨	▨	▨		▨	▨		▨	▨	▨	▨	▨	▨	▨	▨
NO FEEDING	▨	▨				▨							▨											
MUTUAL AVOIDANCE																								
RESIDENT REMAINS IN NEST BOX		▨																						
LATERAL APPROACH	▨						▨										▨							
CHASE	▨																▨							
ATTACK	▨						▨										▨							
ATTEMPTED COPULATION	▨				▨		▨						▨		▨	▨								
SUBMISSIVE TO INTRUDER					▨		▨							▨	▨	▨								
CAGE NUMBERS	1	2	3	4	6	8	1	2	3	4	6	8	1	2	3	4	6	8	1	2	3	4	6	8

intruder and initiated a series of persisten aggressive encounters. When the second colony member was returned for the 10 day test, treated residents appeared capable of some discrimination since no copulatory or aggressive behaviours were displayed and normal activities were resumed.

The results indicate that normal olfactory input is essential to the feral rat for accurate identification of conspecifics. Wild rats, deprived of their olfactory capabilities, show a significant change in respnse pattern to unfamiliar rats while their behaviour towards familiar rats remains largely unaltered. Interlopers are not ignored by anosmic residents, their presence induces investigatory behaviour but these activities do not lead to normal indices of disturbance such as avoidance, cessation of feeding, or overt aggression and are instead followed by the same amicable acts usually displayed toward familiar conspecifics. This finding is particularly striking in the present experimental context where unfamiliar intruders were visually distinctive in size and coloration. Despite the available visual cues for discrimination of novelty, the residents either failed to recognize unfamiliarity or respond to it normally.

The instances where some aggression was observed between anosmic residents and intruders following the inappropriate copulatory attempts indicates that the effects of the treatment procedure did not eliminate intruder-directed aggression by rendering the rat incapable of performing aggressively but instead seems to have removed a stimulus that acts to specifically evoke and maintain the response.

As was reasoned in the introduction to this experiment, if aggression is normally inhibited by the familiar odors of colony members then anosmic residents would be expected to attack both familiar and unfamiliar rats; but this clearly is not the case. Thus, the results lead to the conclusion that intruder-directed aggression is normally the result of some specific novel olfactory characteristic of the interloper which functions to arouse aggressive tendencies in established residents. The results of this experiment suggest that the identifier is a pheromone that specifically elicits aggressive responses. In the 10 day test, most treated residents appeared capable of discriminating between members of the resident pair and unfamiliar intruders since they behaved differentially to the introduction of these individuals. Yet, 4 of the 5 treated residents who showed the differential reaction nevertheless failed to behave appropriately toward interlopers and persisted in copulatory attempts and not in normal intruder-directed activities. Such data could be accounted for by postulating a stage of incomplete recovery in which reaction to a specific olfactory cue is present but the recipient perceives a distorted message and displays behaviour characteristic of another motivational system.

SUMMARY and GENERAL DISCUSSION

The present experiments represent a preliminary investigation of the cues, both olfactory and behavioural, important in the elicitation of intraspecific aggression in feral Rattus norvegicus. The data indicate that olfactory cues are undoubtedly the most critical for the determination of colony identity and in recognition processes, but previous notions concerning the lability of specific odorant identifiers (Eibl-Eibesfeldt, 1970; Lorenz, 1966; Steineger, 1950) should be modified. The results of Experiments 1 and 2 imply that the rats' odoriferous colony-specific identity is highly resistant to alteration and is not substantially affected by the relatively vigorous regime of odorant manipulation employed here. The olfactory identity of the rat appears to be represented by a constellation of odors greater than the simple range of cues contained in the nesting matter of a colony. In Experiment 3, residents confronted with two visually identitical "intruders", showed a clear pattern of differential reaction to the odor of a rat contained in a porous sock compared to another porous sock containing the odors of another colony's nesting material.

In Experiment 5 it was found that the bond of familiarity was maintained outside of the specific territorial context which had supported the clan. This experiment also indicates that territorial affiliation is not functionally related to colonial affiliation since two rats, familiar to the same territory but not familiar to each other, behaved in a manner characteristic of unrelated rats on an established area of a single individual. Experiment 6 reveals the functional role of olfactory stimulation and its mode of control on individual behaviour.

The results of Experiment 6, in which residents were rendered anosmic, indicate that some aspect of an intruder's novel odor functions to arouse and maintain the aggression displayed in the territorial defense of the feral rat and tat, in the absence of olfactory input, the anosmic rat does not display agonistic behaviour in the presence of an intruder. Familiar odors of colony members do not seem to be inhibitors of aggression since untreated residents are not attacked by anosmic rats and are responded to with normal amicable behaviours. For this reason, the odor of a novel male appears to function not only as an identifier but also as a pheromone which arouses or elicits territorial aggression.

Behavioural cues appear to regulate specific interaction following identification; this regulatory function was first suggested by observations made in Experiment 1 in which intruder movement was exactly correlated with initiation of resident attacks. Stereotyped submissive or "cataleptic" postures may actively inhibit attack behaviour. Anesthetically immobilized intruders are sometimes attacked (Experiment 3) but aggression in these instances is topographically atypical. The incidence of inter-resident conflict in the presence of unresponseive intruders was interpretted to imply that ther experimental conditions led to aggressive acts best characterized as "redirection activities" (Bastock, et al., 1953). No evidence was found to suggest that interlopers were identified as unfamiliar by some characteristic behaviour pattern induced by confrontation with a novel environment. If this was the case, then immobile intruders (Experiments 3 and 4), hooded rats that behaved amicably in the novel situation (Experiments 4 and 6) and former residents returned to their homecages (Experiment 5), would have failed to elicit aggression.

The source of the olfactory identifier for rats, however, remains unknown. The sebaceous glands, which are highly developed in rats and other rodents (Montagna, 1962, p. 269; Quay, 1968), are a likely source. Sebaceous secretion is at least partly dependent on androgenic stimulation (Ebling, 1948; Haskin, Lasher & Rothman, 1953). Normal and castrated adult male rats have been shown to possess odors which are discriminably different to rats of either sex (Carr & Caul, 1962; Carr, Loeb & Dissinger, 1965). Barnett (1963) reports that adult male but not female or juvenile rats are subject to intruder-directed aggression. This body of evidence suggests that sexual maturity may be associated with endocrine changes and subsidiary effects that provide the odorant stimuli controlling intruder recognition and the residents' normal aggressive response.

In the strictest sense, pheromones produce specific behavioural responses in the recipient organism (Karlson & Luscher, 1959; Kalmus, 1964) and it may, in the future, be desirable to distinguish these substances from olfactory cues which have non-specific consequences such as general arousal or release of investigatory behaviour. Vertebrate behaviour is sufficiently complex, relative to insect activity, however, to lead most investigators to consider the olfactory signals of mammals as pheromones (Bruce, 1969; Gleason & Reynierse, 1969; Wilson & Bossert, 1963; Whitten, 1966).

Wilson and Bossert (1963) dichotomize pheromones into two types, according to effect. Primer pheromones initiate a series of physiological changes in the recipient, usually in endocrine secretion, of relatively long lasting duration. Immediate behavioural responses are caused by releaser pheromones. Whitten (1966) has pointed out that in some cases the same substance may have both primer and releaser effects.

The present experiments are interpreted to suggest that among the odors of male rats are substances which act as primer pheromones, particuarly in territorial situations where novely is discriminable. A model, illustrated by Figure 3, can account for the two distinct behaviour patterns adopted by established residents following introduction of either familiar or unfamiliar conspecifics in the homecage. Introduction of a familiar or unfamiliar intruder produces a state of general arousal in the resident(s) that leads to normal investigatory behaviours such as approach and investigation. This arousal may be accompanied by, or mediated by a "primer effect" based on known facilitators of excitability such as the adrenal and gonadal hormones. Investigations that provide familiar odorant stimuli would produce a gradual decrease in the general arousal initiated by intruder introduction and in most cases the familiar "intruder" would release or exhibit amicable behaviour and provide accessory stimuli (movement) leading to stereotyped amicable responses. Novel odors enhance the initial arousal and

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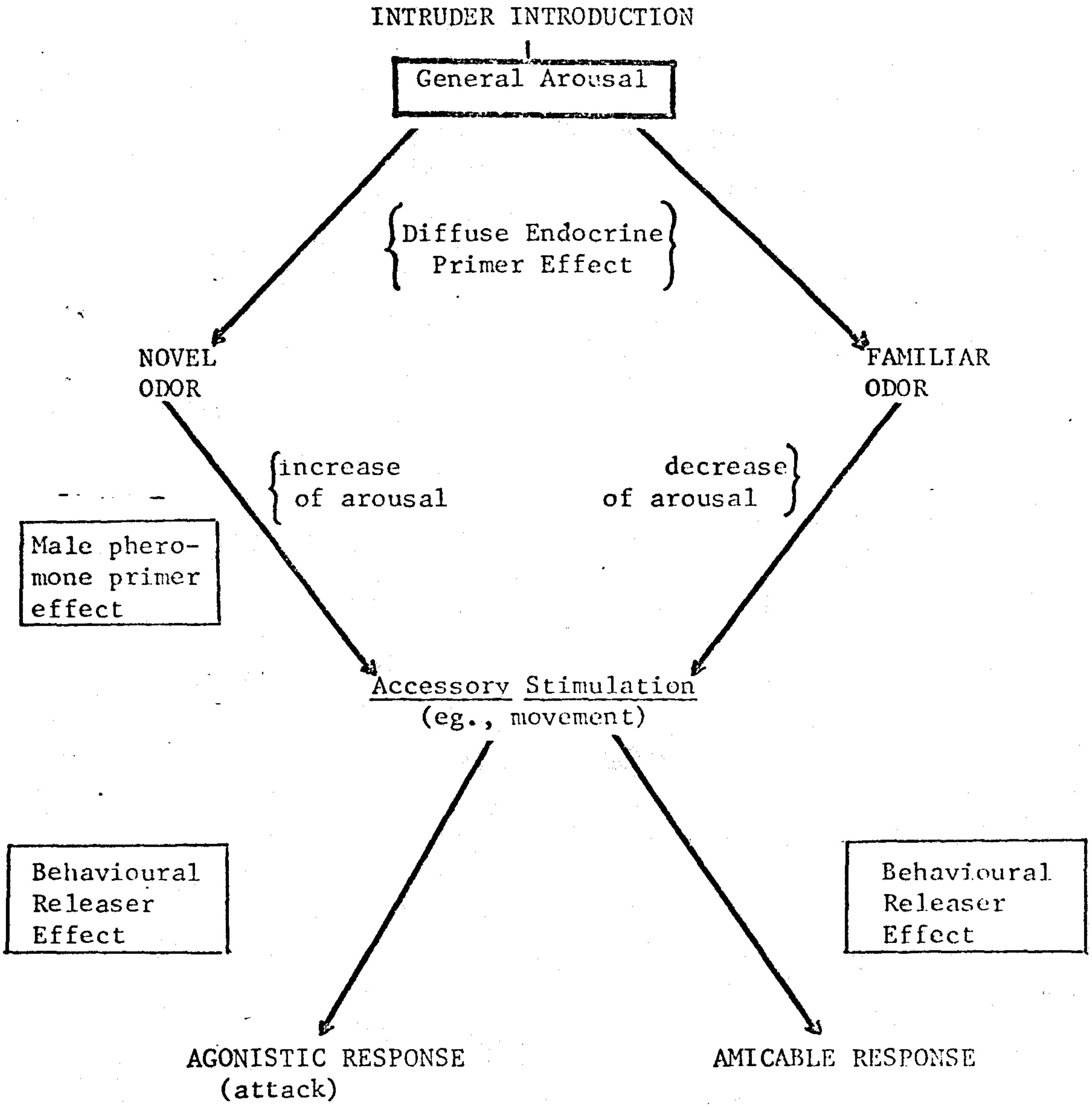
Figure 3 about here

Figure 3. A Model of Behavioural Sequence Reflecting Intraspecific Recognition Processes in a Territorial Situation.


INTRUDER INTRODUCTION
General Arousal
Diffuse Endocrine Primer Effect
NOVEL ODOR
FAMILIAR ODOR
increase of arousal
decrease of arousal
Male pheromone primer effect
Accessory Stimulation
(eg., movement)
Behavioural Releaser Effect
Behavioural Releaser Effect
AGONISTIC RESPONSE
(attack)
AMICABLE RESPONSE

are elaborated by the male pheromone that specifies agonistic response tendencies. Movement cues are now releasers of agonistic responses such as the lateral approach and attack.

In the absence of behavioural stimuli from the intruder, movement of non-arousing colony members can elicit attack; submissive postures actively inhibit aggression. In the absence of olfactory input, the general arousal is not enhanced. Animals who are only partially recovered do not perceive the specific male pheromone but may be aroused to perform a variety of intruder-directed behaviours.

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