

**EFFECTS OF ENVIRONMENTAL ENRICHMENT ON THE BEHAVIOUR OF
MICE IN OPEN FIELD TESTS**

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SUMMARY

Results of open field tests with mice housed under enriched or standard conditions revealed that the locomotor activity of animals from enriched housing conditions is lower than that of animals from standard housing conditions. However, when only the first 3 min of the total test period of 15 min were considered, reversed results were found. The present results seem to indicate that differences in test duration are responsible for the contradicting results in locomotor activity of mice as reported in previously published papers.

It may be concluded that animals from enriched housing conditions habituate faster to the test situation, reflected in a (rapid) decline in exploratory behaviour, whereas control animals longer continued to explore the new environment.

INTRODUCTION

Enrichment of the environment can influence the behaviour of animals. This was first described by Hebb (1947), who reared rats in a free environment (as pets at home). He tested these rats in a learning maze and compared their behaviour with rats with restricted experience. The rats with enriched experience were better at solving the problems in the maze. Since then several different behavioural tests have been used to evaluate effects of environmental enrichment.

The open field test is a classical behavioural test which has been widely used to study the 'emotionality' of laboratory mice and rats. Animals that show high levels of activity (locomotion) and have low defecation scores in this novel environment are regarded as being less emotional than animals that show the opposite (Archer 1973, Walsh & Cummins 1976). Differences between animals from enriched and standard environments in open field test behaviour have been found in both mice and rats. Several authors found that animals from enriched environments had higher activity levels than animals from control environments (Ardila et al 1977; Holson 1986; Manosevitz 1970; Manosevitz & Joel 1973; Manosevitz & Montemayor 1972; Prior & Sachser 1994/95). Other authors, however, found the opposite: mice and rats from enriched housing conditions were less active in the open field test or their activity did not differ from standard housed animals (Denenberg & Morton 1962; Rose et al 1985; Van de Weerd 1994; Van Rijzingen et al submitted).

Among the authors who perform open field tests, a wide variety exists in the physical characteristics of the apparatus used (size, shape and colour of the arena), the procedures (single or repeated testing) and testing environment (amount of lighting and noise levels) (see Archer 1973 and Walsh & Cummins 1976 for reviews). Another factor which differs between studies is the duration of testing, ranging from short tests (2-3 min) to longer tests (8-15 min). Van Rijzingen et al (submitted) found no overall difference in locomotor activity of rats when a time frame of 10 min of open field testing was analysed. However, during the first 2.5 min of the test, rats from the enriched housing conditions showed significantly more locomotion than rats from standard conditions. This suggests that the duration of testing might be an important factor in explaining the differences in locomotor activity found between animals from standard or enriched housing conditions.

In the present study an open field test was performed with mice of two strains, in order to test whether the contradicting findings as reported for the mouse can be attributed to differences in the duration of the test.

ANIMALS AND METHODS

Animals and housing conditions

A total of 64 naive male mice of an inbred strain (BALB/cAaNCrIBR, n=32) and an outbred strain (CrI:NMRI BR, n=32) was used. They were bred without nesting material. At the start of the experiment they were three weeks of age. The mice were housed in a room with controlled photo-period (lights on 7.00-19.00 h, white light 355 lux (at 1 m above the floor), relative humidity (55 ± 5 %) and temperature (22 ± 1 °C), a radio played 24 h a day. The mice were housed per strain in groups of four animals in wire topped Macrolon type III cages (840 cm², UNO Roestvaststaal, Zevenaar, The Netherlands) provided with sawdust (Lignocel S 8/15, Rettenmaier & Söhne, Ellwangen-Holzühle, Germany). Tap water and food-pellets (RMH-TM, Hope Farms, Woerden, The Netherlands) were provided ad libitum.

Per strain half the number of animals (four groups) were randomly allocated to either standard or enriched housing conditions for a period of six weeks. Standard cages contained only sawdust bedding (Lignocel S 8/15). Enriched cages were provided with sawdust bedding and the following objects: a nest box, consisting of a plastic PVC tube (length: 11 cm, \varnothing 7 cm) big enough to give shelter to all animals of a group, nesting material (1 tissue, 25x44 cm, Kim wipers, Kimberly-Clark Corporation[®], Veenendaal, The Netherlands) and a metal climbing grid, vertically attached to the cage lid (size: 16x10 cm, mesh size: 5x5 mm). The location of the objects remained the same throughout the experimental period. The tissues were renewed with weekly cage cleaning. Four days before the start of an experiment the mice were brought in their cages to the test room to acclimatise.

Test system

The animals were introduced individually into a novel environment consisting of a 50x50x50 cm black arena. Light intensity at test cage level was 7.5 lux. Locomotor activity was measured with a Video-track system (type 512, Electronique Lyonnaise, Lyon, France).

Procedure

Mice were tested three times on three consecutive days. Tests were performed between 9.00 and 15.00 h. Duration of a test was 15 min. Groups were tested in random order, the four mice of a group were tested at the same time (in four similar test systems). Between mice, the test systems were cleaned with 1% TEGO 51\15 DL (amfotensiden 90 g/l, TH Goldschmidt NV, Amsterdam, The Netherlands).

The duration (in s) of three levels of activity were measured (based on the speed of movement of the mouse):

high activity = walking and running.

low activity = sitting in one place, including small movements with head or paws.

inactivity = no movements (freezing and resting).

Statistical analyses

Data were analysed per test day with a MANOVA, to detect possible differences between strains and housing conditions. The level of statistical significance was pre-set at $P < 0.05$.

RESULTS

Three weeks before the test one BALB/c mouse (standard housing) was removed from its group, because it showed high levels of aggression, thereby wounding its cage mates badly. The remaining mice were included in the experiment and their performance did not differ from the other standard housed BALB/c mice. Figure 1 depicts the results of total open field activity (top) and activity per 3 min intervals (bottom).

On DAY 1, no differences were found in time spent on the three behavioural categories between mice from the two housing conditions. Significant differences between the strains were found. NMRI mice were more active than BALB/c mice (high activity $P < 0.01$) and showed less inactivity ($P < 0.01$).

On DAY 2 significant differences between mice from the two housing conditions were found in both strains. Mice from enriched environments were less active than mice from standard environments (high activity $P < 0.05$). Strain differences on this day were similar as differences on day 1 (high activity $P < 0.01$, inactivity $P < 0.01$).

On DAY 3 similar differences between mice from the two housing conditions were found in both strains. Mice from enriched environments were less active than mice from standard environments (high activity $P < 0.05$). Significant differences between the strains were found for all three behavioural parameters. NMRI mice were more active than BALB/c mice (high activity $P < 0.01$, low activity $P < 0.01$) and showed less inactivity ($P < 0.01$).

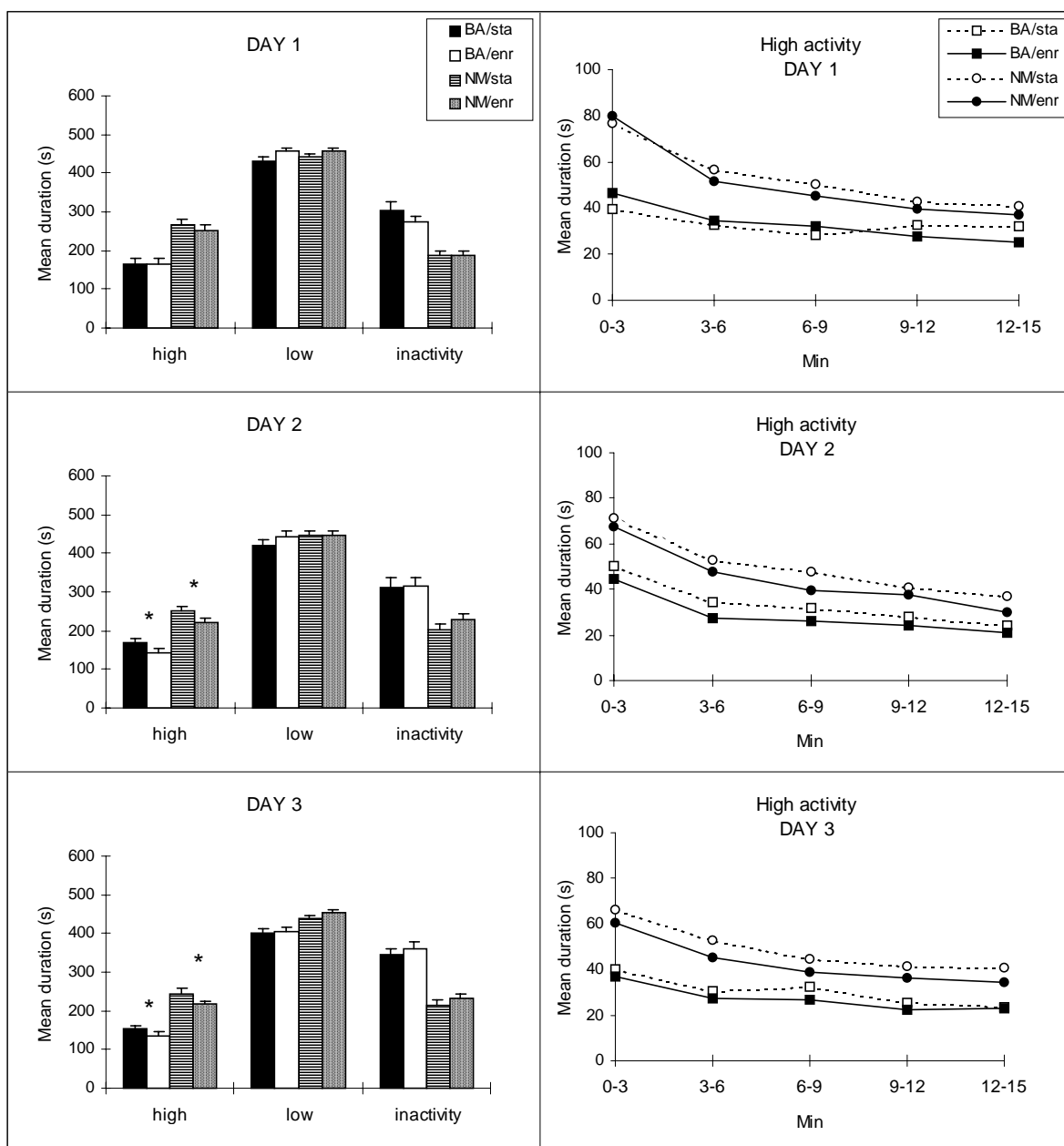


Figure 1 Open field test results of male mice of two strains and two housing conditions ($N=63$). Left: Mean time (and SEM) spent on indicated behaviour during 15 min of testing on three consecutive days. Right: mean time spent on high activity per 3 min intervals. * $p < 0.05$, significant difference between housing conditions.

DISCUSSION

The results indicated a difference in both strains between mice from the enriched housing conditions and the standard conditions. The enriched housed mice were less active on day 2 and 3 of the test. This difference was only found in high

activity (locomotion).

These results are comparable with an earlier study employing the open field test by Van de Weerd et al (1994), in which we found that BALB/c mice from enriched conditions showed less locomotion than standard housed mice during 8 min of open field testing. Similar results were also found in an exploration test (comparable with an open field test) used by Manosevitz & Montemayor (1972) and Manosevitz & Joel (1973). They found that over 5 consecutive daily trials mice reared in enriched conditions explored less (measured as locomotor activity) than mice from impoverished conditions. In other studies, however, contradicting results have been found.

The main difference between studies with opposite results in open field activity scores are the differences in test duration. When comparing the test durations it appeared that in short open field tests (2-3 min trials on consecutive days) mice from enriched conditions were more active than mice from standard or impoverished conditions (Manosevitz 1970; Manosevitz & Joel 1973; Manosevitz & Montemayor 1972; Prior & Sachser 1994/95), whereas in longer open field tests (1 trial for 8-15 min) it was found that mice from standard conditions were overall more active than mice from enriched conditions (Van de Weerd et al 1994; and the exploration test of Manosevitz & Montemayor 1972; Manosevitz & Joel 1973).

The observation that the duration of testing can explain the contradicting results, is supported by the data of the present experiment. When the data are split into 3 min periods, the first 3 min period on day 1 shows that in both strains the mice from enriched conditions were more active than mice from standard conditions. In the following periods the reverse was found (for the NMRI mice in the 3-6 min period, for the BALB/c mice in the 9-12 min period). On day 2 and 3, mice from enriched environments were overall less active. In the study on rats by Van Rijzingen et al (submitted), after the first 2.5 min of the open field test the activity of the enriched housed rats declined fast to levels below those of rats from standard conditions. A decrease in activity over trials on consecutive days has also been reported. Mice from both housing conditions in the exploration test of Manosevitz & Montemayor (1972) and Manosevitz & Joel (1973) showed a reduction in activity over trials, but this decrease was larger in mice from enriched conditions. A similar finding has been reported for rats (Holson 1986).

Overall, the results suggest that animals from enriched conditions habituate faster to the test situation, reflected in a faster decline in exploration activity as compared to control animals, who continue to explore (Manosevitz & Joel 1973). When open field tests are short (3 min or less) this habituation may not be detected. Animals from enriched housing conditions are more competent to control their environment and therefore learn to cope with novel and unexpected changes in their environment (Chamove 1989a; Rose 1994; Wemelsfelder 1994).

As a consequence they might be better able to cope with circumstances during experiments as compared with animals from standard environments. The results of the present experiment, as well as results from other studies, indicate that this might be the case. Animals from enriched conditions can adapt faster to novel environments and thus may be less aroused by the novelty or unfamiliarity of the testing apparatus (Manosevitz 1970, Van Rijzingen et al submitted).

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