

Why Offense Is Reduced When Rats Are Tested in a Strange Cage

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MINK, J. W. AND D. B. ADAMS. *Why offense is reduced when rats are tested in a strange cage.* *PHYSIOL. BEHAV.* 26(4) 567-573, 1981.—Tests of isolation-induced fighting and competitive fighting of rats in various conditions were used to evaluate the hypothesis that there is more offense in a familiar than in a strange cage because of the operation of an "olfactory comparator." The hypothesis was rejected. Although there was more offense in a familiar than in a strange cage, there was not, as predicted, more offense when Petri dishes containing familiar scent-markings were put in the strange test cage, and there was not, as predicted, a difference in the effect between isolation-induced fighting and competitive fighting. The data were consistent with either one or both of two alternative hypotheses: 1) that the strange cage produces fear (neophobia) that reduces offense; or 2) that the strange cage activates exploratory and scent-marking behaviors that compete with offense. The first of these two alternative hypotheses was also consistent with a finding that handling the test animal prior to a test reduces subsequent offense.

Fear Offense Fighting

RATS and other muroid rodents are more likely to show behaviors of offense in a familiar than in a strange environment. The behaviors of offense are apparently under the control of a single motivational mechanism, and they include the motor patterns of bite-and-kick attack, offensive sideways and upright postures, and piloerection [2,4]. In the rat these behaviors are shown more in a familiar environment [6,7], although negative results have occasionally been reported [14]. Greater probability of offense in a familiar environment has also been found in the laboratory mouse [11], *Peromyscus leucopus* [16], and *Notomys alexis* [17], although some negative results have also been reported in the mouse [5,8]. Apparently this increased offense plays a role in the territoriality of muroid rodents, in which dominant animals defend a familiar area against conspecific intruders of the same sex.

The greater probability of offense in a familiar environment led to the hypothesis that offense in male rats might be activated by an "olfactory comparator" mechanism that required simultaneous inputs from odors of an unfamiliar male rat and from odors of a familiar test environment, i.e., the animal's own scent-markings in the home cage [1]. This interpretation of the data was later challenged by Fass, Gutermann, and Stevens [9] who reported that intruder rats also may show offense on some occasions despite the fact that they have been put into an unfamiliar environment, i.e., the home cage of the opponent.

The present set of experiments was designed to test whether or not an "olfactory comparator" mechanism is responsible for the greater probability of offense in a familiar environment. Two types of offense were investigated. In the initial pair of experiments, the isolation-induced fighting of

male rats against an unfamiliar male intruder was tested under various conditions. The main effect was tested by comparing fighting in the home cage to that in a strange cage without familiar environmental stimuli. It was predicted that there would be more fighting in the home cage. The animals were also tested in a strange cage that included one or two Petri dishes containing its own scent-markings, i.e., familiar environmental stimuli. The hypothesis predicted that there would be more fighting in the strange cage condition when the Petri dishes with familiar scent-markings were present. In the last experiment, offense was produced by a different method, competitive fighting, that has been shown to occur in the absence of olfactory stimuli [15,18]. It was predicted that there should be no difference between the home cage and strange cage test conditions in the competitive fighting tests because under these conditions an olfactory comparator would not be involved, at least not as a necessary mechanism.

Other test conditions were also included to control for differences between the home cage and strange cage test conditions. In transporting the rat from the home cage to the strange cage, the experimenter handled the test animals. Therefore, a test condition was added for both types of fighting tests in which the test animals were handled and then replaced and tested in the home cage. Another test condition was also added in which the test animal was transported to the strange cage, but allowed to habituate to that cage for a half hour before being tested. It was predicted from the "olfactory comparator" hypothesis that these conditions should not produce results any different from the other conditions for which these were controls.

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TABLE 1
EXPERIMENTAL DESIGN

Experiments 1 and 2: Isolation-induced fighting							
Test parameter	Test conditions						
	Not handled	Handled	Warmup fight	Habituated	Two dish	One dish	No dish
Cage in which test occurred	Home	Home	Strange	Strange	Strange	Strange	Strange
Petri dishes present	Two	Two	Two	Two	Two	One	None
Test animal handled	No	Yes	Yes	Yes	Yes	Yes	Yes
Test animal habituated to test cage	—	—	No	Yes	No	No	No
Warm-up fight before test	No	No	Yes	No	No	No	No

Experiment 3: Competitive fighting

Test parameter	Test conditions		
	Not handled	Handled	No dish
Cage in which test occurred	Home	Home	Strange
Test animal handled	No	Yes	Yes

METHOD

The experimental animals and housing conditions were similar for all experiments. The animals were highly inbred laboratory rats of the Fischer, WAG-Rij and DA strains or their hybrids. They had not been used in previous experiments. Housing and test cages were 162×25 cm and constructed of wire mesh with a Plexiglas front. Water was always available and, except for specified deprivation periods, rat chow was available in a feeding hopper attached on the outside of the wire mesh wall at one end of the cage. The room was illuminated with white light between midnight and noon, and with red light only between noon and midnight. All testing was done during the dark cycle with red light illumination.

Three experiments were conducted, two of isolation-induced fighting and one of competitive fighting.

Isolation-Induced Fighting, Initial Tests

The subjects were six male Fischer rats, weaned at age 21 days, group housed for three months, and then isolated for one month prior to testing. They weighed between 300 and 400 g when tested. They were called "resident rats," and were the source of the critical data. Other rats, called "intruder rats," were introduced into their cages during testing. Intruder rats consisted of six Fischer or WAG-Rij animals, housed in groups; they also weighed between 300 and 400 g at the time of testing.

In the home cage of each resident rat were two glass Petri dishes that were placed loosely on the wire mesh floor and

left there for the entire experiment. During the course of their isolation the resident rats scent-marked these dishes which thereby became encrusted with an oily brown odorous substance. No attempt was made to control for the fact that some of the rats accumulated more of the substance than others; however, all of the rats had dishes that were encrusted to some extent.

Each resident rat was tested in each of seven conditions, one condition each week. The seven conditions are shown in detail in Table 1. 1) Home cage condition: the intruder was introduced into the subject's home cage, containing two Petri dishes, for 20 minutes. 2) Home cage with handling condition: the resident was removed from his home cage, transported by hand to the far side of the testing room, and returned to the home cage, which included the Petri dishes, at which time the intruder was introduced for 20 minutes. 3) Strange cage with no Petri dish: the resident was transported by hand across the room to a strange cage of the same size and shape as the home cage but which had been cleaned just prior to testing, and the intruder was immediately introduced for 20 minutes. 4) Strange cage with one Petri dish: the resident, along with one of his Petri dishes encrusted with his own scent-marking, was transported by hand across the room to a strange cage of the same size and shape as the home cage but which had been cleaned just prior to testing, and the intruder was immediately introduced for 20 minutes. 5) Strange cage with two Petri dishes: similar to the previously described condition except that two rather than one of the resident rat's Petri dishes were transported with him to the strange test cage. 6) Strange cage with habituation: simi-

lar to the previously described condition except that the resident was allowed to explore the strange test cage for 30 minutes before the intruder was introduced for his 20-minute test. 7) Strange cage with warmup fight: the intruder was introduced into the resident's home cage and the resident was allowed to initiate offense against the intruder (offensive sideways posture), at which time both rats and both Petri dishes were transported to the strange cage and a 20 minute test commenced.

The testing was counter-balanced so that each resident rat was tested in each test condition once and it was tested against each intruder rat only once. Furthermore, the sequence of test conditions was counter-balanced so that there could be no confounding of serial order effects with test conditions. Each intruder was tested only once in each test condition.

Motor patterns of offense and other related behaviors were recorded on a scoring sheet on a minute-by-minute basis for the 20 minute tests. The scoring system was adapted from the postures described by Grant and Mackintosh [10] and was the same as that used by Lehman and Adams [12]. The following acts and postures were consistently recorded: crawl-over-dish (i.e. Petri dish); offensive sideways posture, full aggressive posture, bite-and-kick attack, upright posture, and crouch. The presence or absence of each act or posture was indicated on a minute-by-minute basis without regard to frequency within each minute.

Isolation-Induced Fighting, Replication

In this replication, the testing conditions and environmental conditions were identical to those of the initial tests. The only difference was in the experimental subjects and longer social isolation prior to the testing. The subjects were seven male Fischer/DA hybrids, weaned at age 21 days, isolated immediately and tested after they reached the age of 100 days. A new set of intruder rats was used; as in the initial tests, they consisted of seven group-housed Fischer and WAG-Rij rats.

Competitive-Fighting

The subjects were 42 DA highly-inbred laboratory rats, weaned at age 21 days, group-housed with littermates until four months of age, and then paired with Fischer/DA hybrids. Half of the 42 pairs consisted of two male rats and half of them consisted of two female rats. In previous experiments it has been found that the DA rats have a higher rate of competitive fighting than do the Fischer/DA hybrids [3].

Competitive fighting was elicited by the method employed by Zook and Adams [18]. Prior to testing each pair of animals was deprived of food for 24 hours. Testing was begun by placing a single pellet of lab chow into the food hopper located at one end of the cage. Due to the limited space in front of the hopper, each animal jostled with its cagemate for position at the hopper, and, in some cases, offense motor patterns were elicited. Notice that, in contrast to the procedure used in isolation-induced fighting, neither animal had been socially isolated and each was familiar with each other. In all cases the pair of animals had been cagemates for at least three weeks prior to the first tests for competitive fighting. All tests were 20 minutes in duration, with the beginning of the test defined as the time when one or both of the animals began to feed consistently at the hopper.

Each pair of rats was tested once a week in each of three conditions. 1) Home cage condition: the food was simply

placed into the hopper and the pair of rats was observed for 20 minutes after feeding began. 2) Home cage with handling condition: each of the rats was removed from the home cage, transported to the far side of the testing room, and returned to the home cage, at which time the food was placed into the hopper and testing begun. 3) Strange cage with no Petri dish: both rats were transported by hand across the room to a strange cage of the same size and shape as the home cage but with the food hopper at the opposite end of the cage, and the food was placed into the hopper and testing begun. In none of the three conditions were there any Petri dishes present.

The testing was counter-balanced so that each pair of rats was tested in each test condition once, and the sequence of test conditions was designed so that there was no confounding of serial order with test condition.

Motor patterns of offense, exploration and other related behaviors were recorded on a scoring sheet on a minute-by-minute basis for each 20 minute test as in the isolation-induced fighting tests.

RESULTS

Offense, defined as the occurrence of bite-and-kick attack or offensive sideways posture, occurred frequently in both the isolation-induced fighting tests and the competitive fighting tests. In the tests of isolation-induced fighting, offense occurred in 47 of 91 tests with a mean latency of 8.4 minutes. All 13 resident rats showed offensive sideways posture on at least one test and only one subject failed to show a bite-and-kick attack. With the exception of one occurrence of offensive sideways posture, the intruders did not show any offense. In the tests of competitive fighting, offense occurred in 81 of 126 tests with a mean latency of 7.7 minutes. In 36 of the 42 pairs of rats, one animal became dominant and showed offensive sideways posture and 31 showed bite-and-kick attack. Under optimal test conditions (home cage, not handled) offense occurred in 85% and 81% of isolation-induced and competitive fighting tests, respectively. There was no observable difference in the form of the offensive sideways posture or bite-and-kick attack in the two kinds of fighting.

Offense was quantified in terms of an "offense score." This score was calculated as the sum of the *frequency* of bite-and-kick attack and the *occurrence* of offensive sideways posture (maximum of one) without regard to the frequency of the latter. This was necessitated by the complex relationship between these two motor patterns of offense which will be considered in the discussion section.

Offense scores were significantly affected by the test conditions in both isolation-induced fighting and competitive fighting. This was determined by a repeated measures analysis of variance for both isolation-induced fighting, $F(6,77)=7.68, p<0.01$, and competitive fighting, $F(2,121)=8.76, p<0.01$.

As predicted, rats in the non-handled home cage test conditions had the highest offense scores and rats tested in a strange cage had the lowest scores. This was true for both isolation-induced fighting and competitive fighting as shown in Fig. 1. In the other test conditions the offense scores were intermediate.

In the isolation-induced fighting tests there were many significant differences in offense scores as a function of test condition. Offense scores were significantly higher in the home cage regardless of handling, as indicated by higher scores in the home cage plus handling condition than in the appropriate control which was the strange cage condition

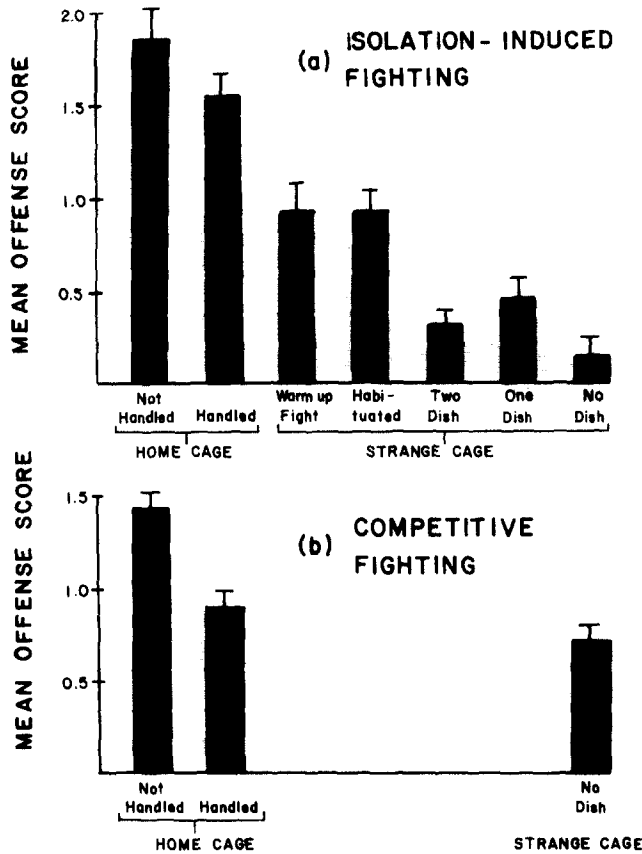


FIG. 1. Mean offense score as a function of test conditions for both isolation-induced fighting and competitive fighting. Data from initial tests and replication of isolation-induced fighting tests are combined since they are similar. Offense score is defined as frequency of bite-and-kick attack plus one if there was an occurrence of offensive sideways posture. Test conditions described in Method section. Standard errors shown by vertical lines.

with two Petri dishes, $t(12)=4.70$, $p<0.01$. The warmup offense condition also increased subsequent offense, as indicated by higher offense scores in that condition than in the appropriate control which was the strange cage condition with two Petri dishes, $t(12)=2.63$, $p<0.05$. Habituation to the test cage also increased offense, as indicated by higher offense scores in the habituation condition than in the strange cage tests with two Petri dishes, $t(12)=2.23$, $p<0.05$. Handling, although it reduced the mean offense score from 1.85 in the home cage without handling to 1.54 in the home cage with handling, was not highly significant in its effect, $t(12)=1.05$, $p>0.10$. There was no significant difference between mean offense score with one or two Petri dishes (on the one hand) and with no Petri dish (on the other) in the neutral cage, contrary to the "olfactory comparator" hypothesis.

In the competitive fighting tests, the non-handled test condition produced offense scores significantly higher than the handled test condition, $t(41)=2.41$, $p<0.02$, but the difference was not significant between the handled home cage test condition and the handled strange cage condition.

The data on latency of offense was complementary to the data expressed in terms of offense scores. We did not include data on latency from tests in which no offense occurred, e.g.,

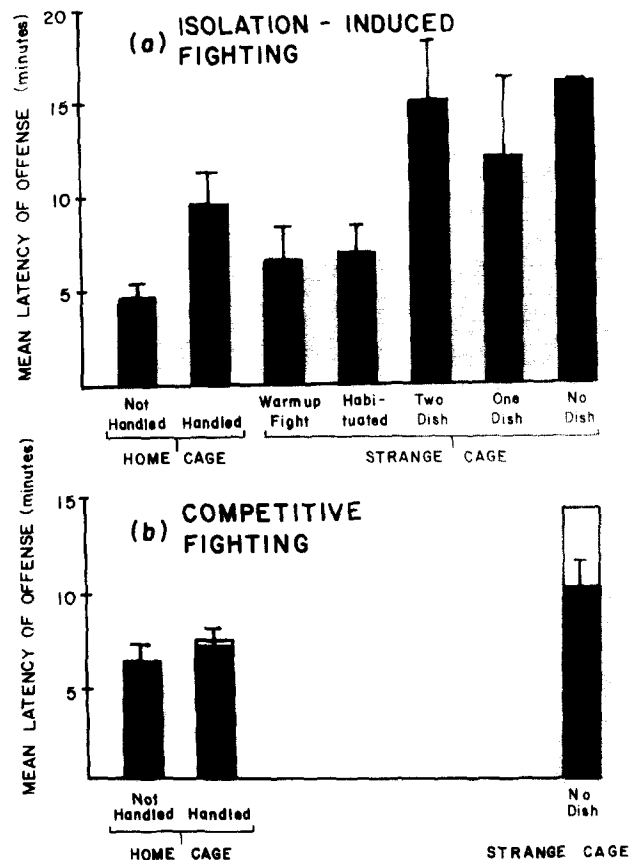


FIG. 2. Mean latency of offense as a function of test conditions for both isolation-induced fighting and competitive fighting. Data from all isolation-induced fighting tests combined. Latency calculated as number of minutes until the first occurrence of bite-and-kick attack or offensive sideways posture. If neither behavior occurred, no latency data are calculated. Test conditions described in Method section. For competitive fighting data each column consists of two portions: the filled portion corresponds to the latency after one or both animals began feeding; the clear portion corresponds to the latency between the time that the piece of food was introduced and one or both animals began feeding. Standard errors shown for latency after feeding.

by counting such data as an arbitrary latency greater than the duration of the test, because such data would have confounded the latency and offense score results. Instead, we calculated latency independently by scoring latency data only on those tests in which some offense occurred. Despite the fact that the data were independent, the results were very similar as may be seen from a comparison of Figs. 1 and 2. In those test conditions with high offense scores there was a low latency for offense and vice versa. One important exception should be noted, however: handling, which had a non-significant effect upon offense scores for isolation-induced fighting in the home cage produced a significant effect upon the latency to offense, $t(9)=4.21$, $p<0.01$. In competitive fighting tests, the latency data were similar whether the start of the test was marked as the insertion of the food (open bars in the figure) or the initiation of feeding (solid portion of bars in figure).

The latency to feeding in the competitive fighting tests also paralleled the latency to offense, as may be seen in Fig.

2. Feeding by the DA rat occurred within the first minute in the home cage, with or without handling, in 80 of the 84 such tests. It was rarely interrupted by exploratory behavior or freezing until after fighting had occurred. In the strange cage test condition, however, feeding did not occur until the fourth minute on the average, and during the first minute in only 14 of the 42 such tests. Instead, feeding was delayed, at least in part, by exploratory locomotion that occurred during those minutes in 20 tests and was apparently related to the fact that the rats were accustomed to feeding at the opposite end of the cage.

Females showed more offense than males in the competitive fighting tests. The mean offense score for females was 1.46 and for males was .56. This difference was significant in an overall repeated measures analysis of variance performed on the competitive fighting data, $F(1,121)=41.37, p<0.01$.

In the isolation-induced fighting tests, there was a slight difference between the two groups of animals tested in the two replication: mean offense scores 6.7 and 8.6 respectively, $F(1,77)=4.15, p<0.05$. The explanation of this difference is complicated by the fact that the members of the first group were Fischers and those of the second group were hybrids, the second group had been isolated longer, and the two sets of tests were done several months apart.

During the course of the experiments there was little change in either offense scores or latency of offense as a function of successive testing. This was the case for both isolation-induced fighting and competitive fighting. Offense scores ranged from .71 to 1.00 over the course of seven weeks of isolation-induced fighting and from .83 to 1.15 over the course of three weeks of competitive fighting. There was no tendency for these scores to increase or decrease over time. Similarly, there were no consistent changes in offense latency over time.

Crawl-over-dish scent-marking occurred regularly in the isolation-induced fighting tests and was significantly affected by test condition. The behavior, which involves a stereotyped lowering of the anogenital region to make contact with an object as the animal passes over it, is easily distinguishable from randomly-directed locomotion. As shown in Fig. 3, scent-marking was most frequent in the condition of testing in a strange cage with two Petri dishes, and was least in the conditions of testing in the home cage. A repeated measures analysis of variance indicated that the effects of test conditions were significant, $F(5,66)=5.76, p<0.01$. Therefore, individual *t*-tests were done on selected pairs of test conditions. Scent-marking was greater in the strange cage than in the home cage, $t(76)=2.42, p<0.02$, when all test scores were considered. Habituation to the test chamber reduced scent-marking, as shown by a paired-comparison *t*-test between the habituated condition and the condition with two Petri dishes, $t(12)=3.04, p<0.02$. There was more marking in the condition with two dishes than with one, as shown by a paired-comparison *t*-test between these two conditions, $t(12)=3.01, p<0.02$. There was also a major difference between the hybrid and Fischer groups, with the latter showing more scent-marking, $F(1,66)=40.30, p<0.01$. The difference between the two groups occurred primarily in the strange test cage conditions, as indicated by a strong significant interaction between group and test condition, $F(5,66)=3.97, p<0.01$.

DISCUSSION

The data contradict the hypothesis that lower rates of offense in a strange cage are due to the operation of an "ol-

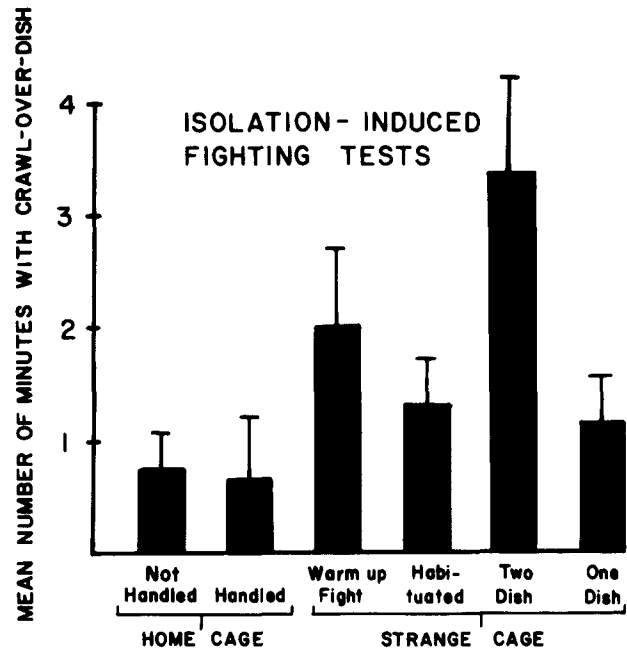


FIG. 3. Mean number of minutes with crawl-over-dish behavior as a function of test conditions for tests of isolation-induced fighting. Data from all isolation-induced fighting tests combined. Test conditions described in Method section. Note that there are no data for the test condition of strange cage testing with no Petri dish; there could be no crawl-over-dish behavior in the absence of the dish. Standard errors shown by vertical lines.

factory comparator" mechanism. First, the hypothesis would predict more offense in those conditions in which the Petri dishes were moved with the animal to the strange cage testing condition, but no difference was found between conditions with and without the Petri dishes. Although there were no associated differences in offense, there were significant differences in scent-marking as a function of the number of Petri dishes which indicates that the test animals were aware of the presence of the dishes. Second, the hypothesis would predict that there would be a decrease in offense in the strange cage test condition in isolation-induced fighting which depends upon olfaction, but not in the equivalent condition in competitive fighting for which olfaction is not necessary. In contradiction to the hypothesis, the decrease in offense in a strange cage was strong in competitive fighting as well as in isolation-induced fighting. Third, there were significant differences in level of fighting as a function of handling and habituation which support alternative hypotheses, but cannot be explained by an "olfactory comparator" hypothesis.

The data are consistent with either of two alternative hypotheses, although they do not make possible a definitive decision as to which (or both) are critical: a fear hypothesis, and an exploration hypothesis.

The fear hypothesis can explain not only the decrease in offense in a strange cage, but also the decreased offense after handling and the higher level of offense in a strange cage after habituation. The hypothesis depends upon the fact that both handling and a strange environment (i.e., neophobia) produce fear, which can be defined as the motivational state of an animal when its defense or submission motivational systems are activated [4]. According to the hypothesis the

activation of these motivational systems (i.e., fear) would result in a decrease in offense behavior by direct inhibition of an offense motivational system [4]. Since habituation to the strange cage would reduce the neophobia, the hypothesis would also predict the higher levels of offense that occur in that condition when compared to the strange cage tests without habituation. The fear hypothesis is also consistent with a recent finding that offense by laboratory rats is reduced when a cat is introduced into the pen where the test is conducted (Blanchard and Blanchard, in press).

The exploration hypothesis can explain the decrease of offense in a strange cage and the higher levels of offense in a strange cage after habituation, although it cannot explain the decreased offense after handling. The hypothesis depends upon the fact that unfamiliar odors, such as those encountered in a strange cage, activate a motivational system that has been called patrol/markings [4] and thereby activate motor patterns that may be incompatible or competitive with those of offense. These motor patterns include exploratory locomotion and scent-marking. Some of the data are consistent with this hypothesis; thus there was an inverse relationship between offense score and crawl-over-dish scent-marking in the conditions of home cage testing, habituation tests, and testing with two Petri dishes, and there was more exploratory locomotion in tests of competitive fighting in a strange cage than in the home cage. However, other data could not have been predicted from an exploration hypothesis; despite the fact that there was no increase in exploration or scent-marking as a result of handling, there were decreases in offense following handling.

It is possible that both the fear and exploration hypotheses are correct and that the results are due to an interaction of the two effects. Further experiments would be required to test this possibility.

The rejection of the hypothesis that the rat uses an "olfactory comparator" to compare an opponent's odor to those of the environment does not address the possibility of another type of comparator mechanism that may compare the opponent's odor to the subject's own body odors, as proposed by Fass *et al.* [9]. Even if such a mechanism exists, however, it could not explain why rats show less offense in a strange cage, since the experimental subjects presumably carried their odors with them equally in all of the test conditions employed here.

The calculation of an "offense score" is an innovation in the present study that is necessitated by the complex relationship of the offense motor patterns of bite-and-kick attack and offensive sideways posture. The former (but not the lat-

ter) may function as a consummatory response that lowers the intensity of the motivational state of offense [2,4]. This is suggested by two sets of data in our previous work. First, there is an apparent refractory period for offense motor patterns during the two or three minutes following a bite-and-kick attack (see Fig. 2 in [1] and Fig. 2 in [12]). Second, the bite-and-kick attack is the *only* motor pattern of an offensive rat that is not followed at a non-random probability by some other act or posture (Fig. 3 in [12]), an effect that would be expected from a consummatory response. Since offensive sideways posture usually occurs earlier and more often than bite-and-kick attack, one may assume that it has a lower threshold of elicitation than the latter. As a result of the preceding considerations one would expect that a test with a high frequency of offensive sideways posture and no bite-and-kick attack paradoxically would reflect a lower motivational state of offense than one in which a bite-and-kick attack occurred and there was little or no subsequent offensive sideways posture. Therefore, it would not be appropriate to measure offense by the sum of the frequencies of the two motor patterns. One way to score offense would be to ignore offensive sideways posture and count only the frequency of bite-and-kick attack. However, the presence of some offensive sideways posture probably reflects more offense than the total lack of it, especially if no bite-and-kick occurs; hence our decision to score one for the presence of this motor pattern.

In criticizing previous studies for finding no offense by intruders, Fass *et al.* [9] seem to have overlooked the important factor of social isolation. They used isolated intruders, which increases the duration and damage of fighting in comparison to socially-housed intruders [13]. Previous studies, like the present one, used socially-housed intruders which have lower levels of offense. As shown by the present results, there is summation of social experience and test-cage familiarity. Highest levels of offense are from isolated males in a familiar cage and lowest are from socially-housed males in an unfamiliar place. Isolated males in an unfamiliar place (such as the intruders of Fass *et al.* and the experimental subjects in some test conditions of the present study) have intermediate levels of offense.

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