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ARTICLES

Behavioral Assessment of Intermittent Wheel Running and Individual Housing in Mice in the Laboratory

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Physical cage enrichment—exercise devices for rodents in the laboratory—often includes running wheels. This study compared responses of mice in enriched physical and social conditions and in standard social conditions to wheel running, individual housing, and open-field test. The study divided into 6 groups, 48 female BALB/c mice group housed in enriched and standard conditions. On alternate days, the study exposed 2 groups to individual running wheel cages. It intermittently separated from their cage mates and housed individually 2 groups with no running wheels; 2 control groups remained in enriched or standard condition cages. There were no significant differences between enriched and standard group housed mice in alternate days' wheel running. Over time, enriched, group housed mice ran less. Both groups responded similarly to individual housing. In open-field test, mice exposed to individual housing without running wheel moved more and faster than wheel running and home cage control mice. They have lower body weights than group housed and wheel running mice. Intermittent withdrawal of individual housing affects the animals more than other commodities. Wheel running normalizes some effects of intermittent separation from the enriched, social home cage.

Housing conditions of animals in the laboratory have been influenced by the knowledge that environmental factors can affect the well being of nonhuman an-

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imals and thereby, the outcome of biomedical research. Redesigning of housing conditions as experimental concept has led to studies investigating the effects of environmental complexity or different degrees of environmental enrichment on behavioral, physiological, and neurobiological parameters (Renner & Rosenzweig, 1986; Will, Galani, Kelche, & Rosenweig, 2004).

Although no single variable of the enriched environmental model has accounted fully for its beneficial consequences, there is a consensus that interaction with the complexity of the environment causes them. From the welfare point of view, one of the goals of an enrichment program is to enhance the animals' well-being and to promote species-specific behavior by meeting the animals' essential needs. Providing enrichment from a human point of view by simply adding extraneous features such as toys to the housing condition may not meet the animals' needs but may increase variations in experimental results or contrive unwanted consequences (Crabbe, Wahlsten, & Dudek, 1999; Wahlsten et al., 2003; Wurbel, 2001). Improvement of the animals' environment by enrichment of the cage or by housing animals in stable social groups results in less-stressed, less-frightened mice who have a broader behavioral repertoire and who are easier to handle (Van de Weerd et al., 2002; Van de Weerd & Baumans 1995). Other studies of mice have shown that lack of physical, social contact because of individual housing resulted in behavioral changes indicative of anxiety and increased aggression (Ferrari, Parmigiani, Rodgers, & Palanza, 1997).

Although running wheels for laboratory mice and rats frequently are used as an enrichment device to provide the animal with a more complex environment, there are discrepancies in rationale for their use. Several disputable hypotheses of wheel running behavior include the replacement for general motor activities, exploration, escape, migration behaviors, physical or stereotypic activity, body weight or energy balance, maintenance, and hormonal control (Beneke, Schulte, & vander Tuig, 1995; Collier, Johnson, CyBulski, & McHale, 1990; Kent, Hurd, & Satinoff, 1991; Koh, Lett, & Grant, 2000; Mason, 1991). Some studies suggest that rodents perceive running as a reward (Sherwin, 1998); other studies have postulated that wheel running is addictive (Werme et al., 2003; Werme, Thoren, Olson, Brene, 1999). Studies examining the effects of wheel running on the brain have shown that wheel running promotes neurogenesis and trophic factors in the hippocampus (Berchtold, Kesslak, & Cotman, 2002; Cotman & Berchtold, 2002; van Praag, Kempermann, & Gage, 2000), and that it is regulated by the brain reward pathways (Werme, 2001; Werme, Lindholm, Thoren, Franck, & Brene, 2002; Werme, Messer, et al., 2002).

Running wheels are intended as an enrichment device for laboratory mice and rats to provide a more complex environment and to stimulate effects of enrichment on brain development and ageing. Therefore, wheel running in laboratory rodents often is used as a model to study exercise-derived effects on physical health and brain function (Berchtold, Chinn, Chou, Kesslak, & Cotman, 2005).

That running regulates brain regions involved in learning and memory as well as the motivational drive highlights how potent running can be in regulating different behaviors. Despite its frequent use with laboratory rodents, a connection between running wheel and animal well-being is not known; and the intuitive value of the running wheel as enrichment device is not clear. Environmental enrichment should consider the animal's needs, be practical, and should not introduce risk to the animals, the staff, or the experiment (Van de Weerd & Baumans, 1995).

This study examines the responses of enriched and standard group housed mice to intermittent exposure to the running wheels. The animals also were tested in an open-field arena to assess exploratory and anxiety-like behaviors. We chose to expose the mice to the running wheels on alternate days to allow each mouse the opportunity to use the running wheel without competition with other cage mates and to assess the effects of different housing conditions on intermittent withdrawal of social contact from cage mates in individual housing condition. This intermittent exposure to wheel running would provide guidelines for the design of exercise and rehabilitation program and be applicable to animal models of humans' exercise habits, which rarely occur daily and consistently but, more often, follow intermittent temporal patterns.

MATERIALS AND METHOD

Animals and Housing Conditions

This study used 48 female mice of inbred strain BALB/c (B&K Universal AB, SCANBUR–BK, Sollentuna, Sweden, Kalamazoo, MI, Brogaarden, Denmark). The mice were shipped at 5 weeks of age. After arrival, they were assigned randomly to 4 mice per cage over 12 cages in standard, transparent, elongated (40.5 × 25.5 × 14.5cm) Macrolon® Type III, (B&K Universal AB, Sollentuna, Sweden). The mice had *ad libitum* access to food (standard laboratory rodent pellets, Beekay Diets, B&K Universal AB, Sollentuna, Sweden) and tap water. Room temperature was maintained at 22ºC ±1, humidity at 45 to 55% and a 12/12 light dark cycle, with lights on at 07:00h. Cages were cleaned once a week. Six groups of mice (n = 24) were housed under standard social condition (S), and six groups of mice (n = 24) were housed under enriched social condition (E).

S-condition consisted of group housing (4 mice/cage) only with 50 ml of wood chips bedding. Under E-condition, the cages were equipped with a shelter (Shepherd Shack, egg-box carton 15 × 9 × 6 cm, Shepherd Specialty Papers, Kalamazoo, MI); two Kleenex® tissues (Kimberly Clark Corp. Sweden); and two gnawing sticks (aspen wood, 1 × 1 × 5 cm, Finn Tapvei, Finland). Enrichment items were renewed as necessary.

Experimental Groups

After 2 weeks of E and S housing conditions, the mice further were assigned to the six following experimental groups, lasting 4 weeks, with seven to eight mice per group:

1. E-control—enriched group housed condition;
2. S-control—standard group housed condition;
3. E-individual—enriched condition and alternate days in individual housing;
4. S-individual—standard condition and alternate days in individual housing;
5. E-wheel—enriched condition and alternate days in individual running wheel cage; and
6. S-wheel—standard condition and alternate days in individual running wheel cage.

PROCEDURES

Wheel Running and Individual Housing

On alternate days, the E-wheel, S-wheel, E-individual, and S-individual groups were put into their assigned cages and returned every other day to their respective home cages with their cage mates. Running wheel cage condition consisted of individual housing in Macrolon Type II cages (25.5 × 19.5 × 13.5cm), each cage equipped with a metal running wheel (39 cm circumference) with rungs (5 cm long and 1 cm apart). Each wheel has an attached magnetic counter that sends signals via the corresponding wire connector on the cage to a matrix for registering of wheel revolutions. Running data were registered automatically every 30 min for 24 hr using customized computer software program (Werme, Lindholm, et al., 2002). The E-individual and S-individual groups likewise were exposed on alternate days to individual housing in Mac II cages (25.5 × 19.5 × 13.5cm) without the running wheels.

Open-Field Test

Exploratory behavior was assessed in the open-field test. The test arena consisted of a gray, PVC circular open field (60 cm diameter) enclosed by a wall (30 cm height). The mice were placed individually in the center of the arena, and the track of the mouse's movements was recorded for 6 min using the EthoVision automated video tracking system (Noldus, The Netherlands). Behavioral parameters measured were locomotor activity and vertical activity, defined as *rearing* (standing on hind legs with forelegs in the air or against the wall). At the end of each test session, fecal boli were counted and removed; the arena was cleaned between each mouse with 20%

ethanol and water. For data analysis, additional zones were defined as *peripheral* and *central zones* of the open field. Using EthoVision's computer software, the following parameters were calculated:

1. In the whole arena—distance moved and mean velocity;
2. in the central zone—distance moved, frequency and total time spent in zone; and
3. in the peripheral zone—distance moved, frequency and total time spent in zone.

Body Weight

As general health monitoring, the body weight was measured twice per week throughout the study. Mean body weight was analyzed for all groups.

Statistics

All results were analyzed statistically using computer software SPSS for Windows (Version 11.0) to determine significant differences between the groups. Data were analyzed using a two-way analysis of variance (ANOVA) with condition (enriched, standard), experiment groups (wheel, individual, control) as between-subject factors and day as within-subject factor. Frequency of wheel running and body weight were analyzed using general linear model with repeated measures. Significant interaction effects were analyzed further with Scheffé's post hoc tests. Open-field data were analyzed using one-way ANOVA (factors being experimental groups at six levels = S- and E- housing conditions for control, individual, and wheel groups), followed by multiple-comparison test (Tukey HSD) to compare between groups with different housing conditions. The level of statistical significance was preset at $p < .05$ for all parameters. Data are presented as group mean values ± SEM (standard error of the mean).

All experimental procedures in this study were approved by the Northern Stockholm Animal Ethics Committee. Treatment and care of animal subjects followed the animal ethics committee's guidelines to minimize pain or suffering and to minimize the number of animals used.

RESULTS

Running Wheel Activity

Running patterns were similar between the groups; all mice showed running entrainment to the dark period. Running pattern was entrained by the light/dark cycle. There was no difference in running about between the groups within the 48 time windows of 30-min. Time windows' data were collapsed into 24-hr running activity. During the first 3 weeks of alternate-day access to running wheels, the mice habituated to the running-wheel cages, and wheel-running revolutions per day served as baseline running levels (Figure 1). Baseline running levels for E-wheel and S-wheel groups were analyzed using a Condition × Day ANOVA with repeated measures for day. There was no overall significant main effect, $F(1, 13) = 4.18$, $p < .10$; or interaction effect, $F(11, 143) = 0.13$, $p < .10$. However, enriched mice showed a tendency to run less than did mice from standard cage condition.

Open Field

All groups were exposed to the open-field arena for 6 min. Statistical analyzes were carried out for each of the dependent variables: locomotor activity, rearing, and fecal boli counts. No difference was found between the groups in rearing or fecal boli counts in the open-field test (data not shown).

Figures 2 and 3 illustrate the mean distance moved and velocity of locomotion in the whole open-field arena. Significant main effects on total distance moved in open-field arena was observed, $F(4, 40) = 3.56$, $p < .01$. Post hoc Tukey test revealed that E-housed mice exposed to individual cages (E-individual) moved a greater distance in the open-field than did the S-housed mice exposed to running wheel (S-wheel; $p < .05$) and those control mice group housed in standard condition (S-control; $p < .01$).

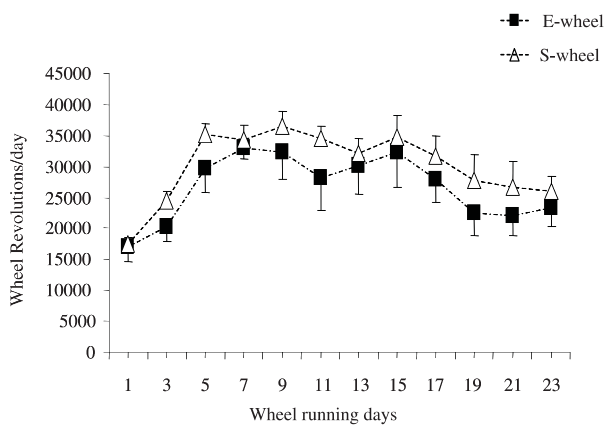

FIGURE 1 Effects of housing conditions on alternate-day wheel running behavior in BALB/c mice. Enriched mice (dark squares) showed similar frequency of wheel running as mice housed in standard condition (open triangles) over 3 weeks (Days 1 to 23 of the experiment) of alternate-day access to running wheel cage. Data are expressed as group mean number of wheel revolutions per day ± standard error of the mean. Enriched (E) wheel: $n = 7$; standard (S) wheel: $n = 7$.

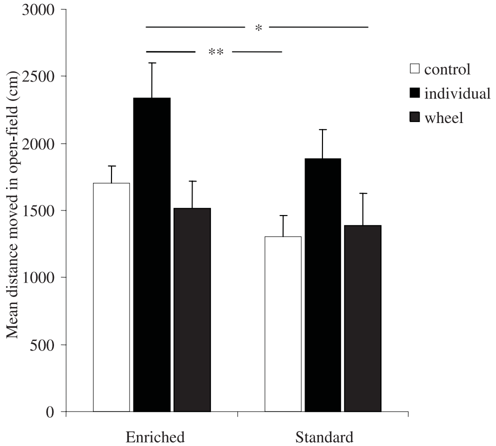

FIGURE 2 Distance moved in the open-field arena during 6-min observation for enriched and standard group housed mice in the control groups (white bars) (enriched control group: $n = 8$; standard control group: $n = 8$), in groups that were exposed to individual cage (black bars) (enriched individual: $n = 8$; standard individual: $n = 8$), and in groups that were exposed to running wheel cage (horizontal stripes bars; enriched wheel: $n = 7$; standard wheel: $n = 7$). Enriched individual mice moved more distance in the open field than the standard control and standard wheel groups (*$p < .05$, **$p < .01$). Error bars indicate group means ± standard error of the mean.

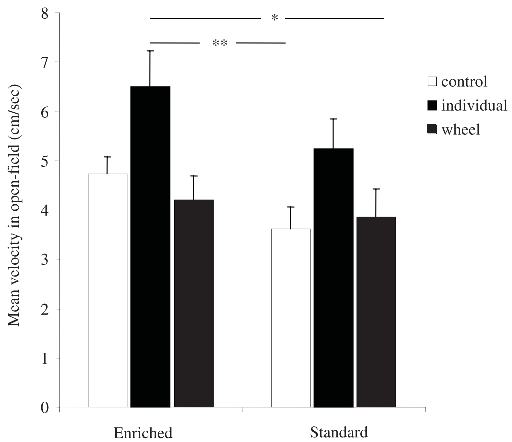

FIGURE 3 Mean velocity of locomotion in the open field of enriched or standard group housed mice exposed to individual cage is higher compared with other groups. Between the groups that were exposed to individual housing condition, the enriched mice (E-individual) also moved faster in the open-field arena than the standard control and the standard wheel (*$p < .05$, **$p < .01$). Error bars indicate standard error of the mean.

Figure 3 shows mean velocity in the open-field arena. A significant difference between the groups was found in the velocity of open-field locomotion, $F(4, 40) = 3.55$, $p < .01$. Tukey post hoc multiple comparisons between the groups indicated that the E-individual mice had higher ambulatory velocity in the open field than the S-control mice, and the standard-housed mice exposed to individual running wheel cages ($p < .01$, $p < .05$, respectively).

Locomotion in Central and Peripheral Zones

The circular open-field arena was defined further with central and peripheral zones to assess for more specific zone exploratory behavior. ANOVA performed on experimental groups and zones for the dependent measures of frequency in zones, time spent, and distance moved in zones showed no significant difference between the groups in either frequency or time spent in central and peripheral zones of the open field (Figures 4 through 7).

A significant difference was found between the groups on distance moved in the central zone (Figure 8), $F(5, 40) = 2.51$, $p < .05$. Tukey test indicated that E-individual group moved a greater distance in the central zone than the S-individual group ($p < .05$). Distance moved in the peripheral zone also revealed significant group effect (Figure 9). Enriched, housed mice intermittently exposed to individual housing without a wheel (E-individual) moved more in the peripheral zone of the open-field as compared to mice in S-control and S-wheel groups (Tukey post hoc test $p < .01$, $p < .05$, respectively). Intermittent changes from group housing to individual housing conditions affected open-field behavior in the mice housed in socially enriched condition more than they affected the S-housed mice.

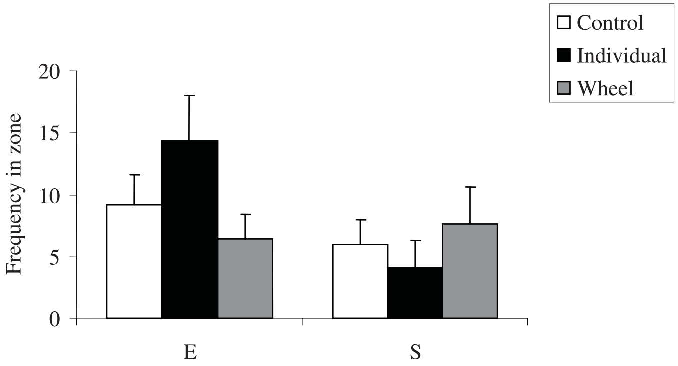

FIGURE 4 Mean frequency in the open-field zones of enriched (E) or standard (S) housed mice in control groups (white bars), in groups that were exposed to individual cage (black bars), and to running wheel cage (horizontal stripes bars). Error bars indicate standard error of the mean.

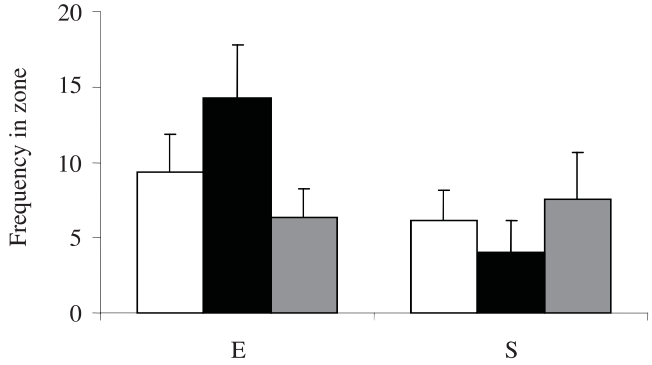

FIGURE 5 Mean frequency in the open-field peripheral zone of enriched (E) or standard (S)s housed mice in control groups (white bars), in groups that were exposed to individual cage (black bars), and to running wheel cage (horizontal stripes bars). Error bars indicate standard error of the mean.

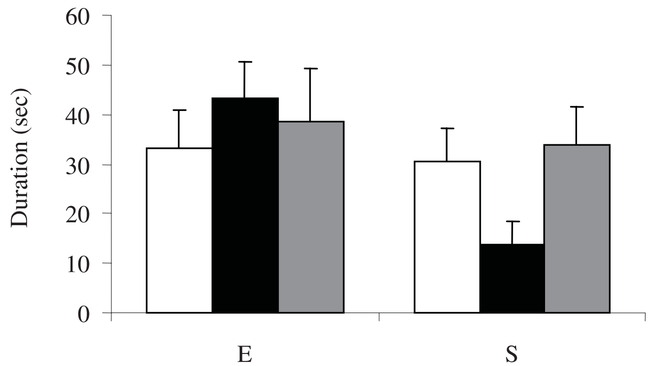

FIGURE 6 Mean time spent in the open-field central zone of enriched (E) or standard (S) housed mice in control groups (white bars), in groups that were exposed to individual cage (black bars), and to running wheel cage (horizontal stripes bars). Error bars indicate standard error of the mean.

Body Weight

Mice exposed to intermittent individual housing conditions (E-individual, S-individual) have lower body weight than the other groups. Figure 10 shows the mean body weights of the mice over the course of the study. ANOVA with repeated measures revealed significant overall effect on body weight between the groups, $F(5,40) = 3.18\, p < .05$. Group mean body weights were not significantly different between the housing conditions of enriched and standard. There was a significant inter-

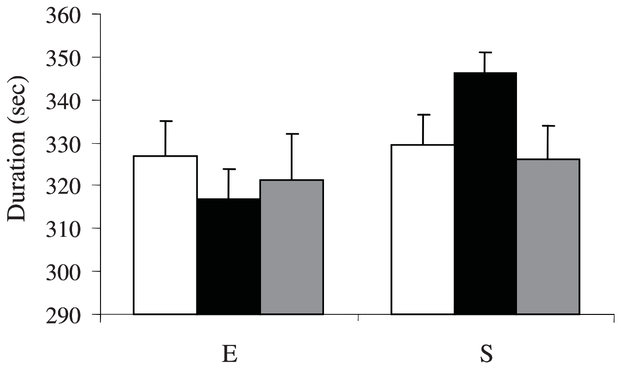

FIGURE 7 Mean time spent in the open-field peripheral zone of enriched (E) or standard (S) housed mice in control groups (white bars), in groups that were exposed to individual cage (black bars), and to running wheel cage (horizontal stripes bars). Error bars indicate standard error of the mean.

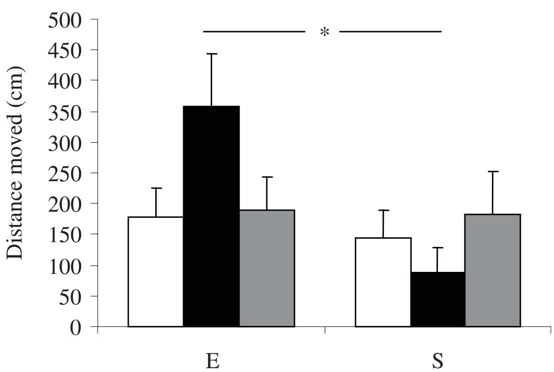

FIGURE 8 Mean distance moved in the open-field central zone of enriched (E) or standard (S) housed mice in control groups (white bars), in groups that were exposed to individual cage (black bars), and to running wheel cage (horizontal stripes bars).Error bars indicate standard error of the mean.

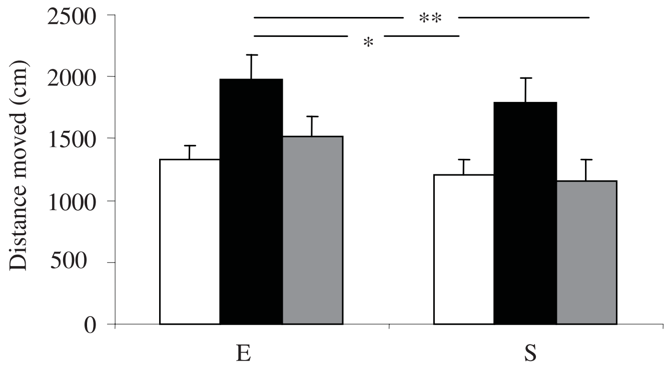

FIGURE 9 Mean distance moved in the open-field peripheral zone of enriched (E) or standard (S) housed mice in control groups (white bars), in groups that were exposed to individual cage (black bars), and to running wheel cage (horizontal stripes bars). Error bars indicate standard error of the mean.

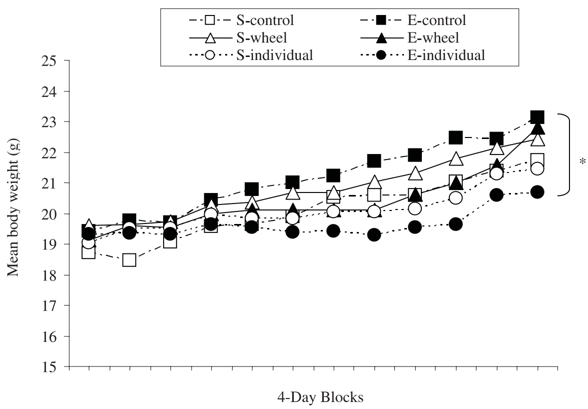

FIGURE 10 Body weight comparisons between enriched (filled symbols) and standard (open symbols) housed mice subdivided in three experimental group conditions: Control groups (enriched [E]-control; standard [S]-control), intermittent exposure to individual housing condition (E-individual; S-individual) and intermittent exposure to running wheel condition (E-wheel; S-wheel). Data represent group mean body weights taken every 4-day blocks over 7 weeks. Overall the groups differ significantly in body weight (*$p < .05$). E-individual mice have significantly lower body weight than the E-control mice (*$p < .05$).

action effect between housing conditions and the experimental groups, $F(2, 40) = 4.05$ $p < .05$. Post hoc Scheffé's test showed that intermittent housing in individual cages without running wheels resulted in significantly lower body weight in the E-individual compared to E-control groups ($p < 0.05$).

DISCUSSION

In this study, BALB/c mice were housed in two different environments: an enriched condition and a standard housing condition. Furthermore, the mice were xdivided into three treatment groups to assess the effects of wheel running on behavior in an open field as compared to exposure to individual housing and stable group-housing conditions. In addition, effects of enriched and standard housing conditions on wheel running activity were determined. Mice exposed to a running wheel on alternate days developed a running pattern within 3 days. For many species, wheel running usually occurs within a few minutes of access to the wheel; in laboratory mice, a steady pattern of wheel running is reached after about 6 days of running (Festing & Greenwood, 1976; Werme, 2001).

In our study, a stable pattern of wheel running that remained throughout the experiment was reached on Day 3 of exposure to the wheel, which actually was Day 6 of the experiment because the mice only had access to the running wheels on alternate days. Thus, alternating day wheel running seemed to build up the behavior performance, such that intermittent removal from wheel running was compensated by faster acquisition of the behavior. Intermittent access to a rewarding stimulus such as alcohol or cocaine also potentiates the development in rats of high preference to the reward (Heyser, Moc, & Koob, 2003; Heyser, Schulteis, & Koob, 1997; Holter & Spanagel, 1999; Nocjar & Panksepp, 2002; Taylor & Jentsch, 2001).

A study of rats—when wheel running was provided on alternate days compared to daily wheel running—found that the alternate-day wheel group increased its wheel running during the 3 hr following re-exposure to the wheel: There was no difference in the overall wheel running activity between the daily and the alternate-day wheel exposure (Mueller, Loft, & Eikelboom, 1997). Differences in wheel running behavior because of the genetic makeup were found, and alterations of genotypes of mice by selective breeding or genetic engineering have large effects (Lightfoot, Turner, Doves, Vordermark, & Kleeberger, 2004; Sherwin, 1998; Werme et al., 2003; Werme, Lindholm, et al., 2002).

In this study, we assessed whether housing conditions for BALB/c mice that provide for species-behavioral needs—such as hiding in shelter, nest building, and gnawing juxtaposed to the basic needs of food, water, and social contact with conspecifics—would influence their voluntary wheel running level and whether wheel running would influence other behaviors. If wheel running results from lack of stimulation from living in minimal, standard housing conditions, it might be performed less frequently in enriched mice. Other studies have shown that mice run in running wheels in complex environments (Brant & Kavanau, 1964; Kavanau & Brant, 1965; Sherwin & Nicol, 1998). We compared intermittent wheel running in socially enriched and nonenriched group housed mice; no significant differences were found.

In our study, the mice were placed in cages containing running wheels on alternate days, so that availability of running wheels was not in competition with other cage mates or contingent on running level or on food/water.

Joseph and Gallagher (1980) also showed there was no difference in running activity between rats reared in enriched and barren housing conditions. However, there are substantial differences in their study compared to our study concerning environmental conditions, duration of wheel running period, and species. Our results indicate that wheel running was performed consistently in both the enriched group and in the standard-housed group with a trend of enriched mice running less (Figure 1).

Similarly, a study with Syrian hamsters showed that cage enrichment did not affect daily frequency of wheel running. Only slightly less running activity resulted when the required number of cages connected by a tunnel to reach the running wheel was increased (Reebs & Maillet, 2003). We emphasize that the standard cages, although not provided with physical enrichment, provided social stimuli from the cage mates, which is a major enrichment in itself (Augustsson, van de Weerd, Kruitwagen, & Baumans, 2003; D'Arbe, Einstein, & Lavidis, 2002). This partial enrichment effect could account for the similarity in frequency of alternating day wheel running between the E-wheel and S-wheel groups. It also could suggest that gaining access to the wheel every other day provided the same novelty for both groups; therefore, the wheel—rather than a response to a basic physiological state—determined the frequency of wheel running.

Intermittently, the mice were exposed to individual cages with a running wheel; therefore, it was necessary to assess the influence of enriched and standard housing conditions on alternate-day exposure to an individual cage condition. A substantial effect of exposure to individual housing in both enriched and standard housed animals was found. In the open field, enriched mice showed increased locomotor activity. There was a trend in more time spent in the central zone by enriched animals. Mice exposed to individual housing only (E-individual, S-individual) showed higher levels of locomotor activity; they moved more and faster than running wheel exposed (E-wheel, S-wheel) and home cage control groups (E-control, S-control). This increase in locomotion may lead to the development of stereotypies (Wurbel & Stauffacher, 1996).

The individually housed mice in this study showed a behavioral pattern similar to the type of isolation induced hyperactivity observed in rodents (Garner & Mason, 2002; Geyer, Wilkinson, Humby, & Robbins, 1993; Jones, Marsden, & Roberts, 1991; Varty & Higgins, 1995; Varty, Paulus, Braff, & Geyer, 2000). The responses of both enriched group and standard group housed mice to alternate day, single housing condition suggest that even intermittent social separation in individual housing has behavioral consequences for the mice. When individual housing conditions lack motor and sensory stimulations, mice develop anxiety-like behavior, similar to isolation-induced stereotypy (Sherwin, 1996, 2004). This increase in locomotor activity also could be shown in the open-field test: Alternate day exposure to individual housing without a wheel caused an increase in locomotor activity. Thus, access to running wheels may alter or minimize the negative effects of individual housing.

In this study, no difference in body weight between enriched and standard conditions was found, although we observed that enriched animals were affected more by intermittent separation from their cage mates and housed in individual condition because over time they have the lowest body weight compared to the standard-housed and the control groups. Other researchers have reported significant increases in body weight for enriched, housed mice (Chapillon, Manneche, Belzung, & Caston, 1999; Van de Weerd, Van Loo, Van Zutphen, Koolhaas, & Baumans, 1997; Van de Weerd et al., 2002). However, this might result from a different degree of complexity of the enrichment, different gender, and/or to the use of different strains of mice. The body weight of the mice intermittently exposed to individual housing condition was significantly lower than in the wheel running and control group housed mice. The energy balance might explain this difference. In single housing conditions, the animals were exposed repeatedly to a negative energy balance state to maintain body temperature. Moreover, exposure to social isolation stress also might have caused the lower body weight as demonstrated by studies correlating body weight to stress (Harris et al., 2002; McLaren, Mathews, Fell, Gelling, & Macdonald, 2004). wheel running mice may be able to restore their energy balance by increasing their activity and/or their food intake. This is supported by other findings, which showed increased food intake in wheel running animals but similar body weight, compared to animals who do not run in wheels. Thus, although energy expenditure for wheel running was high, the animals compensated for energy loss by consuming more food (Mueller et al., 1997).

CONCLUSIONS

It can be concluded that both socially and physically enriched environments do not influence wheel running frequency and pattern in laboratory mice. Intermittent, individual housing of mice, however, seems to affect their locomotor activity in the open-field test and their body weight. Mice are social, living animals; group housing likely is an important factor for the development of a normal, behavioral repertoire. It was shown that intermittent individual housing could lead to increased locomotor behavior in the open field. Having access to physical activity in the running wheel could normalize this increase in activity. Moderate or intermittent physical activity has known positive effects on health and lifespan. Therefore, the idea of moderate physical fitness could be considered in enriching the environment of captive animals to improve their well being.

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