# Circling behavior in mice and rats: possible relationship to isolation-induced aggression

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*Summary.* The relationship between circling behavior (rotation), isolation, and aggression was investigated in normal male mice and rats. Initially the animals were tested for spontaneous nocturnal rotation, then conspecific aggression and muricidal behavior was observed for mice and rats respectively. Subsequently, animals were paired on the basis of net nocturnal rotations and either group-housed or individually housed. Four weeks later all animals were retested for the same behaviors. Spontaneous nocturnal rotation increased significantly for the isolated mice but not for the group-housed animals. Moreover, 9 of the 10 isolates became aggressive and their net rotations were significantly and positively correlated with the number of biting attacks. None of the group-housed mice became aggressive. Rats, on other hand, showed a decrease in rotation and a relationship between rotation and muricidal behavior was not evident. The possible relationship between circling behavior, aggression, and territoriality is discussed.

*Key words.* Rotation; isolation; conspecific aggression; muricide.

## *Introduction*

Aggression in rodents can be elicited by a variety of simple procedures[24]. Housing rodents individually for several weeks has been shown to induce conspecific aggression in mice[1,30] and mouse killing (muricidal) behavior in rats[20,36] that is accompanied by aberrant neurochemical changes[11,22,27,31,32,34] and other behavioral disturbances[1,24,30]. We (unpublished) and others (e.g. Fentress[5]) have observed that mice kept in isolation will frequently turn in circles or rotate within their cages. However, these anectotal observations in mice have not been quantitatively investigated, although isolation-induced rotation in rats has been reported recently[6].

Rodents with unilateral lesions of the nigrostriatal system also display, what appears to be, a similar behavior when administered drugs which differentially activate these bilateral dopaminergic pathways[2,9,25,28,29]. However, circling behavior is more than merely a surgically and drug-induced anomaly. It appears to be a consistent feature of a normal animal's motor repertoire because normal rodents demonstrate significant *spontaneous* rotation[8,14-16] which also is associated with a bilateral imbalance in dopaminergic function[17-19,26,35]. However the meaning of circling behavior has not yet been elucidated. Since the nigrostriatal system, which primarily mediates circling behavior, has also been implicated in agonistic behavior in mice[13,27], we reasoned that perhaps an association existed between rotation and isolation-induced aggression.

## *Methods*

20 Swiss-Webster (BS-1) male mice averaging 26 g and 20 Sprague-Dawley male rats with a mean weight of 136 g were obtained from Blue Spruce Farms Inc., Altamont, NY. Initially the mice were housed 10 per cage (47.0 × 27.5 × 13.5 cm deep) and the rats 5 per cage (41.5 × 24 × 17 cm deep) and allowed ad libitum access to food and water. Approximately one week later all animals were individually tested (test 1) for spontaneous rotation (circling behavior) during the dark phase (18.30–06.30 h) of their diurnal cycle. The apparatus or 'rotometer', previously described[15], consisted of a covered acrylic hemisphere or bowl in which the animal rotated. A soft flexible wire harnessed the animal to a rotor, and as the animal turned, the rotor activated direction sensing counters mounted on top of the removable cover. Each animal was harnessed and placed in the apparatus, and following a short (15–20 min) period of acclimation, complete (360°) left, right, and net rotations were separately recorded; net rotations (i.e. rotations to the left minus rotations to the right) were also independently calculated by a graphic method[15].

Approximately one week later all animals were tested for aggression between 10.30 and 14.00 h. The mice were tested for conspecific aggression toward a stimulus mouse which had received a bilateral olfactory bulbectomy under ether anesthesia according to an established procedure[3]. The bulbectomized mouse elicits attack behavior from the aggressor but does not display agonistic behavior of its own. Therefore it appears to be a relatively constant test stimulus[3]. Each test mouse was placed in a plexiglas cage (47.0 × 25.5 × 20.5 cm deep) containing wood shavings. Following a 10-min period of acclimation, the stimulus mouse was introduced and the latency to attack was recorded. The number of tail flutters and biting attacks were observed for 50 min. The rats were tested for mouse killing behavior using a similar procedure except that a normal mouse was introduced into the test cage following the 10-min acclimation period; the test ended 50 min later.

Subsequently, the animals of each species were paired according to their number of net spontaneous nocturnal rotations and either group-housed as previously described or isolated (1 per cage) for 4 weeks in cages 30.0 × 27.0 × 13.5 cm deep and 24.5 × 18 × 18 cm deep for mice and rats respectively. All animals were then retested (test 2) for spontaneous rotation, and after another week the aggression test was repeated according to the procedures described.

The grouped and isolated rats were respectively housed for an additional 2 weeks, food deprived for 3 days, then tested a 3rd time for spontaneous nocturnal rotation and muricide behavior.

The data were analyzed, for the most part, by two-factor analyses of variance (repeated measures) followed by multiple comparisons using the Newman-Keuls procedure[33] when appropriate.

## *Results*

For the data obtained from mice in table 1, a two-factor analysis of variance (housing × test) with repeated measures across tests indicated that the main effect of test on net nocturnal rotations was not statistically significant [$F(1.18) = 2.54$, $p > 0.1$] and the effect of housing just missed the 0.05 level of significance [$F(1.18) = 4.33$ vs 4.41]. However there was a significant housing × test interaction [$F(1.18) = 4.73$, $p < 0.05$]. Similarly the main effects for total nocturnal rotations observed in mice were not significant [housing, $F(1.18) = 3.43$, $p > 0.05$; test, $F(1.18) = 2.39$, $p > 0.1$], but the housing × test interaction was [$F(1.18) = 7.38$, $p < 0.025$]. Multiple comparisons (table 1) indicated that the isolated mice demonstrated significantly greater net and total nocturnal rotations during test 2 (i.e. after isolation) than the group-housed animals.

During the first test of aggression, neither grouped nor isolated mice demonstrated agonistic behavior. After differential housing, however, 9 of the 10 isolated mice attacked the stimulus mouse at least twice during the 50-min test session whereas none of the grouped animals did. Linear regression analysis indicated that there was a significant correlation [$r(8) = 0.6476$, $p < 0.05$] between the number of net rotations for the isolated mice and the number of biting attacks (mean ± SD = 8.6 ± 5.6). However there was not a similar association between the number of tail rattles or the latency to attack.

A similar two-factor (repeated measures) analysis of variance for the data obtained from rats in table 2 indicated that only the effect of test on *total* rotations was significant [$F(1.18) = 9.03$, $p < 0.01$]. There were significantly *fewer* total rotations for both groups following differential housing. Net rotations also declined, but the change was not statistically significant. Of all the rats, only 3 of the isolates became muricidal at this time. Following an additional 2 weeks of differential housing and 3 days of food deprivation, a total of 5 isolates were now muricidal, but so were 3 of the group-housed rats. Two-factor analysis of variance (repeated measures over test) indicated that the present net and total rotations for both grouped and isolated rats were not significantly different from test 2 ($p > 0.1$). Two-tailed t-tests indicated that there were not significant differences in net and total rotations ($p > 0.1$) between the rats (grouped and isolated combined) which became muridical and those which did not show this behavior.

## *Discussion*

Previous work indicated that normal rats displayed spontaneous rotation that could be pharmacologically augmented[8,14–16]. This present report demonstrates that rotation in mice can be increased by isolation without any pharmacological or surgical manipulations. There also appears to be some relationship between rotation and aggression in mice. Whereas agonistic behavior was not observed for any of the group-housed animals, isolation increased rotation and provoked aggression. Moreover the frequency of biting attacks of the isolates was significantly correlated with their number of net nocturnal rotations. However, none of the other measures of aggression could be related to circling behavior. In contrast to the results obtained with mice, the type of housing rats received did not differentially affect circling behavior. In fact both grouped and isolated rats showed no change or a significant *decrease* in rotation. Our results are also opposite to those of others[6] who found increased circling following isolation. This discrepancy may be due to the different methods of determining 'rotation'. Gentsch et al.[6] did not continuously measure complete rotations, per se, but recorded instead the number of rotational 'bouts' during several 15-min periods.

Although some studies indicate that isolation increases the locomotor activity of mice[1] and rats[32], it has been shown that a positive correlation between locomotor activity and rotation does not exist in these two species[10,14]. Therefore the increased rotation in isolated mice cannot be attributed solely to a greater level of general activity. Furthermore, the isolated rats in this report actually showed a *decrease* in spontaneous rotation.

In contrast to conspecific aggression, there does not appear to be a relationship between rotation and mouse killing behavior. The magnitude of rotation in muricidal rats was not different from that observed in normal animals. Muricide and conspecific aggression are very different behaviors, and species differences undoubtably exist. But, additionally, these two forms of 'aggression' appear to have dissimilar neurochemical bases. Muricidal activity is reputed to involve mainly serotonin[31], whereas dopamine seems to be of primary

Table 1. Mean (± SD) net and total nocturnal rotations for grouped and isolated mice

| Rotations | | Test 1 | Test 2 |
|---|---|---|---|
| Net | Grouped | 37.2 ± 26.8 | 24.4 ± 20.4 |
| | Isolated | 36.6 ± 26.5 | 119.8 ± 135.2* |
| Total | Grouped | 75.0 ± 50.9 | 53.6 ± 40.2 |
| | Isolated | 73.6 ± 36.5 | 151.4 ± 121.2* |

Mice were tested for spontaneous nocturnal rotation and aggression (test 1), paired on the basis of net rotation and either group-housed or isolated. 4 weeks later they were retested (test 2). *Significantly greater than grouped and isolated for test 1, and grouped for test 2 ($p < 0.01$, Newman-Keuls).

Table 2. Mean (± SD) net and total nocturnal rotations for grouped and isolated rats

| Rotations | | Test 1 | Test 2 |
|---|---|---|---|
| Net | Grouped | 43.4 ± 56.5 | 18.6 ± 18.8 |
| | Isolated | 37.8 ± 31.8 | 19.2 ± 27.4 |
| Total | Grouped | 71.8 ± 63.5 | 26.2 ± 18.6 |
| | Isolated | 54.6 ± 40.7 | 29.0 ± 33.9* |

Rats were tested for spontaneous nocturnal rotation and aggression (test 1), paired on the basis of net rotation and either group-housed or isolated. 4 weeks later they were retested (test 2). *Total rotations for both grouped and isolated decreased significantly from test 1 ($p < 0.01$).

importance in rotation and conspecific aggression – hence the association.

The relationship between isolation, rotation, and aggression in general is not clear, and the actual meaning of rotation in rodents still remains elusive. For the mouse, however, it is possible that circling may serve, among other functions, as an aggressive display behavior. Drug-dependent animals (and humans) can become aggressive during abstinence[24], and in this respect it may be significant that rotation increases in mice undergoing morphine withdrawal[23]. Therefore it is possible that rotation in male mice could be interpreted as an indicator of aggression.

As an extension of this idea, territorial defense may involve circling. Mice scent mark territorial boundaries to define their home range to conspecifics[12,24]. Another rodent species – the gerbil, also frequently scent marks, and this behavior, like intraspecific aggression, is also under androgen control in males[21]. Although these two species scent mark the environment differently (i.e. the mouse, probably with urine[4]; the gerbil, with a ventral sebaceous gland[21]) they both display similar conspecific confrontations[7] and significant spontaneous rotation[16]. Therefore, perhaps circling behavior in mice may be an orderly way of scent marking territorial boundries to circumscribe a home range to conspecifics.

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