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Resting Sites Affected by Social Interactions between Male Laboratory Mice

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ABSTRACT—Conditions which affected resting sites of laboratory mice (*Mus musculus*) were examined. Pairs of mice which encountered one another after living in separate home cages for 2 days, established dominant-subordinate relationships. About half of the dominants observed rested in their home cages solitarily and the remainder rested in the home cage of the subordinate gregariously. The subordinates always rested in their home cages. Removing or enclosing one of the paired males after physical contact had no effect on the resting site of the remaining mouse. When one male was removed before physical contact, the other always rested in its home cage. When one male was enclosed before physical contact, the other rested in its home cage or in the cage of the enclosed male: the former being referred to as an active male, and the latter being to as an inactive male. Active males were apt to become dominant and inactive males were apt to become subordinate. The preferences of active and inactive males changed after physical contacts. I conclude that resting sites after physical contact show the social relations between the males, and resting sites before physical contacts show the aggressiveness and cautiousness of each mouse.

INTRODUCTION

Territory formation has been studied in natural and semi-natural conditions (Lidicker, 1976; Singleton, 1983; Singleton and Hay, 1983). A number of laboratory experiments have shown that mice form a single male territory which is indicated by exclusive occupation of some area of experimental apparatus (Mackintosh, 1970, 1981; Poole and Morgan, 1976; Butler, 1980; Mainardi *et al*., 1986). On the other hand, in a restricted area a despotic male dominates the other mice which are generally confined to a corner (Brain and Benton, 1977; Mackintosh, 1981). However, few reports have dealt with territoriality and social dominance simultaneously.

Urine gives information about the social status of the discharger to conspecific animals. The urine of dominant males (Jones and Nowell, 1989; Hurst, 1990, 1993) or territory owners (Gosling and Mckay, 1990; Gosling *et al*., 1996) has a repelling effect on other males. The repelling effects of odors have been studied over a short observation period from 3 (Hurst, 1990) to 20 (Jones and Nowell, 1989) minutes. The response of mice may change with acclimatization to the odors and after social interactions with the discharger. However, such change has not been studied. On the other hand, experiments dealing with territory formation usually contain long observation periods from 10 days (Poole and Morgan, 1976) to 3 weeks (Mackintosh, 1970) but a demographic change as appearance or disappearance of neighbors is not always considered.

Hayashi (1996) has observed that dominant mice occupy exclusively an area including their home cages and that such territorial behavior is indicated by a resting pattern. In the present experiment the resting sites of two male mice were investigated with special focus on effects of physical contact and the presence or absence of the neighbor.

MATERIALS AND METHODS

Animals

The subjects were male mice of ICR-JCL strain bought from Clea Japan at 28 days of age. They were housed in groups of 5 or 6 in plastic cages (24 × 17 × 12 cm) until the test. They were paired with an unfamiliar male and used as subjects only once from 4 months to 9 months old. They had food and water *ad libitum*. Light (on at 8:30 and off at 20:30), temperature (23 ± 2°C) and humidity (50 ± 3%) were controlled. One or two parts of their fur were stained by picric acid for identification.

Apparatus and five states

The apparatus comprized three plastic cages (24 × 17 × 12 cm). The two cages housing the mice were connected to a center cage from both sides by bridges. The bridges were made of transparent PVC plate, 9 cm long, with a cross section of 5 × 5 cm. The center of the bridge was located 8.5 cm above the floor.

The tests were combinations of five states which were defined as inhabiting, observing, open, enclosed and removed states. Inhabiting and observing states were included in every test. (1) Inhabiting state: neither male could cross the bridge because of a sliding door set in the middle of the bridge (Fig. 1a). (2) Observing state: the mice could cross the bridge and enter the center cage but they could not have tactile interaction with each other because of a 5 mm mesh stainless steel net (0.7 mm thick) in the center cage (Fig. 1b). (3) Open state: each mouse could go to any cage except half of the cen-

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ter cage because of a wire mesh wall which was parallel to the short side of the center cage (Fig. 1c). (4) Enclosed state: the two side cages were each separated into two similar compartments by a wire mesh wall which was parallel to the long side of the cage (Fig. 1d). One of the paired mice was used as a neighbor and enclosed in the half of its home cage. (5) Removed state: as in the open state but one of the paired mice was used as a neighbor which was removed from the apparatus.

Four tests

The inhabiting state began at about 16:30 and continued about 48 hr, after which the mice were assigned to removed, enclosed, fight-free removed or fight-free enclosed tests. The four tests are described schematically in Fig. 2. The open state began at 8:30 with the beginning of the light phase. Removed and enclosed states began at 15:30 and ended at 18:00. Each pair experienced the enclosed or removed state twice. Each mouse was used as a subject once and used as a neighbor once.

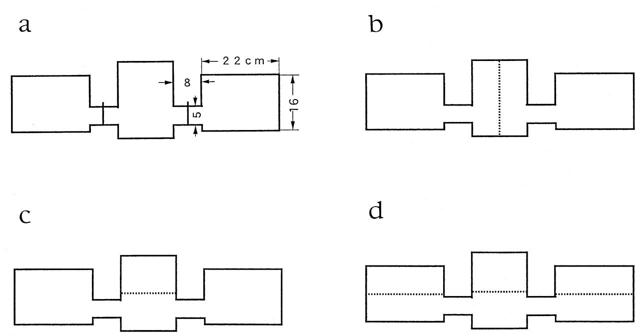

Fig. 1. Schematic top views of the apparatus used in five states. Dotted lines indicate wire mesh walls. (**a**) Inhabiting state; the solid lines in the center of the bridges indicate sliding doors. (**b**) Observing state. (**c**) Open and removed states. (**d**) Enclosed state.

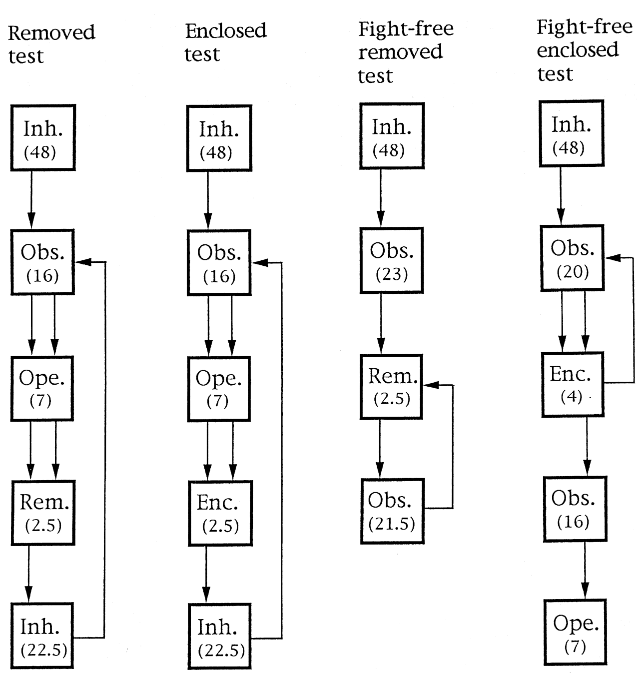

Fig. 2. Sequence of states in the four tests. Inh., Obs., Ope., Rem. and Enc. refer to inhabiting, observing, open, removed and enclosed states, respectively. The numerals in parentheses mean duration (hr).

Aggressive incidents were recorded for 20 min from 1 hr after the beginning of the open state to confirm dominant-subordinate relationships. There were two kinds of aggressions, mutual attack and one-way attack. Aggressive incidents gradually changed from mutual attacks to one-way attacks. The mice which showed one-way attacks were regarded as dominant.

Resting sites of the mice were observed during the last 20 min of the open, enclosed and removed states. The resting site was defined as the cage where each mouse rested without moving for 10 min or more. In a preliminary experiment, it was confirmed that the preference of the resting site was stable for more than 2 days ($P < 0.01$, χ^2 one-sample test).

Forty-four pairs were prepared for the removed and enclosed tests to allot a similar number of gregarious and solitary pairs to each test. Sixteen pairs were used in the removed test and 15 pairs were used in the enclosed test. The mice used in the fight-free removed and the fight-free enclosed tests were 10 and 19 pairs, respectively. In the fight-free removed test a further eight males were tested as controls without a neighbor.

The enclosed state of the fight-free enclosed test was longer than those of other tests because the mice took more time to rest. After the second enclosed state the mice underwent the observing and open states to confirm the dominant-subordinate relationship and the resting pattern. The mice experienced the 1st and 2nd enclosed states and the open state for 3 successive days as in Fig. 2. When dominant males were not detected during the observation periods of the open state, the observing and open states were repeated to confirm dominance.

RESULTS

Decision of the resting site

Resting sites were easily determined because most mice rested motionlessly. A number of typical resting mice piled saw dust around them. However, there were a few exceptions, where either or both males moved around incessantly. They were mentioned below and were not included in the analyses.

Social dominance and two resting patterns

Every pair in the removed and enclosed test established a dominant-subordinate relationship. Nine pairs in the fight-free enclosed test did not establish such a relationship during the first open state.

There were two kinds of dominant males. Twenty-four dominant males rested solitarily and twenty dominant males rested gregariously. No dominant males used the center cage as a resting site. The subordinate mice rested in their home cages ($P < 0.001$) and they did not show any aggressive attitude towards the dominant males. Patrolling dominants sometimes attacked the subordinate which was resting in its home cage. Subordinates which happened to enter the center cage or the home cage of the dominant were attacked vigorously. These facts suggest that the dominant males occupied their home cages exclusively.

Removed test

Each pair was used twice, although the removed mouse was exchanged. The results of the second test which was conducted 2 days after the first test were basically similar to those of the first test. Therefore, the results of the two removed states were analyzed together.

Sixteen pairs were tested (Table 1). In the open state nine dominants were solitary and seven were gregarious. Immediately after removing, almost all mice investigated the center and two side cages. About 2 hr after the removal of subordinates every solitary dominant rested in its home cage ($P < 0.01$, binomial test), and every gregarious dominant rested in the cage of the subordinate ($P < 0.01$). After the dominants were removed, the subordinates investigated the whole area briefly and rested in their home cage ($P < 0.01$). Therefore, every mouse kept the resting site preference in the open state after removal of the neighbor.

Table 1. Number of mice which rested in each cage in the removed test

	Cages		
	Home	Center	Neighbor
<u>Open state</u>			
Dominant	8	0	8
Subordinate	16	0	0
<u>Removed state</u>			
Solitary dominant	8	0	0
Gregarious dominant	0	0	8
Solitary subordinate	8	0	0
Gregarious subordinate	8	0	0

Enclosed test

Thirty males of 15 pairs were tested. The results of the two enclosed states were analyzed together as in the removed test. One pair changed from solitary to gregarious and one pair from gregarious to solitary between the 1st and the 2nd open states. On the whole there was little change of resting cage in the enclosed states ($\chi^2 = 9.1$, df = 1, $P < 0.01$) (Table 2). The enclosing influenced the gregarious dominant males most, with half of them changing their preference. Most subordinates preferred their original cages ($\chi^2 = 5.4$, df = 1, $P < 0.05$) but 2 subordinates preferred to rest near the dominant males. Two dominants moved around incessantly.

Table 2. Number of mice which rested in each cage in the enclosed test

	Cages		
	Home	Center	Neighbor
<u>Open state</u>			
Dominant	7	0	6
Subordinate	15	0	0
<u>Enclosed state</u>			
Solitary dominant	6	0	1
Gregarious dominant	2	1	3
Solitary subordinate	6	0	1
Gregarious subordinate	6	1	1

Fight-free removed test

The results of the 1st and the 2nd fight-free removed tests were identical. Every mouse preferred its home cage ($P < 0.01$). Every male of the control group also preferred its home cage ($P < 0.01$).

Fight-free enclosed test

The results are shown in Table 3. Thirty-five males (active males) rested near enclosed neighbors. The other 32 males (inactive males) rested in their home cages.

These data could be analyzed in depth by considering the results from the open state which they underwent on the day following the 2nd enclosed test. All the subordinates rested in their home cages. Nine pairs showed only mutual attacks during the observation period, and they showed a territorial resting pattern. They then underwent the observing and open states again, and dominant males were identified.

Active males were apt to become dominant ($\chi^2 = 5.4$, df = 1, $P < 0.05$) and inactive males were apt to become subordinate ($\chi^2 = 6.1$, df = 1, $P < 0.01$). Active subordinates caused a territorial resting pattern ($P < 0.01$, binomial test). Subordinates of gregarious pairs were inactive ($P < 0.01$).

Table 3. Number of pairs which showed the indicated combinations of resting patterns

<u>Enclosed state</u> Dominant	Subordinate	<u>Open state</u> Resting pattern	
Active	Active	Gregarious	1
Active	Inactive		9
Inactive	Active		0
Inactive	Inactive		2
Active	Active	Solitary	6
Active	Inactive		4
Inactive	Active		1
Inactive	Inactive		2
Active	Active	Solitary (2nd)*	0
Active	Inactive		5
Inactive	Active		3
Inactive	Inactive		1

* Subordinates did not emerge until the 2nd open state.

DISCUSSION

The gregarious dominants rested in the home cages of the subordinates not only when the subordinates cohabited but also when the subordinates were removed. On the other hand, all the mice in the fight-free removed test rested in their home cages. In the fight-free enclosed test, all the pairs that showed mutual attack were solitary. Therefore, a dominant-subordinate relationship was necessary for gregarious pairs.

Inactive males predominated in gregarious subordinates, and active males showed a solitary resting pattern when they became subordinate. These results suggest that the active responses of subordinates cause the dominants to refrain from gregarious resting. The suggestion seems to be sustained by the findings that aggressiveness is reciprocal and changes according to the reaction of the opponent (Cairns and Scholts, 1973; Parmigiani and Pasquali, 1979). If the neighbor is a castrated male, normal mice rest with it in its home cage (unpublished data).

In the fight-free removed test, every mouse rested in its home cage. This is explained by two factors. One is the repelling odor of a strange male (Jones and Nowell, 1989; Hurst, 1990, 1993). The repelling effects were nil when the discharger or the neighbor was confirmed to be a subordinate as shown by the removed test. The other factor is suggested by the control mice which were more attached to a familiar area than an unfamiliar area. It is reasonable that mice select a secure resting site because they are defenseless and vulnerable while resting. The gregarious dominants of the removed test seemed to confirm the security of the whole area during the open state.

All the mice preferred the home cage in the fight-free removed test. The difference between these males and active males was the presence or absence of the neighbor. The active males do not seem to avoid social interactions. They might get a benefit from social interactions because male mice which meet competitors and fight periodically become sexually more attractive than those which lack such chances (Hayashi, 1990).

The prediction of social dominance has been studied (Hilakivi-Clarke and Lister, 1992; Gosling *et al*., 1996). In the present experiment active mice were likely to become dominant when they were paired with an inactive mouse. However, it was not clear whether they could choose between active and inactive tactics according to their opponent.

Nine pairs in the fight-free enclosed test could not establish a dominant-subordinate relationship and each mouse seemed to hold its own territory on the first open state. There may be three reasons. (1) It is a “dear enemies” effect (Temeles, 1994). Familiarity during the fight-free enclosed test reduced aggressiveness during the open state. (2) The mice underwent confronting and enclosed states twice. It may be similar to the circumstance where two male mice establish adjacent stable territories. The territory owner as a resource holder may be an aggressive opponent, and the subjected mice may be deterred from intruding (Corridi *et al*., 1993). (3) The mice in the enclosed test had to fight until one of them gave up fighting before they settled. In the fight-free enclosed test, however, each mouse could confirm the whole situation before physical contacts, and they could withdraw into their home cages after only a few fights.

In the present experiment the mice were derived from a closed colony and observed in a laboratory. Their territorial interactions may be different from those exhibited by wild mice. However, aggression and related ambivalent behavior in laboratory mice have been reported to be similar to that in wild mice (Smith *et al*., 1994). Social interactions of wild mice are difficult to observe directly in their natural habitat. It is hoped that the present investigation will serve to elucidate an aspect of the social system in mice.

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