Resident rats' aggression toward intruders

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A study was performed in which attacks by four different types of "resident" rat (males housed with fertile females, males housed with sterile females, paired males, and isolated males) on six different types of intruder (isolated males, grouped males, castrated males, isolated females, grouped females, and ovariectomized females) were investigated. The objective was to study features of resident and intruder rats that would allow the designing of an aggression test that used a minimum of animals and produced a rapid behavioral response. In some combinations of residents and intruders, attack was generated within a 10-min test period. Isolated resident males attacked as much as males housed with females; however, paired rats showed only low incidences of attack. The fertility of the female partner did not influence the male's aggressiveness. Most male attacks were directed towards like-sexed intruders. Only isolated males differentiated between the different treatment types of male intruder, attacking group-housed and castrated rats less intensely than isolates. Of the females, only those that were fertile produced significant amounts of attack behavior and almost exclusively attacked female intruders. Group-housed intruder females received more attacks than isolates. The results suggest optimal conditions for generating two models of attack behavior in the laboratory rat.

Laboratory rat strains show relatively little spontaneous fighting; Lockard (1968) pessimistically attributed this to their retention of only vestigial remnants of the attack and defense patterns of their ancestors. However, recent studies suggest that isolation (which is the common method of generating fighting in mice) is not the optimal strategy for generating high levels of intermale fighting in rats: colony-housed wild-strain rats (*Rattus norvegicus* L.) have been found to direct vigorous attack towards intruder animals (Barnett, 1958, 1969; Calhoun, 1962). Blanchard, Fukunaga, Blanchard, and Kelley (1975) and Flannelly and Lore (1977) also describe how small mixed-sex colonies of rats consistently attack "intruder" animals. The intensity of attack in this intruder-resident model of aggression appears to be influenced by a range of variables including the presence of females (Barnett, Evans, & Stoddart, 1968) and whether the intruder has been isolated previously (Flannelly & Lore, 1975; Luciano & Lore, 1975).

Although there has recently been success in inducing spontaneous intraspecific attack in laboratory strains of rat, the situation is still rather complex and the onset of attack is relatively slow; for example, Flannelly and Lore (1975) record that fighting does not appear until at least 1 h after the introduction

of a strange rat into a colony. The objective of the present study was to examine features of residents and intruders that would allow the designing of an aggression test that uses the minimum number of resident animals and produces a faster behavioral response than in previous studies.

METHODS

Animals

One hundred and two adult Lister hooded rats bred in the animal facilities of The University College of Swansea and aged 100 days at the start of the experiment were used in this investigation. A reversed lighting schedule was employed, with white fluorescent lights on between 2230 and 1030 h. The temperature of the facility was regulated between 18° and 22°C, and the animal cages were regularly supplied with fresh sawdust litter. Rodent food (Pilsbury's Breeding diet) and water were freely available, except at the time of intruder tests.

Residents

"Resident" animals received the other animals (the "intruders") into their home cages, which were wire-topped, opaque plastic cages measuring 56 × 38 × 18 cm. Four different categories (N = 6) of resident were produced: (1) one intact male with one intact female partner (these animals bred freely, but litters were always removed at birth); (2) one intact male with one female partner whose fallopian tubes were sutured (obviously, such animals never produced young but frequent mating activity was observed; it should be noted that these animals would become pseudopregnant and therefore come on to heat about half as often as those which were allowed to go through pregnancy); (3) paired intact males; and (4) individually housed intact males.

Intruders

Six different categories (N = 10) of intruders were employed, housed in wire-topped plastic cages measuring 45 × 28 × 22 cm: (1) a group of 10 intact males; (2) a group of 10 intact females;

The authors thank Professor E. W. Knight-Jones for facilities in Swansea, and Messrs. Roger Guntrip and James Young for care of animals. Please note that D. Benton is at the Department of Psychology; the other authors are in the Department of Zoology.

(3) individually housed, sham-castrated males; (4) individually housed, sham-ovariectomized females; (5) individually housed, castrated males; and (6) individually housed, ovariectomized females.

The animals in different categories were marked with fur dye to facilitate individual recognition. They remained together for 95 days to ensure that the social organizations within residential cages were established firmly.

Aggression Test

Ninety-five days after the groups of residents were established, they received a 10-min encounter with an intruder on 6 consecutive days. Each resident group received an intruder of a different type on each day. The order in which particular cages of residents received particular types of intruder was balanced in such a way as to counteract the effects of previous behavior on that subsequently observed. Encounters took place under dim red lighting in the early "dark" portion of the reversed lighting schedule (i.e., 1030-1400 h). Cages of resident animals were cleaned the day before the first test and immediately after each subsequent encounter to prevent any buildup of behavior-modifying odors. As the major aim of this study was simply to identify features of residents and intruders associated with unequivocal attack behavior, relatively conservative measures of overt attack were recorded. These included (1) a simple record of whether overt biting attack on the intruder was observed within the test, and (2) the number of bouts of biting attack on the intruder; these were discrete attacks, separated by activities such as exploration, grooming, threat, mounting attempts, etc. (3) The accumulated

attacking time (AAT) in seconds, that is, the total duration of time within an encounter in which the resident/residents directed biting attack towards the intruder, was also recorded.

The differences in the proportion of cages in which fighting was observed were compared using Fisher's exact probability test. The number of attacks and accumulated attacking times are comparisons between these measures and were made using the Mann-Whitney U test (Siegel, 1956).

RESULTS

The responses of the four categories of resident to the different types of intruder are presented separately in Tables 1 to 4. It should be noted that responses to a particular type of intruder were not altered by previous experience with other types of intruder. To facilitate the description of results, initially the effects of different types of intruder to the different categories of resident are described, and then the categories of resident are contrasted.

Comparisons Between Intruders

Isolated male and grouped male intruders were attacked more frequently and with a higher intensity than female counterparts (Tables 1, 2, and 4) by

Table 1
Aggressive Behavior of a Cohabiting Intact Male and an Intact Female Rat Directed Toward Different Types of Intruder

Type of Intruder	Male Resident					Female Resident				
	P	Number of Attacks		AAT (in Seconds)		P	Number of Attacks		AAT (in Seconds)	
		Median	Range	Median	Range		Median	Range	Median	Range
Isolated Male	6/6**	6.5††	3-13	2.90††	1.3-18.2	0/6	0†	0-0	.00†	0-0
Grouped Male	4/6*	6.0‡	0-10	1.45‡	0-3.4	0/6*	0‡‡	0-0	.00‡‡	0-0
Castrated Male	4/6	4.5	0-20	1.40	0-3.3	1/6	0	0-6	.00	0-1.8
Isolated Female	0/6	.0	0-0	.00	0-0	3/6	1‡	0-3	.25‡	0-9
Grouped Female	0/6	.0	0-0	.00	0-0	5/6	4	0-6	1.10	0-2.2
Ovariectomized Female	1/6	.0	0-1	.00	0-3	4/6	2	0-7	.60	0-1.9

Note—P = proportion of cages showing fighting. The number of attacks and accumulated attacking time (AAT) each represent the product of six observations per cage. *Differs from grouped female ($p < .05$) by Fisher's exact probability test. **Differs from isolated female ($p < .005$) by Fisher's exact probability test. †Differs from isolated female ($p < .03$) by Mann-Whitney U test. ††Differs from isolated female ($p < .001$) by Mann-Whitney U test. ‡Differs from grouped female ($p < .05$) by Mann-Whitney U test. ‡‡Differs from grouped female ($p < .008$) by Mann-Whitney U test.

Table 2
Aggressive Behavior of Cohabiting Intact Male and Sterile Female Directed Toward Different Types of Intruder

Type of Intruder	P	Male Resident				P	Female Resident			
		Number of Attacks		AAT (in Seconds)			Number of Attacks		AAT (in Seconds)	
		Median	Range	Median	Range		Median	Range	Median	Range
Isolated Male	5/6 *	5.5†	0-32	1.8†	0-12.5	0/6	0	0-0	0	0-0
Grouped Male	6/6	4.0††	1-10	1.3††	.3-2.6	0/6	0	0-0	0	0-0
Castrated Male	5/6	1.5	0-9	.9	0-3	0/6	0	0-0	0	0-0
Isolated Female	1/6	.0	0-1	.0	0-3	1/6	0	0-5	0	0-1.3
Grouped Female	0/6	.0	0-0	.0	0-0	0/6	0	0-0	0	0-0
Ovariectomized Female	2/6	.0	0-2	.0	0-6	2/6	0	0-4	0	0-1.5

Note—P = proportion of cages showing fighting. The number of attacks and accumulated attacking time (AAT) are each the product of six observations per cage. *Differs from isolated female ($p < .05$) by Fisher's exact probability test. †Differs from isolated female ($p < .05$) by Mann-Whitney U test. ††Differs from grouped female ($p < .001$) by Mann-Whitney U test.

Table 3
Aggressive Behavior of Paired Male Rats Directed Toward
Different Types of Intruders

Type of Intruder	P	Number of Attacks		AAT (in Seconds)	
		M	R	M	R
Isolated Male	2/6	0	0-2	0	0-1.8
Grouped Male	0/6	0	0-0	0	0-0
Castrated Male	1/6	0	0-1	0	0-3
Isolated Female	1/6	0	0-1	0	0-3
Grouped Female	0/6	0	0-0	0	0-0
Ovariectomized Female	0/6	0	0-0	0	0-0

Note—P = proportion of cages showing fighting; M = median; R = range. The number of attacks and accumulated attacking time (AAT) are each the product of six observations per cage. None of these comparisons reached significance.

Table 4
Aggressive Behavior of Individually Housed Male Rats
Directed Toward Different Types of Intruder

Type of Intruder	P	Number of Attacks		AAT (in Seconds)	
		M	R	M	R
Isolated Male	6/6**	3.0‡	1-8	1.80†‡	.9-1.7
Grouped Male	4/6	1.5	0-5	.40	0-2.2
Castrated Male	3/6	.5	0-2	.15	0-1.1
Isolated Female	0/0	.0	0-0	.00	0-0
Grouped Female	0/0*	.0	0-0	.00†	0-0
Ovariectomized Female	1/6	.0	0-1	.00	0-3

Note—P = proportion of cages showing fighting; M = median; R = range. The number of attacks and accumulated attacking time (AAT) are each the product of six observations per cage.

*Differs from grouped male ($p < .05$) by Fisher's exact probability test. **Differs from isolated female ($p < .005$) by Fisher's exact probability test.

†Differs from grouped male ($p < .03$) by Mann-Whitney U test. ‡Differs from castrated male ($p < .01$) and isolated female ($p < .001$) by Mann-Whitney U test.

all male residents except those that had been paired (Table 3). Male residents failed to make any other distinctions between intruders except in the case of the individually housed category (Table 4), where residents directed more attack towards isolated male intruders than towards either group housed or castrated counterparts. Intact females in the mixed-sex residential cages directed more attacks towards isolated females than towards isolated male intruders (Table 1). Group-housed female intruders were also attacked more than group-housed male counterparts. Interestingly, the intensity of attacks directed towards group-housed female by intact female intruders was greater than that inflicted on isolated counterparts.

Comparisons Between Residential Categories

The first point to note is that paired residents attacked less than other categories. Even when no correction is made for the fact that the data gener-

ated by pairs referred to summated activities of two animals, isolated residents (Table 4) differed ($p < .05$, Fisher exact probability test) from pairs (Table 3) in terms of the numbers of attacks on isolated male and grouped male intruders. The number of attacks and accumulated attacking times on these two categories of intruder also differed significantly ($p < .008$ and $p < .03$, respectively, for isolated and group-housed intruders, Mann-Whitney U test).

No significant differences were evident between the male residents housed with intact (Table 1) or sterile females (Table 2), but intact females directed more attacks ($p < .005$, Fisher exact probability test) at higher intensities ($p < .008$ for both numbers of attacks and AATs) to group-housed female intruders than did sterile residents.

DISCUSSION

The results largely confirm those features which previous workers have identified as important for generating high incidences of attack in laboratory rats, including isolation and the presence of females (Barfield, Busch, & Wallen, 1972; Blanchard, Blanchard, Takahashi, & Kelley, 1977; Blanchard et al., 1975; Flannelly & Lore, 1977; Luciano & Lore, 1975). They also, however, demonstrate a number of experimental conditions which have advantages compared with those previously used.

First, considerably fewer animals are used in the "resident" cages—a factor of economic importance and one that enables easier quantification of fighting behavior. The residential arenas are simple rat cages rather than the expensive (in terms of finance and space) colony caging described elsewhere. Males housed alone, with an intact or a sterile female, proved suitable residents in this type of test. In fact, the presence of a female resulted in relatively little improvement in the fighting capacity of males. Aggressiveness was not influenced by the reproductive condition of the male's female partner. These results are in contrast to those reported by Flannelly and Lore (1977), who found that the presence of an intact female (but not ovariectomized females or males) increased attack in resident rats on male conspecifics. One may note, however, that they employed only a week of cohabitation and animals were tested before and after housing with females. They related the increased fighting to mating behavior enhancement of testosterone secretion.

The present data contrast with a number of other studies employing "isolated" residents; indeed, the findings were unexpected. The results obtained when intermale fighting is compared in isolated and group-housed rats are somewhat ambiguous. Bernstein and Moyer (1970) and Garattini, Giacalone, and Valzelli (1967) both noted that fighting behavior is little

modified by even 7 weeks of individual housing. In contrast, a number of reports (Bevan, Bloom, & Lewis, 1951; Seward, 1945; Sigg, Day, & Colombo, 1966; Yen, Day, & Sigg, 1962) have utilized isolation to generate intermale aggression. These latter papers, however, report boxing rather than actual fighting in individually and group-housed rats. Perhaps strain differences, duration of housing conditions, or the type of intruder employed account for these differences? It is also worth suggesting that individually housed males have advantages compared with males housed with females in that they seem to discriminate between the types of male intruder (i.e., castrated or group-housed intruders produced lower responses than intact isolates).

It is interesting that intact female residents directed more attack to female intruders than did sterile residents, for this arrangement seems to have obvious biological utility in reducing competition for resources. One may comment that the lower incidence of attack in the sterile females (about 50% of that in intact animals in some comparisons) may be related to their reduced incidence of coming into behavioral estrus. It is likely that this form of attack is related to pregnancy/lactation in female residents; intact females appeared either pregnant or had just given birth to litters prior to tests. A subjective impression was that the intensity of attack was greatest in females that had just given birth: this form of attack seems related to the "maternal" aggression described elsewhere (Erskine, Barfield, & Goldman, 1978; Erskine, Denenberg, & Goldman, 1978; Noirot, Goyens, & Buhot, 1975; Svare, 1977). Erskine, Barfield, & Goldman (1978) reported that lactating rats showed maximal attacks on strange adult male intruders on Day 9 of milk production. In addition, removal of the litter for 4 h on Day 9 or Day 10 of lactation suppressed this form of attack, whereas hypophysectomy on Day 5 did not disrupt behavior. These findings can be taken as evidence that maternal aggression is strongly situation dependent and that, once instigated by lactation/suckling activity, hormones have little influence on this activity. It is interesting to note that both maternal and intermale fighting can be studied in colony-housed animals by the choice of appropriate intruders.

Unlike Flannelly and Lore (1975) and Luciano and Lore (1975), it was not consistently found that isolated male intruders generated more attack than group-housed counterparts; this only occurred when isolated male residents were employed. Again, this may, reflect the differences in the duration of the test, the size of the enclosure, etc. Males from mixed-sex groups, in fact, appeared relatively indiscriminate in their attacks on male intruders—housing conditions and gonadectomy of the latter animals had relatively little influence. It is interesting to speculate

about the reasons underlying the increased attack of intact female residents on grouped female intruders. Perhaps grouped animals have a colony odor, or attacks may be influenced by the estrous state of the intruder (grouped females are likely to be in anestrus)?

A striking feature of the present study is that the recorded fighting behaviors were generated in the course of 10-min tests. This is considerably faster than that described in most other studies [where at least 1 h is generally required, although Barfield et al. (1972) employed a 30-min observation period].

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(Received for publication March 22, 1979;
revision accepted July 28, 1979.)