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Short communication

Group housing of mice increases immobility and antidepressant sensitivity in the forced swim and tail suspension tests

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Abstract

The forced swim test and tail suspension test are often used in laboratory practice to identify compounds that possess antidepressant-like activity. This experiment was conducted to determine whether housing conditions per se influence the response of mice in these antidepressant screening procedures. Male NIH Swiss mice were housed individually or in groups (five per cage) for 8 weeks prior to testing. After 8 weeks, the animals were exposed to the forced swim and tail-suspension tests. Group housed mice displayed high levels of immobility in the forced swim and tail suspension tests. Desipramine injection 60 min prior testing, in doses 7.5 and 15 mg/kg, produced significant reductions in the immobility time in forced swimming and tail suspension tests. Individually housed mice, when exposed to these tests, displayed lower levels of immobility with a magnitude comparable to the effect of desipramine in group housed mice. Desipramine given to individually housed mice did not reduce the duration of immobility either in the forced swim test or in the tail suspension test. These results indicate that both tests are sensitive to housing conditions. This observation suggests that long lasting group housing may be critical to the behavioral response in these preclinical screening procedures in mice. © 2001 Published by Elsevier Science B.V.

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1. Introduction

The forced swimming test and tail suspension test are non-escapable stressful situations (Porsolt et al., 1977a; Steru et al., 1985) and are widely used for screening substances for potential antidepressant effects. Briefly, when mice are forced to swim or are hung upside down by the tail in an inescapable situation, they tend to become immobile after initial vigorous activity. Moreover, substances that decrease immobility often have antidepressant properties in humans (Porsolt, 1979; Porsolt et al., 1977a, 1978a) This immobility has been described as a symptom of "behavioral despair" (Porsolt et al., 1977a), and both tests have been suggested as animal models of human depression, although this is open to considerable argument. Nonetheless, these tests are predictive of antidepressant-like activity, and hence are widely employed.

Stressful events are considered an important factor in the development of central nervous system disorders such as depression (Brown, 1993), and a single experience of a stressful event enhances the vulnerability to stress-related disorders (Koolhaas et al., 1997). In situations where an animal has no control over its environment, the stress response may be inadequate and may initiate the development of behavioral and physiological changes, ultimately leading to a pathological state (Meerlo et al., 1997). We hypothesized that long lasting group housing in mice may constitute a stressful environment, where defeat of a potentially aggressive male by a dominant male could be equivalent to loss of control, and might result in adaptive changes necessary to achieve an antidepressant-sensitive behavioral response.

Now we report that group housing results in comparatively high levels of immobility in vehicle-treated mice in forced swim and tail suspension tests, and that desipramine injection reverses this effect. In contrast, single housing results in a comparatively low level of immobility which is unaffected by desipramine treatment. The duration of

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immobility in single-housed animals treated with either vehicle or imipramine is comparable to that of desipramine-treated, group housed animals. These finding are consistent with the hypothesis that group housed mice are more vulnerable to acute inescapable stress than individually housed mice. Further, group housing of mice may, of itself, constitute an inescapable stressful environment necessary to the antidepressant sensitivity of these tests.

2. Materials and methods

2.1. Animals and drug administration

The study was carried out on male NIH Swiss–Webster mice weighing 20–25 g on arrival. The animals were housed for 8 weeks either in single cages or in groups of five per cage, under standard colony conditions in an Association for Assessment of Animal Laboratory Animal Care (AAALAC) approved facility, with a 12-h light/dark cycle (lights on at 07:00) and with free access to food and water. All animal care and experimental procedures were in accordance with AAALAC Guidelines and the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, and were approved by the University of Mississippi Medical Center Animal Care and Use Committee. On the day of testing, mice were removed from the colony and allowed to acclimate to a sound-attenuated behavioral testing room for 2–3 h prior to testing. All testing was conducted between 12:00 and 17:00 h. Both the forced swim and tail suspension tests were carried out in the same animals. After the first test (forced swim or tail suspension), animals were returned to their home colony for 1 week and then were processed in the other test. The groups were counterbalanced for testing order.

Desipramine was obtained from Research Biochemicals (Natick, MA) and was prepared fresh on the day of testing in saline (0.9% NaCl). Desipramine at doses of 7.5 or 15 mg/kg was given i.p. in a volume of 0.2 ml/mouse, 60 min prior to testing. We employed two doses of desipramine (7.5 and 15 mg/kg) based on the literature (see (Borsini and Meli, 1988) for a review) and our own previous experience. A single drug injection is the norm for the mouse forced swim and tail suspension tests (see Porsolt et al., 1977b, 1978b; Steru et al., 1985; Borsini and Meli, 1988) and is the standard approach in this laboratory (see Harkin et al., 1999).

2.2. Forced swim test

The test was conducted using a modification of the method of Porsolt (Porsolt et al., 1977a). Briefly mice were individually placed in 10 cm of ambient temperature water (~22°C) in 2000-ml glass beakers. Mice were allowed to swim for 6 min and their activity was videotaped. The videotaped behavior was subsequently analyzed by 1–2 raters who were blind to the animals' housing conditions. Duration of immobility defined as the absence of active, escape-oriented behaviors, such as swimming, jumping, rearing, sniffing or diving, was recorded during the last 240 s of the test using the PORSOLT data collection program (Infallible Software, Res Tri. Pk., NC).

2.3. Tail suspension test

This method is based on the observation that a mouse suspended by the tail shows alternate periods of agitation and immobility, similar but not identical to that observed in the forced swim test (Steru et al., 1985). Animals were moved from the housing colony room to the testing area in their own cages and allowed to adapt to the new environment for 2–3 h before testing. Mice were suspended on the edge of a shelf 58 cm above a tabletop by adhesive tape, placed approximately 1 cm from the tip of the tail. The mouse was 15 cm away from the nearest object and both acoustically and visually isolated. Animals were allowed to hang for 6 min and duration of immobility was recorded during the last 240 s of the test using the PORSOLT data collection program (Infallible Software, Res Tri. Pk.). Mice were considered immobile only when they hung passively and completely motionless.

2.4. Statistical analysis

Data were initially analyzed by two-factor (housing condition × drug treatment) analysis of variance using SPSS 10.0 (Chicago, IL). Differences between treatment groups were assessed with the Bonferroni-corrected *t*-test. Data were deemed significant when $p < 0.05$.

3. Results

Rodents housed in groups (five per cage) displayed high levels of immobility in both the forced swim test and the

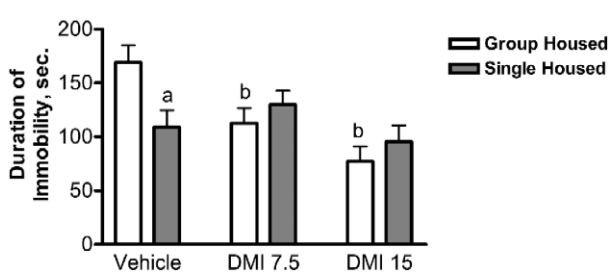

Fig. 1. The effect of housing conditions on behavior in the forced swim test. Experiment was carried out 60 min after saline/desipramine injection. Values represent the mean ± S.E.M. of 10 subjects/group. Analysis of variance revealed a significant main effect of drug treatment ($F(2,54) = 6.367$, $P = 0.003$), no main effect of housing ($F(1,54) = 0.459$, $P = 0.501$), and a significant drug treatment × housing interaction ($F(2,54) = 4.444$, $P = 0.016$). (a) $P < 0.05$ vs. paired group housed control; (b) $P < 0.05$ vs. vehicle treatment (Bonferroni-corrected *t*-test).

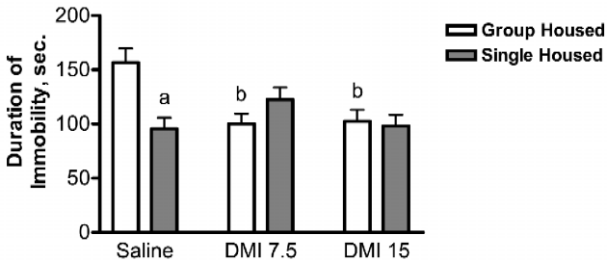

Fig. 2. The effect of housing conditions on behavior in the tail suspension test. Experiment was carried out 60 min after saline/desipramine injection. Values represent the mean ± S.E.M. of 10 subjects/group. Analysis of variance revealed no significant main effects of either drug treatment or housing environment ($F(2,54) = 2.526$, $P = 0.118$ and $F(1,54) = 2.852$, $P = 0.067$, respectively) but did reveal a significant drug treatment × housing interaction ($F(2,54) = 7.548$, $P = 0.001$). (a) $P < 0.05$ vs. paired group housed control; (b) $P < 0.05$ vs. vehicle treatment (Bonferroni-corrected *t*-test).

tail suspension test (169 ± 16 and 157 ± 13, respectively —Figs. 1 and 2). Desipramine dose-dependently reduced immobility in the forced swim test (−33% and −55% at 7.5 and 15 mg/kg, respectively—Fig. 1). Likewise, desipramine reduced immobility in the tail suspension test, however, no evidence of dose dependency was observed in this test (−35% at 7.5 and 15 mg/kg, respectively—Fig. 2).

In contrast, single housed mice displayed a significantly lower level of immobility in forced swim test and tail suspension test when treated with saline, compared to group-housed mice (109 ± 16 and 96 ± 10 s, respectively —Figs. 1 and 2). Moreover, desipramine treatment of single housed mice did not alter the immobility of mice in either the forced swim or tail suspension tests compared to saline-treated controls (Figs. 1 and 2). In fact, the immobility of all single-housed mice, regardless of treatment, was comparable to that of group-housed mice treated with 15 mg/kg desipramine.

4. Discussion

The forced swim and tail suspension tests are widely employed for the detection of antidepressant activity (Porsolt et al., 1977a,b; Steru et al., 1985). Antidepressant drugs modify the balance between activity and immobility in these paradigms in favor of activity and at the expense of energy. The present results demonstrate significant differences in both baseline response to inescapable stress and in the behavioral response to an antidepressant treatment in these paradigms. Here, we report that both tests are highly sensitive to housing conditions. After a period of 8 weeks of group housing, the immobility time in the forced swim and tail suspension tests was significantly greater than that observed in single housed mice. Moreover, desipramine significantly reduced immobility in the group but not in single housed animals.

For this initial study, we chose to focus on housing status per se rather than *duration* of housing status and to house the animals long enough to (in our estimation) reduce to insignificance the effect of housing conditions in the breeding facility (which most certainly are group housing conditions). However, in this vein, it is worth noting that the basal and desipramine-induced immobility scores reported for our group housed animals are quite consistent with the data from the literature and in our laboratory (Borsini and Meli, 1988; Harkin et al., 1999). Thus, prolonged housing in our colony did not, of itself, appear to affect immobility in these tests.

The rat and mouse versions of the forced swim test are both based upon the observations of Latz et al., (1966, 1967) and the learned helplessness models of Maier et al. (Maier et al., 1972; Seligman et al., 1968). This is reflected in Porsolt's original description of the immobile behavior of rodents in these tests as "behavioral despair" (Porsolt et al., 1977a, 1978a). However, as noted by Borsini and Meli, it has never been clear why the version of the test in rats requires a relatively long (15 min) conditioning swim prior to the test swim, whereas the mouse version does not (Borsini and Meli, 1988). Porsolt and others have suggested that the initial few minutes (2–3) of the forced swim in mice provides the same experience of uncontrollability that the 15-min conditioning swim does in rats; however, this has never been systematically examined (Nomura et al., 1982; Porsolt et al., 1977a).

Rats and mice are routinely housed in small groups for convenience and based upon the knowledge that they can be successfully maintained in relatively stable social groups. However, while rats often live in social groups in the wild, mice are usually territorial and tend to remain solitary except for reproduction (Brain, 1975). Moreover, in contrast to the rat, mice in social groups form simple dominance hierarchies based on the emergence of a "despotic individual" with little distinction among subordinate mice (Mondragon et al., 1987). These dominance hierarchies are such, that one "despot mouse" emerges in a relatively stable position claiming most of the territory and resources. In contrast, the hierarchy among the remaining subordinate mice is unstable, with frequent agonistic encounters between subordinates (subdominant and submissive) as well as between subordinates and the established "despot mouse." In addition, the behavioral reaction of mice to agonistic interactions with other mice is very much dependent upon strain (Mondragon et al., 1987; Siegfried et al., 1984; Strozik and Festing, 1981) and housing environment (Haemisch et al., 1994).

Unlike rats, mice in the wild tend to be behaviorally isolated, do not tolerate a mature male on their territory, and are consequently rarely subjected to the stress of defeat. Thus, it may be argued that housing mice in small cages in groups results in a situation where defeated subordinates are unable to escape from the territory of the

dominant (Brain, 1975). Based on these observations, the present experiment was an attempt to resolve whether differences in housing conditions result in differential response to behavioral challenge in the forced swim and tail suspension tests.

It has previously been reported that isolates are more active in a novel situation than group housed animals, but that such animals are less active in their home cages when not disturbed (Brain, 1975). This was confirmed by Wilmot and coworkers, who also noted increased intermale fighting among individually housed mice (Wilmot et al., 1989). These two behaviors of the "isolation syndrome" have been associated with increased reactivity of monoaminergic systems relative to the group housed mice (Hodge and Butcher, 1975; Welch and Welch, 1971). The most consistent of the findings concerning the effect of housing on brain neurotransmitters is the observation that in isolated mice, the turnover rate for serotonin and norepinephrine is lower than that found in group housed mice. This has been indicated by the presence of lower levels of 5-hydroxyindoleacetic acid (5-HIAA) and norepinephrine in the brain of mice isolated for at least 14 weeks (Welch and Welch, 1968).

This is supported by the results from several laboratories indicating differences in the behavioral response of mice in the forced swim test, elevated plus maze and locomotor activity chamber depending on prior housing status (Hilakivi et al., 1989b). Specifically, individually housed mice engage in more exploratory and escape-oriented behaviors than do group-housed mice. This is paralleled by the observation that dominant males within a group are much more active in the forced swim test than either non-dominant cage mates or controls (Hilakivi et al., 1989a). This has led at least one group to postulate group housing in mice as a potential murine analog of human depression (Kudryavtseva et al., 1991).

Based on these data, it is reasonable to ask whether there was behavioral evidence of different behavior between despotic and subordinate mice under group housing conditions, in either the forced swim or tail suspension tests in the present study. Only one group, the vehicle-treated group housed mice, might be expected to show an outlier that might correspond to a "despot mouse." We did not detect such an outlier in this group, but whether this is due to the relatively uniform sample distribution with both lower and upper tails, the small sample available for examination ($n = 10$), the insensitivity of this measure to detect such an animal or other reasons, is unknown. Further studies with larger samples and specific dominance testing will be required to resolve this issue.

One further possibility should be considered in interpreting this study. Namely, that desipramine did not show an effect in single-housed mice because of reaching a floor response. This is highly unlikely inasmuch as a true floor effect of zero second of immobility can be demonstrated in mice by administration of psychostimulants, such as D-amphetamine or scopolamine (see (Borsini and Meli, 1988) for a review). It is also possible that single housing has orthogonal effects that both reduce the basal (vehicle-injection) duration of immobility *and* the potency desipramine to affect immobility. While we believe this to be unlikely, we cannot exclude the possibility in this initial study.

In summary, individually housed adult mice do not display antidepressant-sensitive immobility in the two most common preclinical screening procedures for antidepressant activity. In contrast, prolonged group housing results to increased immobility and antidepressant sensitivity. Based on our results, we conclude that group housed mice are more vulnerable to inescapable stress than individually housed mice. Clearly, accurate interpretation of the results of these tests will require close attention to the housing conditions of mice prior to testing.

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