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The effects of environmental enrichment on depressive- and anxiety-relevant behaviors in socially isolated prairie voles

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Abstract

Objectives—Social isolation is associated with depression, anxiety and negative health outcomes. Environmental enrichment, including environmental and cognitive stimulation with inanimate objects and opportunities for physical exercise, may be an effective strategy to include in treatment paradigms for affective disorders as a function of social isolation. In a rodent model – the socially monogamous prairie vole – we investigated the hypothesis that depression- and anxiety-related behaviors following social isolation would be prevented and remediated with environmental enrichment.

Methods—Experiment 1 investigated the preventive effects of environmental enrichment on negative affective behaviors when administered concurrently with social isolation. Experiment 2 investigated the remediating effects of enrichment on negative affective behaviors when administered following a period of isolation. Behaviors were measured in 3 operational tests: open field; forced swim test; and elevated plus maze.

Results—In isolated prairie voles, enrichment prevented depression- (immobility in FST, group × housing interaction, $P=0.049$) and anxiety-relevant behaviors (exploration in open field, group × housing interaction, $P=0.036$; exploration in elevated plus maze, group × housing interaction, $P=0.049$). Delayed enrichment also remediated these behaviors in isolated animals (immobility in forced swim test, main effect of housing, $P=0.001$; exploration in open field, main effect of housing, $P=0.047$; exploration in elevated plus maze, main effect of housing, $P=0.001$), and was slightly more effective than physical exercise alone in remediating anxiety-relevant behaviors.

Conclusions—These findings provide insight into the beneficial effects of an enriched environment on depression- and anxiety-relevant behaviors using a translational rodent model of social isolation.

Keywords

anxiety; depression; environmental enrichment; exercise; microtus; social behavior

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INTRODUCTION

Both human and non-human animal studies indicate that social stressors, such as social isolation, influence the development of emotional and physiological disturbances (1–7). In humans, individuals with smaller social networks display increased depressive symptomatology compared to those with larger networks (8). Additionally, individuals who have less diverse social networks (lower levels of social integration) have an increased risk of several physical health-related problems and mortality, versus individuals who are more socially integrated (9). Further, in a recent study, social isolation was associated with an increased risk of mortality in older men and women (10).

Studies with animal models support the role of social isolation in mediating behavioral and physiological dysfunction. Rats living in social pairs display lower levels of depressive behaviors and improved cardiac function following social defeat stress versus those housed individually (11). The consequences of various social stressors, including (but not limited to) social isolation, also have been investigated using prairie voles, which are rodents that display socially monogamous behaviors similar to humans, such as forming long-term social bonds and living in family groups (12,13). Social isolation from family members or opposite-sex partners in this species produces depressive and anxiety behaviors including anhedonia, helplessness, and altered exploration; and physiological dysfunction such as exaggerated autonomic and endocrine reactivity to novel stressors (14–17). These studies suggest that the prairie vole is a valuable translational model for investigating the integration of social isolation with behavioral and physiological variables.

Given the detrimental effects of social isolation on health, treatments for emotional consequences of social isolation deserve greater consideration. A potential method for treating affective disturbances associated with social isolation is environmental enrichment (EE). Originally conceived by Hebb (18), EE provides environmental and cognitive stimulation with inanimate objects, the potential for physical activity, and (sometimes) increased social contact. This form of positive environmental stimulation has gained attention in the context of the *Healthy Brain Initiative* (19), with suggestions for ensuring healthy cognitive functions in aging populations. As reviewed elsewhere, exposure to EE improves several conditions, including degenerative diseases (Huntington's, Alzheimer's, Parkinson's), epilepsy, stroke, traumatic brain injury, fetal alcohol syndrome, anxiety, and depression (20–24). EE has recently been adapted to improve nervousness and social interactions in autistic children (25).

EE promotes a host of adaptive cognitive and behavioral outcomes, and has been demonstrated to promote both physical and psychological health (20,26–28). Although the details of each EE paradigm vary, they are hypothesized to engage similar neuroplastic mechanisms to influence behavior, and previous attempts have failed to attribute the effects of EE to any single variable (24,29). Both EE and voluntary exercise alone reduce anxiety- and depression-like behaviors in a variety of animal models (27,30–33). In the social domain, mice and rats exposed to EE exhibit increased sociability compared to control animals (34,35). Male mice with EE show heightened resilience to stressors such as social defeat (36). Interestingly, some group-housed mice given EE display exacerbated responses

to social stress versus individually-housed counterparts (37). It was suggested by the authors of this study that group housing in mice might facilitate social disturbances. Social behavioral variations among different rodent species may therefore interact with the responses to EE.

EE has not been explored as a treatment for the negative consequences of social isolation using prairie voles. The use of this animal model may inform an important question of whether additional environmental stimulation provided via EE is sufficient to replace the social stimulation that is lost when animals are socially isolated. To this end, the present study investigated the hypothesis that EE will improve depression- and anxiety-relevant behaviors in socially isolated prairie voles. Experiment 1 focused on the preventive effects of EE on behaviors when administered concurrently with social isolation; and Experiment 2 focused on the remediating effects of EE on behaviors when administered in delayed fashion following a period of social isolation.

METHODS

Animals

69 adult female prairie voles, descendants of a wild stock caught near Champaign, Illinois, had a mean ($\pm$ standard error of the mean; SEM) age of 99 ± 7 days and a body weight of 34 ± 1 grams. All animals were maintained on a 14/10 h light/dark cycle (lights on at 0630h), with a mean $\pm$ SEM ambient temperature of $25 \pm 2^\circ\text{C}$ and relative humidity of $40 \pm 5\%$. Animals were allowed food (Purina rabbit chow) and water ad libitum. Offspring were removed from breeding pairs at 21 days of age and housed in same-sex sibling pairs until the commencement of the experiment. For all procedures, only one animal from each sibling pair was studied. All procedures were conducted according to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and approved by the Northern Illinois University Institutional Animal Care and Use Committee.

Experiment 1

Experiment 1 investigated the potential preventive effects of EE, using a design of social isolation or pairing (control) with concurrent EE or standard housing (Figure 1A). 32 female prairie voles were randomly divided into paired ($n=16$) or socially isolated ($n=16$) groups. Paired control animals were continually housed with the siblings for 4 weeks, while isolated animals were separated from their respective siblings and housed individually without visual, auditory or olfactory cues. The paired and isolated prairie voles were assigned to either a standard cage ($n=8$ per group) or an EE cage ($n=8$ per group) (32,38), concurrent with 4 weeks of pairing or isolation. The standard cage was $12 \times 18 \times 28\text{cm}$ in size, consisting of food, water and bedding. The EE cage was $25 \times 45 \times 60\text{cm}$ in size, consisting of food, water, bedding, and the following items: a running wheel (4in diameter), a wood block, a rubber die, a wood jack chew toy, a mini straw hat, a cardboard toilet paper roll, a tin foil ball, a piece of cotton nesting material, a plastic bowl with small food pellets, 2 plastic toys (1 hanging from cage top and 1 inside the cage), 2 marbles, and a plastic igloo house. Items were placed randomly inside the cage, and sanitized or replaced each week. Handling, cage changing, and body weight measurements were matched among all groups.

Following this 4-week period, all animals were exposed to 3 behavioral tests, each separated by 48 hours (see below): (a) 20-minute open field test for the investigation of exploratory and anxiety-relevant behaviors; (b) 5-minute forced swim test (FST) for the investigation of depression-relevant behaviors; and (c) 5-minute elevated plus maze (EPM) test for the investigation of anxiety-relevant behaviors.

Experiment 2

Experiment 2 investigated the potential remediating effects of either EE or physical exercise in isolated prairie voles, using a design of social isolation followed by delayed access to either EE, physical exercise alone, or standard housing (Figure 1B). 37 female prairie voles were socially isolated from their same-sex siblings for 4 weeks using procedures described in Experiment 1. Following this 4-week period, all animals were randomly divided into either standard housing (n=17), EE housing (n=9), or exercise housing (n=11) for an additional 4 weeks. The standard and EE cages were identical to those described in Experiment 1. The exercise housing cage was 25×45×60cm in size, consisting of food, water, bedding, and a running wheel (4in diameter). Handling, cage changing, and body weight measurements were matched among all groups.

Following this 8-week period (4 weeks of social isolation and 4 weeks of additional isolation in standard, EE, or exercise housing), all animals were exposed to 3 behavioral tests (see below) as described in Experiment 1. Analysis of body fat was conducted at the end of the experiment (see below), 48 hours following the last behavioral test.

Open Field

Investigation of behaviors in the open field was used as an index of exploratory and anxiety-relevant behaviors (39). The animal was placed in a transparent Plexiglas box (40×40×40cm) for 20 minutes. A red outline on the bottom of the arena (18×18cm) delineated the center section. The arena was cleaned thoroughly prior to testing each animal. The animal was replaced in its home cage immediately following the test.

Behaviors during the open field were recorded using a digital video camera, and scored manually by trained, experimentally-blind observers. Behaviors were defined as: (a) time spent in the center vs. surrounding section; (b) time spent autogrooming; (c) number of rears; and (d) number of crosses into the center section of the arena. The animal was coded as entering a section of the arena when all 4 paws crossed into the respective section. Number of crosses into the center section of the arena was used as an index of general activity; reduced exploration of the center section, coupled with altered grooming and/or rearing, was defined as anxiety-relevant behavior (39).

Forced Swim Test

Investigation of swimming behavior in the FST was used as an index of a depressive phenotype (15,40). A clear, cylindrical Plexiglas tank (46cm height; 20cm diameter) was filled to a depth of 18cm with tap water (20–24°C). The animal was placed in the tank for 5 minutes. The tank was cleaned thoroughly and filled with clean water prior to testing each

animal. Each animal was returned to its home cage immediately following the test, and allowed access to a heat lamp for 15 minutes.

Behaviors during the swim test were recorded using a digital video camera, and scored manually by trained, experimentally-blind observers. Behaviors were defined as: (a) swimming, movements of forelimbs and hindlimbs without breaking the surface of the water; (b) struggling, forelimbs breaking the surface of water; (c) climbing, attempts to climb the walls of the tank; and (d) immobility, no limb or body movements (floating) or using limbs solely to remain afloat without corresponding trunk movements. Swimming, struggling, and climbing were summed to provide one index of active coping behaviors; immobility was used as the operational index of depressive (helpless) behavior (15,40).

Elevated Plus Maze

Anxiogenic behaviors were measured in the EPM (41). The maze (57cm height) consisted of two opposing open arms of clear Plexiglas (49.5×10cm), two opposing closed arms of black Plexiglas with an open roof (49.5×10×30.5cm), and a center section of clear Plexiglas (10×10cm). The animal was placed in the center in a brightly-lit room, and allowed to explore freely for 5 minutes. The maze was cleaned thoroughly prior to testing each animal. Animals were returned to the home cage immediately following the test.

Behaviors were recorded using a digital video camera, and scored manually by trained, experimentally-blind observers. Behaviors were defined as (a) duration of time spent in each the open, closed, and center sections of the maze; and (b) number of crosses into the center section. The animal was coded as entering a section of the maze when all 4 paws crossed into the respective section. Number of crosses in the center section of the maze was used as an index of general activity; reduced exploration of the open arms was used as an index of anxiety-relevant behavior (41).

Body Fat Analysis

To ensure that animals in the physical exercise condition used the running wheels, body fat mass was analyzed at the end of the study in this condition, compared to a randomly-selected sample of standard-housed animals. The following fat pads were removed, cleaned of connective tissues, and weighed: (a) parametrial white adipose tissue (pWAT); (b) inguinal white adipose tissue (iWAT); and (c) retroperitoneal white adipose tissue (rWAT) (42). Total body fat and percentage of body fat, based on the total weight of these fat stores, were calculated.

Data Analyses

Data are presented as means $\pm$ SEM for all analyses and figures. A value of $P < 0.05$ was considered to be statistically significant. The data were analyzed with single-factor or two-factor independent-groups analyses of variance (ANOVA) to compare group (paired or isolated) and housing (standard or EE in Experiment 1; standard, exercise, or EE in Experiment 2), followed by a priori Student's *t*-tests with a Bonferroni correction for multiple comparisons. All statistically significant results reported throughout the reference section include actual probability values; however the results are only reported for multiple

comparisons when they reached statistical significance after the Bonferroni correction was applied.

RESULTS

Experiment 1

Open Field—Exploration of the center section in the open field was reduced as a function of social isolation, and EE prevented this alteration (Figure 2A). Grooming behavior was increased as a function of EE but not social isolation (Figure 2B). General activity and rearing behavior were not affected by either social isolation or EE (data not shown). The ANOVA for exploration in the center of the open field yielded a group $\times$ housing interaction [$F(1,28)=4.85$, $P<0.036$]. The isolated + standard housing group explored the center section of the open field arena significantly less than the isolated + EE group [$t(14)=4.85$, $P=0.001$] and paired groups [$t(14)=2.24$, $P=0.010$ versus paired + standard; $t(14)=3.61$, $P=0.001$ versus paired + EE]. There were no significant differences in duration of time spent in the center section among the isolation + EE, paired + EE, or paired + standard housing groups ($P>0.05$ for all comparisons).

The ANOVA for autogrooming duration yielded a main effect of housing [$F(1,28)=5.06$, $P=0.033$]. Animals in the EE conditions spent more time autogrooming than the standard-housed conditions [$t(30)=2.31$, $P=0.014$]; but there was no effect of group (paired or isolated) on autogrooming behavior ($P>0.05$). The ANOVA for number of rears did not yield any significant effects ($P>0.05$ for all comparisons); no follow-up tests were conducted. The ANOVA for number of crosses into the center section of the open field arena yielded no significant effects ($P>0.05$ for all comparisons); no follow-up tests were conducted.

Forced Swim Test—Social isolation led to an increase in immobility during the FST, and EE prevented this behavior (Figure 3). The ANOVA yielded a main effect of group [$F(1,28)=11.59$, $P=0.002$], a main effect of housing [$F(1,28)=6.93$, $P=0.014$], and a group $\times$ housing interaction [$F(1,28)=3.52$, $P=0.049$]. The isolated + standard housing group exhibited significantly greater amounts of immobility versus the isolated + EE [$t(14)=3.17$, $P=0.004$] and paired groups [$t(14)=3.17$, $P=0.004$ versus paired + standard; $t(14)=3.20$, $P=0.003$ versus paired + EE]. There were no significant differences in duration of immobility among the isolation + EE, paired + EE, or paired + standard housing groups ($P>0.05$).

Elevated Plus Maze—Social isolation led to a decrease in exploration of the open arms of the EPM, and EE prevented this alteration (Figure 4). General activity in the EPM did not differ as a function of either social isolation or EE (data not shown). The ANOVA for open arm duration yielded a main effect of group [$F(1,28)=5.71$, $P=0.020$], main effect of housing [$F(1,28)=7.73$, $P=0.011$], and a group $\times$ housing interaction [$F(1,28)=4.77$, $P=0.049$]. The isolated + standard housing group spent significantly less time in the open arms of the maze versus the isolated + EE [$t(14)=1.74$, $P=0.050$] and paired groups [$t(14)=2.70$, $P=0.009$ versus paired + standard; $t(14)=2.10$, $P=0.015$ versus paired + EE]. There were no

significant differences in duration of open arm exploration among the isolation + EE, paired + EE, or paired + standard housing groups ($P>0.05$ for all comparisons).

The ANOVA for closed arm duration did not yield any significant effects ($P>0.05$). No follow-up tests were conducted (data not shown).

The ANOVA for center section duration yielded a group $\times$ housing interaction [$F(1,28)=6.51$, $P=0.017$]. The isolated + standard housing group spent slightly more time in the center section than the paired + standard housing group [$t(14)=2.29$, $P=0.050$], and slightly, but non-significantly, more time than the EE groups ($P>0.05$ for both comparisons; data not shown). The ANOVA for number of crosses in the center section of the maze yielded no significant effects ($P>0.05$ for all comparisons); no follow-up tests were conducted.

Body Weight—There were no significant differences in body weight among the four groups at any point during the experiment ($P>0.05$; data not shown).

Experiment 2

Open Field—In isolated prairie voles, both EE and exercise alone were associated with increased center section exploration of the open field, relative to standard housing (Figure 5A). Autogrooming behavior was increased in the EE group, relative to the exercise and standard-housed group (Figure 5B). General activity and rearing behavior did not differ among the three groups (data not shown). The ANOVA for exploration in the center of the open field arena yielded a main effect of housing [$F(2,34)=3.34$, $P=0.047$]. Animals housed in standard cages spent less time in the center section than those in EE [$t(24)=1.78$, $P<0.020$] and exercise housing conditions [$t(26)=3.00$, $P=0.003$]. The amount of time spent in the center section did not differ between EE-housed and exercise-housed conditions ($P>0.05$).

The ANOVA for autogrooming behavior yielded a main effect of housing [$F(2,34)=5.60$, $P=0.008$]. Standard-housed animals spent less time grooming than EE-housed animals [$t(24)=1.97$, $P<0.021$]. Animals in the exercise condition also spent less time grooming than EE-housed animals [$t(18)=2.98$, $P=0.020$]. The amount of time spent grooming did not differ between standard-housed and exercise-housed conditions ($P>0.05$). The ANOVA for number of rears during the open field did not yield any significant effects ($P>0.05$); no follow-up tests were conducted. The ANOVA for number of crosses into the center section of the open field arena did not yield a significant effect ($P>0.05$); no follow-up tests were conducted.

Forced Swim Test—In isolated prairie voles, both EE and exercise alone were associated with increased active coping behaviors and decreased immobility during the FST, relative to standard housing (Figure 6). The ANOVA for immobility yielded a main effect of housing [$F(2,34)=10.30$, $P=0.001$]. Animals in a standard cage displayed greater levels of immobility than those in an enriched cage [$t(24)=3.15$, $P=0.003$] and those in the exercise housing condition [$t(26)=2.95$, $P=0.004$]. There was no significant difference in immobility duration between EE-housed and exercise-housed conditions ($P>0.05$).

Elevated Plus Maze—In isolated prairie voles, housing in an enriched cage was associated with increased exploration of the open arms of the EPM versus both standard housing and exercise housing (Figure 7), however general activity did not differ among the three groups (data not shown). The ANOVA for open arm duration yielded a main effect of housing [$F(2,34)=27.04$, $P=0.001$]. Isolated animals in standard cages spent significantly less time in the open arms of the maze versus EE-housed animals [$t(18)=2.60$, $P=0.011$]. However, the open arm duration of animals in the exercise condition did not differ significantly from either standard or EE housing ($P>0.05$), indicating an intermediate response in this condition.

The ANOVA for closed arm duration yielded a main effect of housing ($F(2,34)=8.29$, $P=0.001$). Animals in the enriched housing condition spent less time in the closed arms versus both standard [$t(24)=3.51$, $P=0.009$] and exercise housing conditions [$t(18)=3.03$, $P=0.003$]. Animals in the standard and exercise housing conditions did not differ in the amount of time spent in the closed arms ($P>0.05$; data not shown).

The ANOVAs for center section duration and number of crosses into the center section yielded no significant effects ($P>0.05$ for both comparisons; data not shown). No follow-up tests were conducted.

Body Weight and Body Fat—There were no differences in body weight among the three groups at any point during the experiment ($P>0.05$; data not shown). The exercise group had significantly less absolute body fat mass [$F(1,20)=5.21$, $P=0.034$], and significantly lower percentage of body fat relative to body weight [$F(1,20)=5.60$, $P=0.029$], versus the standard housing group (Table 1).

DISCUSSION

The current study demonstrates that EE both prevents and remediates depressive and anxiety-relevant behaviors associated with long-term social isolation in female prairie voles. Social stressors – including social isolation, the disruption of established social bonds, and acute social environmental changes such as crowding – have repeatedly been shown to produce detrimental consequences in prairie voles (14,17,43–45), contributing to the argument that this species is an excellent model for the study of the psychological and physiological effects of social experiences. More specifically, both depression- and anxiety-related behaviors have been demonstrated in prairie voles following various forms of social stressors, including, but not limited to, social isolation (14,15,45). The present study is the first to describe the effects of EE in prairie voles exposed to social isolation, and provides insight into the protective and remediating effects of this strategy on affective behaviors.

The present findings indicate that EE is capable of preventing both depression- and anxiety-related behaviors in socially isolated prairie voles. The EPM, open field, and FST were employed here given the demonstrated validity of these behavioral tests for the investigation of affective behaviors in rodents, including exploration of potentially threatening environments (anxiety), and immobility and helpless behavior (depression) (39–41). Similar to previous research with long-term social isolation in prairie voles (15), 4 weeks of

isolation in a standard cage was associated with increased immobility during the FST, as well as reduced exploration in both the EPM and open field. In contrast, isolated animals in an enriched cage were protected from these depressive and anxiety-related behaviors. The levels of immobility and exploratory behaviors in the isolated group with EE were comparable to those of paired animals both with and without EE. Similarly, exposing other rodents to EE has been shown to improve learning and memory functions, reduce anxiety- and depression-like behaviors, and improve stress-coping abilities (20–22,46,47).

An important distinction was observed between paired and isolated prairie voles with exposure to EE, suggesting the specific nature of the beneficial effects of an enriched environment. EE was effective as a replacement for the lack of social stimulation in isolated prairie voles, whereas paired animals with EE did not show any improvements in behaviors over standard-housed animals. Hence, social pairing is sufficient to protect prairie voles against some negative affective states, as measured in the present study by the FST, EPM and open field. Social pairing may be a useful form of enrichment in prairie voles in some circumstances; additional protective mechanisms to aid in stress coping do not appear necessary for animals that have access to appropriate social buffering from a cagemate. This suggestion is paralleled by previous results showing that the administration of oxytocin – a peptide involved in social behavior and stress reactivity – also attenuated depressive behaviors in isolated prairie voles, whereas this same treatment had no effect on the behavior of paired animals (48). Taken together, these findings lend support to the evidence from human studies which points to the detrimental effects of social isolation on health (7,10).

The findings from Experiment 1 were extended in a second experiment focused on the potential remediating effects of EE. Given that EE had neither a positive nor a negative effect on behaviors of paired prairie voles in Experiment 1, Experiment 2 focused on isolated prairie voles exclusively. The results indicate that delayed EE was effective at remediating depressive and anxiety behaviors in isolated prairie voles. Compared to animals with EE, isolated animals in a standard cage displayed significantly greater levels of immobility in the FST, and significantly lower levels of exploration in the EPM and open field, similar to the isolated-standard housed animals in Experiment 1. Exposure of isolated prairie voles to EE in a delayed manner (i.e., after 4 weeks of social isolation) was investigated specifically to be consistent with the most likely time course of dysfunction and the subsequent seeking of treatment in humans experiencing social stress and/or affective symptoms (i.e., after a problem or potential problem has occurred). The current results indicate that it is not necessary for EE to be presented at the beginning of a period of social isolation to achieve positive effects on behavior.

To determine the beneficial effects of the complexity of EE on depressive and anxiety behaviors, this treatment was also compared to physical exercise alone. Physical exercise was comparable to EE in its ability to attenuate depressive behaviors (i.e., immobility in the FST) in isolated animals. However, exercise did not have the same pattern of effects on anxiety-related behaviors. Physical exercise was not as effective as EE at increasing open arm exploration in the EPM. Further, the amount of time the animals engaged in center section exploration of the open field was comparable in the physical exercise and EE

conditions, however grooming was increased in the EE group only (relative to the physical exercise and standard housed groups). Interestingly, this grooming behavior mirrors the pattern observed in Experiment 1; grooming also was increased as a function of EE, regardless of social housing condition. Studies with other rodent models indicate that both EE and voluntary exercise can reduce depressive and anxiety behaviors (27,30–33). Indeed, exercise also has been shown to have both antidepressant and anxiolytic properties in humans (49–51). In the operational measures employed here, physical exercise may serve a similar function as EE in the context of depressive behaviors following social isolation, but does not appear to be as effective as EE at remediating anxiety-relevant behaviors.

The neurobiological mechanisms that underlie the beneficial effects of EE and exercise are not well defined. EE has several interactions with brain function (23,24,52,53), and may improve conditions such as motor disorders, dementias, brain injury, and affective behaviors (20–24). However, the influence of EE and exercise in the brain may have dissociable mechanisms. Many components of EE increase overall neurogenesis by decreasing the normal loss of cells, whereas voluntary exercise enhances the proliferation of progenitor cells in addition to reducing cell death (54). A specific investigation of key brain regions that play a role in social behavior, stress, and emotion may shed light on the central mechanisms underlying the beneficial effects of EE and exercise in the context of social isolation. For instance, it is possible that these treatments would influence the production or release of hormones from the hypothalamic paraventricular nucleus, which has been shown to be altered following social isolation in prairie voles (14,43,44), thereby improving stress coping ability.

Considered together, the present findings provide evidence that EE is an effective treatment for the behavioral consequences of long-term social isolation using a translational animal model. This study has demonstrated for the first time that EE can prevent and remediate social isolation-induced depressive and anxiety behaviors in the prairie vole. These results might have implications for humans who experience social isolation. For example, an enriched environment, including exercise, may be a useful treatment strategy to employ in combination with psychological or pharmacological therapies for socially isolated individuals. Further investigation of the specific neurobiological mechanisms underlying the beneficial influence of EE and physical exercise is warranted. Additional experiments using animal models, such as those described here, may contribute to the development of novel treatment strategies and improve the quality of life for individuals experiencing affective disorders as a function of social isolation.

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Abbreviations

ANOVA   analysis of variance

EE	environmental enrichment
EPM	elevated plus maze
FST	forced swim test
SEM	standard error of the mean

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A. Experiment 1 Study Design **B. Experiment 2 Study Design** **Figure 1.**

General study designs used in Experiments 1 (Panel A) and 2 (Panel B). Note: EE = environmental enrichment; EPM = elevated plus maze; FST = forced swim test.

Figure 2.

Mean (+SEM) duration of time spent exploring the center section (Panel A) and autogrooming (Panel B) in a 20-minute open field test in paired and isolated prairie voles with concurrent access to either standard or enriched housing. * $P < 0.05$ vs. isolated housing in a standard cage; ^ $P < 0.05$ vs. paired housing in a standard cage (see text for specific probability values). Note: in Panel A, the remainder of 20-minute period is comprised of exploration of the surrounding section of the open field arena.

Figure 3.

Mean (+SEM) duration of immobility in a 5-minute forced swim test in paired and isolated prairie voles with concurrent access to either standard or enriched housing. * $P < 0.05$ vs. isolated housing in a standard cage (see text for specific probability values). Note: the remainder of 5-minute period is comprised of active coping behaviors.

Figure 4.

Mean (+SEM) duration of time spent exploring the open arms of a 5-minute elevated plus maze in paired and isolated prairie voles with concurrent access to either standard or enriched housing. * $P < 0.05$ vs. isolated housing in a standard cage (see text for specific probability values).

Figure 5.

Mean (+SEM) duration of time spent exploring the center section (Panel A) and autogrooming (Panel B) in a 20-minute open field test in isolated prairie voles with delayed access to either standard, exercise (running wheel only), or enriched housing. * $P < 0.05$ vs. isolated housing in a standard cage; # $P < 0.05$ vs. isolated housing with exercise alone (see text for specific probability values). Note: in Panel A, the remainder of 20-minute period is comprised of exploration of the surround section of the open field arena.

Figure 6.

Mean (+SEM) duration of immobility in a 5-minute forced swim test in isolated prairie voles with delayed access to either standard, exercise (running wheel only), or enriched housing. * $P < 0.05$ vs. isolated housing in a standard cage (see text for specific probability values). Note: the remainder of 5-minute period is comprised of active coping behaviors.

Figure 7.

Mean (+SEM) duration of time spent exploring the open arms of a 5-minute elevated plus maze in isolated prairie voles with delayed access to either standard, exercise (running wheel only), or enriched housing. * $P < 0.05$ vs. isolated housing in a standard cage (see text for specific probability values).

Table 1

Mean ($\pm$ SEM) body fat in isolated prairie voles following either standard or exercise housing conditions.

	Standard Housing	Exercise Housing
Total Fat (mg)	1509.9 $\pm$ 202.8	953.3 $\pm$ 149.6*
Fat Percentage (%)	3.8 $\pm$ 0.4	2.6 $\pm$ 0.3*

* P<0.05 vs. respective standard housing value.