Individually housed rats exceed group-housed animals in rotational movements when exposed to a novel environment

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Summary. Individually and group-housed rats of both sexes were compared in respect to spontaneous rotational movements when exposed to a novel environment. Thereby, individually housed animals showed a higher number of rotational movements than group-housed controls. During an L12:D12 cycle, such movements occurred most frequently at the beginning of the dark phase, when locomotor activity was highest. It is assumed that these rotations are part of the hyperreactivity toward a novel environment induced by long-term individual housing.

Long-term individual housing of rats has been shown to induce behavioural alterations, the most prominent being an increase in activity when exposed to a novel environment[2-5]. During previous openfield trials, we observed that rats tended to perform rotational movements, trying to chase their tails. However, these rotations, which seemed to occur more frequently in individually housed rats than in group-housed controls, were often incomplete, interrupted by other activities and were observed only seldom. When looking at the literature, these observations turned out to be in agreement with previous reports[6,7]. For example, Baenninger[6] described in 1967 a behavior in rats termed 'tail-manipulation' (including tail-sniffing, tail-licking and tail-chasing). Such 'tail-manipulation' occurred more frequently in individually housed rats than in group-housed animals. More recently, Day et al.[7] supported Baenninger's observations by reporting a category of non-social behavior (named 'manual self-manipulation'). Again, such behavior was more frequently present in individually housed rats (although not significantly so).

With these clues in mind we decided to have a closer look at such rotational movements in individually and group-housed rats, further considering possible sex-related differences and diurnal variations.

Materials and methods. a) Animals. Full details of the rearing and housing conditions have been described previously[3]. Individually- and group-housed (3 animals to a cage) rats had free access to food and water and were kept in the same animal room with an L12:D12 cycle. All cages of individually housed rats were separated by sheets of cardboard to prevent visual contact, but since isolation was not on an olfactory or auditory basis, we prefer to use the term individually housed, rather than isolated.

b) Test-environments. In most experiments, large macrolon cages (55 × 36 × 20 cm; with covers), furnished with a thin layer of sawdust, served as a novel environment. Previous experiments[4] had indicated that isolation-induced hyperactivity was qualitatively similar in such macrolon cages to that in the wooden openfield used and described previously[3]. In the former environment, however, rotational movements occurred at a higher rate and, additionally, rotational movements were made optically conspicuous by a shiny circle in the layer of sawdust. In addition, 28 individually housed rats were tested for rotational movements while remaining in their home-cages (time-point D + 1) and on the next day, immediately after cage-cleaning.

c) Behavioral observations. Parameters were manually recorded by observers sitting near the cages. For rotational movements, no differrentiation was made between clockwise and counter-clockwise turning. Rotations were counted as one bout of turning, without taking into account whether the animal turned once, twice or several times around itself. Adjacent rotational movements were only counted as discrete bouts provided they were separated by a clearcut rotation-free interval (e.g. separated by rearing or crossing activity). In experiment d, in addition to rotational movements, crossings and wall-rearings, behavioral parameters which have been defined and discussed previously[4] were recorded.

d) Experimental procedure. Adult individually housed rats were observed for 30 min while in their home cage, and for 30 min, immediately after cage-cleaning.

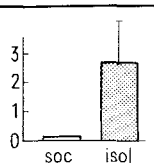
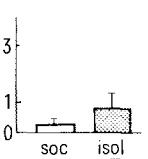
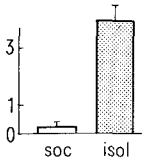
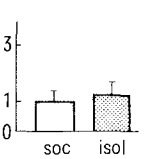
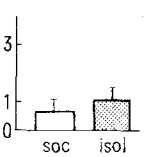
Experiment	Males	Females	Statistics
a			2-way ANOVA Housing $F_{1,20} = 4.6 - p < 0.05$ Sex $F_{1,20} = 1.1$ NS Interaction $F_{1,20} = 1.6$ NS
b			2-way ANOVA Housing $F_{1,44} = 13.8 - p < 0.001$ Sex $F_{1,44} = 3.8$ NS Interaction $F_{1,44} = 10.2 - p < 0.005$
c			Student's t-test $t = 0.86$ NS

Figure 1. Rotational movements in individually and group-housed rats after exposure to a novel environment. Open bars: grouphoused animals; solid bars: individually housed animals; all values as $\bar{x} \pm$ SEM. Experiment a: 15-min, trials: N = 6 per sex and housing condition, after 9 weeks of different housing. Experiment b: 20-min, trials; N = 12 per sex and housing condition, after 10 weeks of different housing. Experiment c: 20-min, trials; N = 12 females per housing condition, after 15 weeks of different housing.

At an age of 12–18 weeks (corresponding to 9–15 weeks, respectively, of different housing), individually and group-housed rats of both sexes were tested for rotational movements after exposure to large macrolon cages. All of these trials were run in the first half of the dark phase (experiments a, b and c). In another group of rats (experiment d), rotational movements, crossings and wall-rearings were recorded at 6 different time-points within the L12:D12 cycle. For each time-point (L+3, L+6, L+9, D+3, D+6 and D+9) naive groups of 5 individually and 5 group-housed male rats were used (differently housed for 7 weeks). All these animals were re-tested after adaptation to the novel environment (again 15-min trials).

e) Statistics. Comparisons between mean values of differently housed groups of rats were done by Student's t-test. For assessing the effects of sex, time of day, and housing, two-way analyses of variance were used.

Results. When individually housed rats were observed in their home-cages (time-point D+1), none of the 28 animals showed any rotational movements. Immediately after cage-cleaning, individually housed rats started with rotations within minutes. When exposing animals to the large, macrolon cages, rotational movements occurred with a frequency (mean of all animals) of 3 bouts per 15 min in individually housed males, and of about 1 bout in females. In group-housed animals of both sexes the corresponding values were less than 1 bout per 15 min. Rotational movements were observed in about 60% of individually housed rats but were almost lacking in group-housed animals (present in less than 20% of the rats tested). It has to be added that rotational movements were observed as early as 10 days after having started individual housing (results not shown here).

Since rotational movements in adult rats occurred at a low rate, we performed different experiments (a–c), each time using naive groups of rats. The results of these experiments are summarized in figure 1. In experiment a), the two-way analysis of variance revealed a significant housing effect ($F(1,20)=4.6$; $p<0.05$), with sex effect and sex × housing interaction not reaching significant levels ($F(1,20)=1.1$ and $F(1,20)=1.6$, respectively). Similar findings were obtained in experiment b), with the housing effect again being significant ($F(1,44)=13.8$; $p<0.001$). Again, no sex-related difference was observable ($F(1,44)=3.8$; not significant); however, in this 2nd experiment a significant housing × sex interaction could be found ($F(1,44)=10.2$; $p<0.005$). This latter finding was due to the uniform number of rotational movements in individually and group-housed females. In the 3rd experiment (experiment c), therefore, another set of differently housed females was compared. Although individually housed animals tended, once again, to show a higher number of rotations than group-housed counterparts, the difference between these 2 groups did not reach a significant level ($t=0.86$).

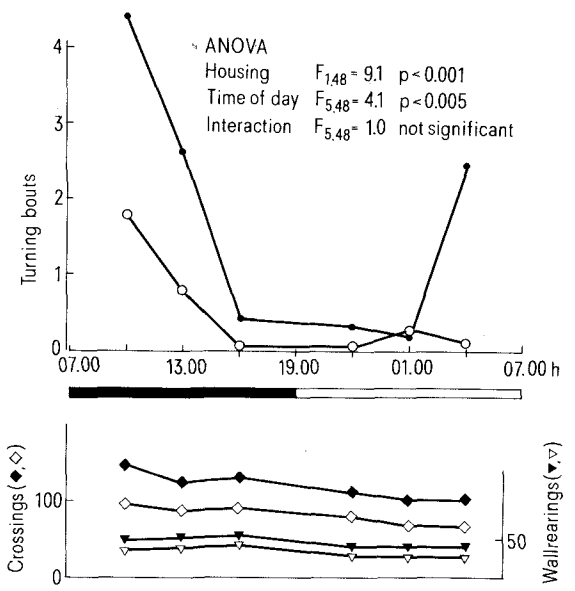


Figure 2. Activities in individually and group-housed rats after exposure to a novel environment, taking into account time-of-day effects. For each time point a different group (each consisting of 5 rats) of naive individually (solid symbols) or naive group-housed (open symbols) animals was used, which had been differently housed for 7 weeks. Upper part of figure: Rotational movements (●,○) per 15-min trial. Lower part of figure: Crossings (◆,◇) and wall rearings (▼,▽) performed within the 15-min trial.

The results of experiment d are shown in figure 2. Rotational movements also occurred more frequently in individually housed male rats here. Furthermore, diurnal variations were observed. The 2-way analysis of variance revealed a significant housing effect ($F(1,48)=9.1$; $p<0.001$) as well as a significant time of day effect ($F(5,48)=4.1$; $p<0.005$). The housing × time of day interaction did not reach significance ($F(5,48)=1.0$). As further indicated in figure 2, rotational movements seemed to parallel general activity (as measured by crossings and wall-rearings), with all 3 parameters being highest at the beginning of the dark phase. After an adaptation to the novel environment, the number of rotational movements decreased, and after 24 h rotations were no longer observed (results not shown here).

Discussion. In confirmation of previous reports[6,7], our experiments have shown that long-term changes in an animal's environment could induce an alteration in the amount of tail directed activity; individual housing intensified a behavioral response toward a novel environment rarely present in group-housed rats; individually housed rats more often contacted, and followed, their tail, leading to rotational movements. These rotational movements are probably linked with the locomotor-hyperreactivity toward a novel environment, since a) highest levels of rotational movements within the L12:D12 cycle were observed at the beginning of the dark phase, when locomotor-activity was highest, b) during habituation to a novel environment, both the number of rotational movements and crossings decreased, being no longer observable after a 24-h adaptation (remaining locomotor-activity represents spontaneous activity) and c) under home-cage conditions no rotational movements where observed (except immediately after cage-cleaning). Since rotational movements were observed as early as 10 days after beginning individual housing, it might be that in adult animals one is looking at a continuation of a 'practised' and 'habituated' behavior. However, it was found that at all developmental stages rotational movements are performed in response to a novel environment.

In attempting to explain rotational movements in individually housed rats, another behavioral approach has previously been considered by Baenninger; when she proposed that for the individually housed rat, the tail is the only stimulus that moves in relation to the environment. She stated: :The responses made to the tail are all responses that are made toward other rats: chasing, sniffing, grooming. It is as if the isolated rat used its tail as a 'cage-mate' surrogate[6]. However, in that case, one would expect individually housed rats to show rotational movements under home-cage conditions, or after a 24-h adaptation period to a novel environment; these conditions are also ones where these animals are without social contact.

For assessing sex-related differences in rotational movements (as hinted in figure 1) further experiments would be required to decide whether females do indeed show fewer turnings than males and whether isolation-induced increases are less pronounced in females. In such studies video-taping should replace the relatively crude "recording-by-hand-technique", as with a more sensitive method it would become possible to obtain more information about the fine structure of rotational movements, and possibly get more quantifiable parameters. Such detailed characterisation was impossible in the present study due to the high velocity of rotational movements.

From a neurochemical point of view, it is tempting to speculate that these rotational movements could be caused by a brain assymetry, an imbalance in the dopaminergic system, for example, as extensive literature exists on rotational behavior and lesions in this neuro-transmitter system. To the best of our knowledge, no reports exist in which determinations of any neurochemical metabolite per hemisphere were carried out in differently housed rats. It would, however, be of interest to test whether individual housing could induce an imbalance. It should be added here, in any case, that we observed that the same individual might rotate in both directions; this finding would not favor a neurochemical explanation for the observed movements.

A final comment should be added in regard to lesion- and pharmacologically induced turning behavior. It has been reported that the magnitude of spontaneous and D-amphetamine-induced rotations in rats were highly correlated[8]. Since we have observed that an animal's environment can influence the amount of rotational movements, attention should be paid to 'post-operative' environmental conditions when examining rotational behavior. Special attention should be given when lesioned animals are individually housed and controls are group-housed, an experimental plan which could possibly, lead to an overestimation of differences in turnings.

In summary, individually housed rats carried out more rotational movements than group-housed animals when exposed to a novel environment. It was assumed that such behavior represented a further facet of the well known isolation-induced hyperreactivity toward a novel environment.

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2 Garzon, J., Fuentes, J., and Del Rio, J., Eur. J. Pharmac. *59* (1979) 293.
3 Gentsch, C., Lichtsteiner, M., Kräuchi, K., and Feer, H., Behav. Brain Res. *4* (1982) 45.
4 Gentsch, C., Lichtsteiner, M., and Feer, H., Behav. Brain Res. *6* (1982) 93.
5 Weinstock, M., and Speizer, Z., Psychopharmacologia *30* (1973) 241.
6 Baenninger, L.P., Anim. Behav. *15* (1967) 312.
7 Day, H.D., Seay, P., Hale, P., and Hendricks, D., Devl Psychobiol. *15* (1979) 47.
8 Glick, S.D., and Cox, R.D., Brain Res. *150* (1979) 149.

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Sexual behavior: influence of avoidance conditioning and of immediate punishment in male rats

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Summary. Mount, intromission and ejaculation latencies are reduced in male rats if they are previously subjected to an avoidance learning session or if the first 4 attempts to mount the female are immediately punished with an electrical shock.

It has been repeatedly observed that painful stimuli peripherally applied (back[1,2], tail[3]) as well as aversive stimulation of selected brain areas[4] pace and accelerate copulatory performance of sexually experienced male rats in the presence of receptive females, or pace copulatory behavior in virgin male rats[5].

We here report the results of a study of the effects of reputedly stressful or frustrating experimental models, such as active avoidance conditioning and immediate punishment, on sexual performance in adult male rats.

Materials and methods. 3-month-old male Wistar rats (220–250 g) were used. They were housed 5 per cage with food and water continuously available in a temperature-controlled colony room (22±0.2 °C; 60% relative humidity) with a 12-h light-dark cycle, with lights-off at 06.00 h. Starting after 1 week of adaptation to the laboratory, all subjects were tested for spontaneous sexual behavior. Tests were conducted 3 h after lights off under a red light. After a brief adaptation period in a semicircular glass mating arena, a stimulus female was presented to the male. (Ovariectomized females had been brought into estrous by administration of a single injection of 15 µg of estradiol benzoate, followed 48 h later by an injection of 1 mg of progesterone).

The following behavioral parameters were recorded: mount-mount with pelvic thrusting; intromission-mount with vaginal penetration; ejaculation-mount with intromission and a final prolonged thrust, slow dismounting and genital grooming; mount latency and intromission latency-period of time to the first mount and intromission, respectively; ejaculation latency-period of time from the first intromission to the ejaculation; postejaculatory interval-time from ejaculation to the first intromission of a new series.

The test was ended if the mount or intromission latency was > 15 min, if the ejaculation latency was > 30 min or if postejaculatory interval was > 15 min.

Only those males satisfying these criteria in at least the last 2 out of 5 preliminary weekly tests were selected for our study and randomly assigned to 2 groups of 12. The rats in the first group were trained for active avoidance conditioning. Shuttle boxes divided into 2 equal communicating compartments were used. The conditioned stimulus was the sound of a buzzer; if the rat did not cross the passage between the compartments within 5 sec, the unconditioned stimulus (an electrical shock of 25 V, 1.8 mA) was delivered through the grid floor of the box. 10 consecutive trials at 40-sec intervals were performed daily (from 08.00 to