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Effects of Social Housing on Hippocampal Dendrites and Behavior in Ovariectomized Rats

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Abstract

Social stress is both species and gender specific. For female rats, individual housing and social instability housing conditions are associated with behavioral indicators of stress and depression. The present study directly compared the effects of six weeks of individual housing, social instability and mixed sex, semi-crowded housing in a visible burrow system (VBS) on ovariectomized female rats. Paired, stable housing was used as the control. Behavioral tests were conducted two, four and six weeks into the housing manipulations and included sucrose consumption, social interest, and activity in the open field. Following a series of four behavioral tests, animals were sacrificed and brains were processed for Golgi impregnation. Basal dendrites of CA3 hippocampal neurons were measured. Results indicate that the individual housing and social instability groups were comparable to the control group for all measures. In contrast, the rats housed in the VBS exhibited reduced activity in open field testing, and alterations in social interest. Dendritic lengths were also reduced in those animals living in the VBS in comparison to the animals housed in pairs. To our knowledge, this is the first report of behavioral and neural effects of VBS housing on female rats. Further research is necessary to determine what facets of the VBS housing are responsible for the behavioral and neural changes.

Keywords

depression; social stress; hippocampal dendrites; open field behavior; anxiety

1. Introduction

Major Depressive Disorder is a global public health issue associated with decrements in physical health (Cassano & Fava, 2002; Moussavi et al., 2007). Although the etiology of depression is complex (Firk & Markus, 2007; Goldberg, 2006), there is now widespread agreement that chronic stress contributes to both behavioral changes and to functional alterations of the hippocampus and other limbic regions (Joëls, Karst, Krugers, & Lucassen, 2007; McEwen, 2007; Sapolsky, 2000).

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The continued progress in research on the neurobiological bases of depression is dependent on the availability of valid, reliable, and relevant animal models (Matthews, Christmas, Swan, & Sorrell, 2005; Frazer & Morilak, 2007). Three such models which have yielded important results include the chronic mild stress (CMS) model (Baker, Kentner, Konkle, Barbagallo, & Bielajew, 2006; Willner, 2005; Willner, Muscat, & Papp, 1992), restraint stress (McLaughlin, Baran, Wright, & Conrad, 2005; McLaughlin, Gomez, Baran, & Conrad, 2007) and exposure to conspecific aggression such as that experienced in a visible burrow system (VBS) (Blanchard & Blanchard, 1990).

Deciding on an animal model must include consideration of gender. Because women are approximately twice as likely to experience depression as men (Ohayon, 2007), a female-specific model of depression is a priority. The etiology of depression for women is complex, with cultural, social, genetic and hormonal factors postulated to account for this gender disparity (Kessler, 2003; Kuehner, 2003). Thus developing a model with construct validity, i.e. one which “follows a plausible explanatory theory for the human condition” is essential (Dalla, Pitychoutis, Kokras & Papadopoulou-Daifoti, 2010, p. 227).

Social stress therefore may be an especially valuable component of an animal model of depression. One form of social stress which is effective for female, but not male, rats is individual housing. Brown and Grunberg (1995) systematically compared corticosterone levels in female and male rats housed singly, in groups, or in crowded cages. Corticosterone levels, taken once after 14 days of this housing, were highest in male rats living in groups of ten under crowded conditions. In contrast, corticosterone levels were higher for the individually housed females compared to all of the grouped housing conditions, regardless of spatial density.

Social instability is another model of inducing depression-like responses in female rats. Haller, Fuchs, Halász, and Makara, (1998) alternated housing gonadally intact female rats (oviducts were ligated to prevent pregnancy) between individual housing and crowded mixed sex housing. Instability was enhanced by changing the group membership each day. Control animals were stable male-female pairs. After 14 days, females living in the social instability condition showed chronic stress-related changes including reduced weight gain, thymus atrophy, adrenal hypertrophy and elevated plasma corticosterone levels. Further studies found that even without the male rats, this social instability paradigm resulted in a reduction in time spent in social behavior, an increase in time spent in aggressive behavior, and a reduction in preference for a sucrose solution and reduced food intake (Baranyi, Bakos, & Haller, 2005; Haller, Baranyi, Bakos, & Halász, 2004; Herzog et al., 2009).

While the social instability model has been effective in inducing behavioral and physiological changes in female rats, a well-documented social stress for male rats is the visible burrow system or VBS (Blanchard & Blanchard, 1990). The VBS consists of a large open field attached to smaller compartments interconnected by a tunnel system. When several males and females are housed together in the VBS, fighting among the males is initially high. Over time, this aggression subsides as one male achieves dominance. Behavioral, physiological, and neural effects of VBS housing on male rats have been reported (Blanchard & Blanchard, 1990; Blanchard et al., 1995; Blanchard, McKittrick, & Blanchard, 2001; Kozorovitskiy & Gould, 2004). An apparent advantage of this model is the semi-natural environment simulated by the VBS.

The present study was designed to determine the effectiveness of three types of social stress for inducing depression in female rats: individual housing (INDIV), social instability (INST) and mixed-sex semi-natural housing (VBS). The VBS model was chosen even though females do not develop the social hierarchies which underlie result in stress for males

housed in this condition (Tamashiro et al., 2004). Nonetheless, there are reports that female rats living in the VBS are subjected to high levels of agonistic behaviors from both dominant and subordinate males (Blanchard et al., 2001).

A paired housing control (CON) condition was used as the reference group. All animals were ovariectomized to control for hormone fluctuations across the estrous cycle. Exogenous estrogen was not provided since chronic treatment with estrogen induces receptivity in female rats (Davidson, Smith, Rodgers, & Bloch, 1968), which would be a confounding variable for those animals living in the VBS. We hypothesized that each of the experimental conditions of Individual Housing, Social Instability, and mixed-sex VBS housing would result in anhedonia, reduced social interest, low levels of exploration of the physical environment and retraction of dendrites of hippocampal CA3 neurons.

2. Materials and methods

2.1. Animals

Female Sprague-Dawley rats, bred in the vivarium and between 49 and 70 days of age, were anesthetized with ketamine (75 mg/kg)-xylazine (5 mg/kg), and bilaterally ovariectomized. After the surgery, animals were housed two per cage and allowed 14 days to recover before being randomly assigned to experimental groups. Body weights were taken each week. All animals were housed under a reversed light-dark cycle with the lights on 21:00–9:00 h.

Experimental conditions—Rats were randomly assigned to one of four housing conditions ($n = 8$ per condition). Shoe-box style polycarbonate cages were used for all animals except for those in the VBS. INDIV subjects were housed singly (33 cm $\times$ 30 cm $\times$ 18 cm) and CON animals were housed two per cage (50 cm $\times$ 38 cm $\times$ 21.5 cm). Animals assigned to the INST group alternated between individual and grouped housing phases. For the grouped housing period, the INST subject was introduced into socially-established groups of three non-experimental, gonadally-intact female rats (50 cm $\times$ 38 cm $\times$ 21.5 cm). After 24 hours, they were returned to an individual cage for 24 hours. Four different established groups were used and the INST animals were rotated through these groups.

A diagram of the VBS is shown in Figure 1. A large open space contained food and water. This space was interconnected via clear tunnels to two smaller darkened chambers. The VBS housed four experimental females and four intact male rats of approximately the same age. The composition of each VBS was stable throughout the experimental period.

2.2. Behavioral tests

Four different behavioral tests were administered once per week for four weeks, beginning two weeks after animals were placed in experimental conditions. All behavioral experiments were performed under red lighting, between 1200 h and 1500 h. The timeline for all tests and experimental procedures is presented in Figure 2.

2.3.1. Sucrose consumption tests—During the week prior to the start of behavioral testing, all animals were given three sucrose training sessions. Briefly, the animals were placed in individual cages with a bottle of 1% sucrose solution and given ad libitum access for 1 hour before being returned to their home cage. Prior to the actual sucrose consumption tests, animals were water deprived for 12 hours. At the beginning of the dark phase, they were placed in a clean individual cage and presented with a pre-weighed bottle of 1% sucrose solution. Following the 1 hour test, the bottle was again weighed. The total amount of sucrose solution consumed, measured in grams, was recorded.

2.3.2. Open field tests—For the 10 minute open field tests, rats were placed in a darkened open field apparatus (35.5 cm × 35.5 cm) and data were collected using an automated open-field locomotor activity apparatus (Digiscan, Omnitech Electronics, Columbus, Ohio). Activity measures recorded included the total moving time, total distance, time spent in the corners of the open field, and horizontal movements.

2.3.3. Social interest tests—For the social interest tests, the test rat was placed in a clear Plexiglas chamber (51.5 cm × 36.5 cm × 27 cm). Smaller chambers (18 cm × 11.5 cm × 16 cm) were affixed to either end of the main chamber and a 2.2 cm diameter hole was cut in the partition between the main chamber and the small chambers. For each test, an ovariectomized female rat was placed in one small chamber and an intact male was placed in the other, with the placement alternated each week.

The tests began when the experimental rat was placed in the large chamber and lasted for 10 minutes. Behaviors recorded during the test included the number and total duration of time the test rat poked her nose into the chamber containing the stimulus male and into the chamber containing the stimulus female. The number of events and duration of activities were recorded and analyzed using JWatcher 1.0 software (Blumstein, Daniel, & Evans, 2006).

2.3. Dendrite Analysis

Following the final behavior test, animals were lightly anesthetized and decapitated. Brains were immediately removed. Using a Rat Brain Blocker (Kopf Instruments), a 5 mm thick section, with the rostral border 1 mm anterior to the optic chiasm, was taken so as to include the entire rostral-caudal and ventro-dorsal dimensions of the hippocampus. These sections were processed according to FD Rapid Golgistain™ Kits (FD NeuroTechnologies, Baltimore, MD). The staining procedure followed previously established methods that successfully stain hippocampal pyramidal cells (McLaughlin et al., 2005). Sections remained in the Golgi-Cox rapid stain for 12 to 14 days. The sections were then cut on a vibrating microtome (Vibratome) and 100 µm sections were mounted on slides and coded for blind analysis.

Dendrite analysis was conducted using the methods of Cook and Wellman (2004) and Pawluski and Galea (2006). Briefly, with the experimenter blind to condition, the hippocampal CA3 region was visualized under 20X magnification using a Leica DM E microscope. Cells were chosen for Golgi analysis based on the following criteria: (1) the cell body and dendrites were fully impregnated, (2) the cell was relatively isolated from surrounding neurons, and (3) the cell was located in the CA3 region of the hippocampus. Six cells from each animal were photographed using QCapture software from QImaging. CA3 neurons and basal dendrites were viewed on a computer monitor and digitally traced and measured using ImageJ software package (NIH). Total dendritic length for each of the six identified labeled neurons was determined, as was the number of dendritic branches. The means for the six cells were used as the unit of analysis for each animal. Drawings of a neuron from animals in each housing condition are shown in Figure 3.

2.4. Data Analysis

Means and standard error of the means ($M \pm SEM$) were calculated for all variables. One-way analyses of the variance (ANOVA) were used to compare the dendrite lengths and the number of dendritic branches across the four housing groups. If appropriate, post hoc Dunnett's tests compared each of the experimental groups to the CON group. Body weights and all behavioral measures were analyzed by repeated measures analyses of variance (ANOVA) with tests as the repeated factor and Housing Condition as the independent

factor. Effects were considered significant for $p < 0.05$. When appropriate, post hoc Dunnett's tests were performed. All analyses were carried out using the Windows version of SPSS software, version 17.0.

3. Results

Rats were weighed weekly. No differences in weights were found across the four groups ($p > .1$) nor was there a significant interaction between weight and time ($p > .1$). Mean weight gain for the groups over the four week testing period ranged from 11.0 ± 3.3 g for the CON group to 22.2 ± 5.1 g for the VBS group.

3.1. Dendrite analysis

Figure 4 shows the mean total length of CA3 basal dendrites for each of the four housing conditions. There was a difference in this measure based on housing conditions, $F(3,28) = 3.72$, $p < .05$. Dunnett's post hoc tests revealed that only those females living in the VBS had reduced dendritic lengths in comparison to the control-housed animals, $p < .05$. Although there was a similar trend for the number of branch points for the CA3 neurons, this did not reach significance, $p = .12$.

3.2. Behavior Tests

3.2.1. Sucrose consumption—Sucrose consumption was expressed as a percentage of body weight [$100 \times (\text{sucrose solution consumed (g)}/\text{body weight (g)})$]. No differences were found across the four housing condition groups, $F(3,28) = 1.36$, $p = 0.27$. Likewise, no difference was found across the tests ($p > .1$) nor was the Tests $\times$ Groups interaction significant ($p > .1$).

3.2.2. Open field tests—As shown in Figure 5A, a significant main effect of Housing Condition was found for the horizontal activity measure, $F(3,28) = 5.12$, $p = .006$. Dunnett's *post hoc* comparisons indicated that the VBS group mean was significantly lower than the CON group mean ($p < .05$). There was no difference between the INDIV or the INST groups compared to the CON group. There was a significant difference in horizontal activity across the four tests, $F(3,84) = 3.29$, $p = .02$, but the Test $\times$ Housing Condition interaction was not significant.

Similar results were found for the total distance traveled by the rats during the 10 minute tests, with a significant main effect of Housing Condition, $F(3,28) = 4.61$, $p = .010$. Dunnett's *post hoc* comparisons showed that the VBS group mean was significantly lower than the CON group mean ($p < .01$). Again, no difference was found between for the INDIV or INST groups in comparison to the CON group. The effect of tests was significant, $F(3,84) = 3.12$, $p = .03$, while the Test $\times$ Housing Condition interaction did not approach significance. These results are shown in Figure 5B.

There was a strong trend towards significance for the main effect of Housing Condition for the amount of time the rats spent in the corners of the apparatus, $F(3,28) = 2.74$, $p = .06$ (see Figure 5C). Dunnett's *post hoc* comparisons showed that the VBS animals spent more time in the corners than did the CON animals ($p < .05$). Neither the INDIV nor the INST group means differed from the CON group mean for this measure. Overall, there was a difference across the four tests for the amount of time the rats spent in the corners, $F(3,84) = 4.75$, $p = .004$, but there was no Test $\times$ Housing Condition interaction. Similar results were found for time spent in the center of the apparatus, but this failed to reach significance ($p = .14$).

As shown in Figure 5D, time spent immobile increased across the tests, $F(3,28) = 10.01$, $p < .001$). There was also a significant main effect of Housing Condition for immobility time, $F(3,28) = 6.474$, $p < .002$. The Dunnett's *post hoc* comparisons showed that the VBS group spent significantly more time immobile than the CON group ($p < .05$). Neither the INDIV nor the INST group differed from the CON group, nor was there a Test X Housing Condition interaction.

3.2.3. Social exploration—Social interest was defined as the total time that the subject poked her nose into the opening to the male and female compartments combined. There was no difference across groups for this social interest measure ($p > .1$) but there was a difference for the percent of time directed towards the male compared to the female, $F(3, 28) = 12.02$, $p = < .01$. As shown in Figure 6, the VBS animals spent a greater proportion of the social interest oriented towards the stimulus female than did the CON group, $p < .05$. Neither the INST nor the INDIV group differed from the CON group. There was also no difference across tests nor was there a Test X Housing Condition interaction ($p > .1$).

4. Discussion

The purpose of the present study was to determine the effectiveness of three gender-specific social stress models for inducing behavioral and neural changes in female rats. While we hypothesized that each of the three conditions would result in the onset of behaviors associated with stress and depression, our study failed to support the effectiveness of either individual housing or social instability. In contrast, females housed in mixed-sex visible burrow systems did exhibit behavioral changes and retraction of CA3 basal dendrites, which did not diminish over the weeks of testing.

Significant loss of hippocampal volume has been observed in humans diagnosed with unipolar depression (Sheline, 2000; Sheline, Wang, Gado, Csernansky & Vannier, 1996). A leading hypothesis for this loss is the deleterious effect of glucocorticoids on neurogenesis, neuronal death, synaptic changes and dendritic remodeling (Czeh & Lucassen, 2007). According to Czeh and Lucassen, neurogenesis and neuronal death may play a minor role. Retraction of dendrites, on the other hand, has been repeatedly implicated using animal models. For example, chronic mild stress (Vyas, Mitra, Shankaranarayana Rao, & Chattarji, 2002), chronic immobilization stress (Vyas, et al., 2002), chronic exposure to glucocorticoids (Conrad, et al., 2007), and social stress (McKittrick, et al., 2000) have all been reported to be associated with dendritic retraction. According to Conrad (2008), these forms of stress may render the hippocampus vulnerable, via repeated exposure to elevated levels of glucocorticoids. In the present study, we found dendritic retraction of the CA3 basal dendrites only for those animals living in the VBS. This change, coupled with behavioral changes, lends support to the hypothesis that living in mixed sex VBS housing results in stress-induced changes.

Although Brown and Grunberg (1995) have reported that housing female rats in individual cages results in elevated corticosterone levels, such housing was without impact in the present study. As reviewed by Palanza (2001), individual housing effects have not been consistent across time frame, schedules or strain of rat. Krohn, Sørensen, Ottesen, and Hansen (2006) discuss the importance of variables including environmental enrichment, cage size and cage structure and concluded that the overall impact of individual housing may be small.

Likewise the lack of a stress-induced response to social instability in the present study may be due to differences between the procedures used here and those reported by previous researchers (Baranyi et al., 2005; Haller et al., 1998). First, the number of animals and the

spatial density used in the present study were lower and secondly, the instability was created by introducing the experimental female into an established group of female rats. While it was hypothesized that this alternation between individual housing and placement into an already established group of females would be stressful, this did not appear to occur. It may be that the relative low spatial density compensated for the social instability.

Developing an animal model of depression presents numerous challenges. Symptoms of depression overlap with those of anxiety disorders and the two often coexist in humans. Notably, antidepressants are often the drug of choice for both depression and anxiety (Hranov, 2008). Determining behaviors in an animal model which are associated only with depression or anxiety is thus complicated. Reduction in activity and exploration in an open field apparatus have been used as reliable indicators of the impact of social defeat and chronic mild stress protocols (Blanchard, McKittrick, & Blanchard, 2001; Rygula, Abumaria, Flugge, Fuchs, Ruther, & Havemann-Reinecke, 2005). In the present study, the animals housed in the VBS exhibited a reduction in total distance traveled in the open field, increased time spent immobile, increased time in the corners and a trend towards decreased time in the center. These deficits were most evident in the fourth test, with the differences between the VBS animals and the Control animals becoming more pronounced over time.

Sucrose consumption or preference is largely considered a measure of anhedonia and therefore is included as a measure of depression (Katz, 1982; Gronli et al., 2004). In the present study, we failed to find a difference in consumption across groups. Further research should use sucrose preference, which may be more sensitive to the effects of VBS housing (Willner, Towell, Sampson, Sophokleous, & Muscat, 1987).

According to our study, the VBS housing results in behavioral and morphological changes in ovx rats. is somewhat similar to that reported for males. Since the deleterious effects of VBS housing is on subordinate rather than dominant animals, status within the social hierarchy is the presumed mechanism for stress for males (Blanchard & Blanchard, 1990; Blanchard et al., 1995; Blanchard et al., 2001; Blanchard, McKittrick, & Blanchard, 2001; McKittrick et al., 2000; Tamashiro, Nguyen, & Sakai, 2005). Females, however, do not form such hierarchies (Tamashiro et al., 2004). Thus, if females living in the VBS experience stress, it is likely due to a mechanism other than dominate/subordinate interactions.

Further studies should examine whether agonistic encounters, either initiated by males and or females, are instrumental in the behavioral and dendritic changes reported here. Previous studies, conducted to assess the effects of VBS housing on males, have reported that male-on-female aggressive behaviors are common (Blanchard et al., 2001). According to these authors, the most common site of attack was the anogenital region, suggesting that the attacks were actually sexual approaches (Blanchard et al., 2001). When the females were not in estrus, they used defensive posturing and non-aggressive biting to ward off the mounts, thus avoiding injurious attacks. These aggressive interactions were also seen in crowded mixed-sex social instability housing conditions, as reported by Haller and colleagues (Haller et al., 1998).

In addition, frequent female-female aggressive encounters in the crowded social instability and VBS conditions have also been reported (Blanchard et al., 2001; Haller et al., 1998). Reduced litter size and lower pregnancy rates have been reported for females housed in the VBS (Tamashiro et al., 2005), indicating that stressful conditions for the females do exist. Nonetheless, the present study did not directly measure agonistic encounters.

It should be mentioned that the animals living in the VBS are engaged in very different physical and social environments, requiring more complex behaviors than those living in the other conditions. Indeed, the VBS housing results in a both social and physical

“enhancements” similar to those associated with enriched environments (van Praag, Kempermann, & Gage, 2000). Rather than resulting in dendritic retraction, the enriched environments have been reported to protect hippocampal dendrites (Hutchinson, et al., 2012) and hippocampal neurogenesis (Veena, Srikumar, Raju, & Shankaranarayana Rao, 2009) from the effects of chronic restraint stress.

While an increased risk of depression for women compared to men is a consistent epidemiological finding, the underlying factors for this disparity have not yet been resolved (Altemus, 2006; Goldberg, 2006; Kessler, 2003; Kuehner, 2003; Ohayon, 2007; Piccinelli & Wilkinson, 2000). A considerable body of research exists on women’s vulnerability to depression during the perimenopausal period (Deecher, Andree, Sloan, Schechter, 2008; Harsh, Meltzer-Brody, Rubinow, & Schmidt, 2009.) Yet the role of estrogen in depression is far from clear.

Interestingly, McLaughlin, Baran, Wright and Conrad (2005) found that chronic restraint stress resulted in dendritic retraction in ovariectomized female rats regardless of whether they were treated with estrogen post-ovariectomy. Indeed no differences were found for any of their morphological or behavioral measures based on hormonal status. Nonetheless, it is important to study the effects of stress across the various transitional points of the female reproductive life cycle.

Conclusions

Differential exposure and increased vulnerability to stressful life events have been posited as major contributors to the increased risk, yet alternative explanations stress the critical role played by psychological characteristics of the stress (Kessler 2003; Kuehner, 2003). The stressors most clearly associated with depression are those that result in a loss of status, psychobiological defeat, or submission (Bebbington 1996; Honkalampi et al. 2005; Kendler et al. 2003). Assaults, especially when committed by someone close to the individual, are particularly potent events (Tjaden & Thoennes 2000).

There is a need to develop an animal model of depression that is relevant to humans and that uses situations that are regularly encountered (Palanza, 2001). Palanza further notes that for females, special attention to the social environment and the use of contextually relevant conditions are critical. In this light, the VBS may be a valid animal model of female-specific stress associated with depression in women. The results presented here indicate that living in mixed-sex group housing in the VBS results in behavioral changes and dendritic atrophy. Further research on the impact of VBS housing on females must include hormonal manipulations, since the impact of chronic restraint stress on dendritic morphology in the medial prefrontal cortex has been shown to be dependent on the presence of estrogen (Garrett & Wellman, 2009).

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Highlights

► Social stress is both species and gender specific. ► We explored the impact of three social housing conditions on behavior and hippocampal morphology in ovariectomized female rats. ► Semi-crowded, mixed sex housing in a visible burrow system was associated with behavioral and neuronal compromise.

Figure 1. Diagram of the VBS. The open space: 62 cm × 62 cm × 45 cm; tunnels: 9.5 cm H × 9.5 cm W; darkened chambers: 25 cm × 21 cm × 18.5 cm and 17.5 cm × 14.5 cm × 18.5 cm. Food and water were constantly available.

Figure 2. Schedule of procedures. Ovx: ovariectomy; S: sucrose consumption tests; OF: open field tests; SI: social interest tests; Ex: exploration of novel objects tests.

Figure 3. Representative ImageJ drawings of Golgi-stained CA3 cell bodies and basal dendrites from an animal in each of the four housing conditions.

Figure 4.

Analysis of CA3 hippocampal basal dendrites (A) Mean combined length of dendrites ($\mu\text{m} \pm \text{SEM}$); (B) Mean number of dendritic branches ($\pm \text{SEM}$). Results of one-way ANOVA given in text; significance levels of Dunnett's tests shown on figure: * $p < 0.05$.

Figure 5.

Analysis of behavior in the Open Field Tests. Mean scores ($\pm$ SEM) for (A) number of horizontal activities within the 10 minute test; (B) total horizontal distance; (C) time spent in the corners of the apparatus; and time spent immobile. *symbols indicate significant differences ($p < .05$) between the VBS and CON animals.

Figure 6.

Analysis of behavior in the Social Interest Tests. Mean scores ($\pm$ SEM) for (A) the total time the subject spent with the nose in the opening to either the male or female compartment.