Published in final edited form as:

Behav Brain Res. 2009 August 12; 201(2): 257–264. doi:10.1016/j.bbr.2009.02.019.

Strain, Sex, and Open-Field Behavior: Factors Underlying the Genetic Susceptibility to Helplessness

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Abstract

Learned helplessness represents a failure to escape after exposure to inescapable stress and may model human psychiatric disorders related to stress. Previous work has demonstrated individual differences in susceptibility to learned helplessness. In this study, we assessed different factors associated with this susceptibility, including strain, sex, and open-field behavior. Testing of three rat strains (Holtzman, Long-Evans, and Sprague-Dawley) revealed that Holtzman rats were the most susceptible to helplessness. Holtzman rats not only had the longest escape latencies following inescapable shock, but also showed spontaneous escape deficits in the absence of prior shock when tested with a fixed-ratio 2 (FR2) running response. Moreover, when tested with fixed-ratio 1 (FR1) running—an easy response normally unaffected by helplessness training in rats—inescapable shock significantly increased the escape latencies of Holtzman rats. Within the Holtzman strain, we confirmed recent findings that females showed superior escape performance and therefore appeared more resistant to helplessness than males. However, regression and covariance analyses suggest that this sex difference may be explained by more baseline ambulatory activity among females. In addition, some indices of novelty reactivity (greater exploration of novel vs. familiar open-field) predicted subsequent helpless behavior. In conclusion, Holtzman rats, and especially male Holtzman rats, have a strong predisposition to become immobile when stressed which interferes with their ability to learn active escape responses. The Holtzman strain therefore appears to be a commercially available model for studying susceptibility to helplessness in males, and novelty-seeking may be a marker of this susceptibility.

Keywords

learned helplessness; strain differences; sex differences; post-traumatic stress disorder; exploratory behavior; escape response

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1. Introduction

Learned helplessness (LH) represents a failure to exhibit an escape response after exposure to inescapable stress [26]. This paradigm serves as a useful tool to model stress-induced psychopathology, such as depression or post-traumatic stress disorder (PTSD) [6,28,29,31]. However, there are individual differences in the response to stress because most individuals do not develop psychopathology in response to psychological stress or trauma [2,16,17,25]. Therefore, there is a need to identify biological factors which confer vulnerability to stress-induced psychopathology. Identifying these factors in humans is difficult because most human studies have examined individuals only after stress has taken its toll. Animal models provide a convenient way to investigate the predisposing factors underlying susceptibility to helplessness. One successful strategy has been to study rat lines selectively bred for behaviors which model human psychiatric disorders [10,32]. For example, rats selectively bred to display LH show neurological and behavioral signs similar to those seen in humans with depression and PTSD [32,36,38]. However, selective breeding protocols can take years to develop and can be burdensome to maintain. Therefore, it would be beneficial if there were a commercially available strain with increased susceptibility to LH. While there are a few reports of increased anxious or depressed behavior in some inbred rat strains such as the Wistar-Kyoto [1], to our knowledge only one study has assessed different susceptibilities to LH among different outbred strains. Wieland et al. [41] found that Holtzman rats were twice as likely to develop learned helplessness as Sprague-Dawley rats. Our first objective was to see if this susceptibility difference between strains reported over 20 years ago was still present today. In addition, we assessed the susceptibility of Long-Evans rats, which, to our knowledge, have never been evaluated in the learned helplessness paradigm.

As our results will show, we verified that the Holtzman line still shows the most helpless behavior following inescapable shock. Our next objective was to evaluate whether the escape deficits exhibited by Holtzman rats were in fact induced by prior exposure to inescapable shock, or whether they reflect a baseline deficit in escape learning (i.e., learned vs. spontaneous helplessness). For example, after multiple generations of selective breeding for learned helpless behavior, the majority of rats show spontaneous escape deficits regardless of whether they are exposed to inescapable shock beforehand [11]. Rats from this selected line also show a number of other behavioral differences, such as hyperactivity specific to novel environments [32,38]. We have interpreted these behaviors as reflecting a temperament of high novelty-seeking, which in humans has been associated with risk of developing PTSD [30,39]. However, to our knowledge, only one study has examined whether individual differences in temperament can predict the development of helplessness in rats, and this study came to essentially the opposite conclusion that reluctance to explore a novel open field predicted vulnerability to helplessness [23]. Therefore, our third objective was to use linear regression to evaluate whether open-field activity before inescapable shock could predict escape deficits after inescapable shock.

Our final objective was to assess sex as a risk factor for helplessness. Despite consistent reports of increased incidence of PTSD and depression in women [2,40], there are conflicting reports of sex differences in the development of learned helplessness in rats. One study found that female rats expressed more helpless behavior than males depending on estrus phase [13], while another study found less helpless behavior in females, independent of gonadal hormones [4]. To address this issue, we evaluated both males and females with measures of temperament and helplessness and assessed whether the phase of estrus cycle contributed to the expression of helpless behavior.

2. Materials and Methods

2.1. Subjects

Subjects were 41 male Holtzman, 10 male Sprague-Dawley, 10 male Long-Evans and 41 female Holtzman rats obtained from Harlan (Madison, WI) at postnatal day 30 (P30). Animals were housed 2–3 per cage and maintained on a 12h/12h light/dark photoperiod in a facility accredited by the Association for the Assessment of Laboratory Animal Care International. Food and water were available *ad libitum*. Subjects were handled and weighed for five minutes every day for one week prior to starting behavioral experiments. Open-field experiments occurred between 0900 h and 1200 h. Inescapable shock training occurred between 0900 h and 1700 h and escapable shock testing was performed between 0800 h and 1900 h. Experiments were done in accordance with NIH guidelines for the use of experimental animals and were approved by the University of Texas Institutional Animal Care and Use Committee.

2.2. Apparatus

The open-field chamber (43.2 × 43.2 cm) consisted of clear plastic sides 30.5 cm high and a white plexiglass floor. Activity was detected by arrays of infrared light beam motion detectors (16 × 16, 2.5 cm apart) at the sides of each chamber, thus creating a detection grid. Two arrays of detectors were located 1 cm above the floor, and another array was located 13 cm above the floor, to detect rearing. The chambers were controlled by the Activity Monitor program, version 5.10 (Med Associates, St. Albans, VT).

Two inescapable shock chambers (30 × 25 × 20 cm) (Med Associates, St. Albans, VT) were enclosed in sound-attenuated boxes and illuminated by a red light. Each apparatus had two sides of aluminum, with clear plexiglass for the front, back, and top. A soapy solution made from Ivory dishwashing liquid (Procter and Gamble, Cincinnati, OH) was placed in the tray beneath the chambers to provide a distinct olfactory cue for the inescapable context. Shocks were delivered through metal bars separated by 1.2 cm forming the floor of the chamber, which was wired to shock generators (Med Associates). The chamber was controlled by MED-PC, version 4 (Med Associates, St. Albans, VT), using a program written in the MEDSTATE language.

The shuttle box (42 × 16 × 25 cm) consisted of two compartments of equal size, separated by a door (11 × 9 cm) that remained open throughout the session. The chamber was enclosed in a sound-attenuated box and illuminated by a white light (10 lux). Two sides of the chamber were aluminum, with clear plexiglass for the front, back, and top. Shocks were delivered through metal bars separated by 1.2 cm forming the floor of the chamber, which was wired to shock generators (Med Associates). The subject's position was detected by eight sets of infrared light beam motion detectors, located 2 cm above the grid floor, spaced 4.4 cm apart from each other, on both sides of the chamber. The chamber was controlled by MED-PC, version 4, using a program written in the MEDSTATE language. This program used beam breaks of the two pairs of beams located at either end of both sides of the chamber as the contingency for terminating shock, to score a complete crossing. A povidone-iodine solution (First Priority, INC., Elgin, IL) was placed in the tray beneath the chamber to provide a distinct olfactory cue for the escapable context.

2.3. Behavioral experiments

Ten separate cohorts (n=102) underwent behavioral testing for 11 weeks. Within each cohort behavioral experiments took place during four consecutive days. All animals were tested for open-field (OF) activity during the first day of experiments (novel OF) on P40 to determine if behavioral characteristics predictive of helplessness were present before learned helplessness training. Each animal was placed in the same corner of the open-field chamber and behavior

was recorded for 10 minutes. The chambers were washed with a diluted Bio-clean detergent solution (Stanbio laboratory, Boerne, TX) between each session. Measures included ambulatory counts (horizontal beam breaks), average velocity, rearing counts (vertical beam breaks), average rearing duration, and thigmotaxis (time spent in the 62% periphery versus the 38% center of the open-field). Measures were automatically scored by a computer using MED-PC software.

On the second day, subjects were re-tested in the open-field (familiar OF). From these two open-field sessions, behavioral measures were calculated reflecting both general activity (novel plus familiar) and novelty-specific activity (novel-to-familiar ratio). Thus, an animal with high exploratory activity in both the novel and familiar open fields would have a high general-activity score but a low novelty-reactivity score. In order to have a high novelty-reactivity score, an animal would have to be very active in the novel open-field but much less active in the familiar open-field. Thus, these two scores help to separate animals which are excited by novel environments from those that are chronically hyperactive.

On the third day, eighty-two subjects (31 male Holtzman, 10 male Long-Evans, 10 male Sprague-Dawley and 31 female Holtzman), were trained in the inescapable shock chamber to observe the helpless phenotype [12]. Each session included 60 trials of 10 s duration with pseudorandom inter-trial intervals ranging from 10 to 110 seconds. To find the minimum shock intensity that would induce helplessness and motivate escape behavior, we tried three different shock intensities: 0.5 (n=11), 0.75 (n=40), and 1.0 mA (n=11). An ANOVA revealed no significant effect of amperage [$F(2,59) = 0.521, p = 0.60$] on escape performance, and the mean FR2 escape latencies for 0.5 mA (17 s), 0.75 mA (20 s), and 1.0 mA (18 s) were virtually equivalent. Therefore, subjects were pooled together for the statistical analyses without regard to amperage. In addition, in order to test the effects of prior shock exposure on escape behavior, 10 male and 10 female Holtzman rats did not receive inescapable shock prior to helplessness testing in the shuttle box.

On the fourth day, subjects were tested with the escapable shock paradigm using a shuttle box to measure escape behavior. First, subjects were tested with five trials of a fixed-ratio (FR) 1 schedule consisting of crossing from one side of the box to the other to terminate the shock. This was followed by 25 trials of an FR2 schedule in which animals had to cross twice; in other words, rats had to return to the compartment where the shock was initiated in order to terminate the shock. The maximum possible footshock duration was 30 seconds, after which the shock was terminated if the subject had not escaped. Pseudorandom inter-trial intervals consisted of durations ranging from 10 to 110 seconds. Amperage was matched to what was delivered in the inescapable session, as discussed above. Number of escape failures, latency to perform the first cross during FR2, and escape latency (time to terminate the footshock) were automatically scored using MED-PC software.

2.4. Procedure for determining phase of estrus cycle

Daily vaginal smears were taken on the days of open field tests, inescapable shock training and escapable shock testing. Tapered-end cotton swabs were immersed in a beaker filled with 10 ml of tap water. Using the moistened swab, samples were taken from the vaginal wall and smeared over gelatin-coated slides. Samples were allowed to dry for 24 hours and vaginal epithelial cells were imaged using an Olympus light microscope at 10x magnification and 0.25 NA. The imaging process was used to classify the reproductive phase of the female rats into estrus, diestrus or proestrus [24].

2.5. Statistical analysis

Strain differences in open-field activity were analyzed using both sums (novel plus familiar) and ratios (novel to familiar) of the two open-field sessions. The former served as an index of general activity, while the latter reflected the proportion of activity that was novelty specific. Analysis of variance (ANOVA) was used to test for an overall effect of strain in these parameters, and Tukey's post-hoc test was used to test for differences between individual strains. Helplessness testing was analyzed with repeated measures ANOVA, using the FR1 and FR2 escape latencies as two separate levels in a within-subject variable. In addition to the final (second cross) escape latencies of the FR2 trials, latencies to make the first crossing were also analyzed with ANOVA, with Tukey's post-hoc test as a follow-up. An analysis of covariance (ANCOVA) tested whether controlling for ambulation in the open-field would have an effect on the escape latency results. Effects of prior shock exposure and sex differences were analyzed with repeated measures ANOVA as described above. ANOVAs were used to analyze sex differences in open-field activity and differences in escape latency related to estrus cycle. A series of linear regressions were calculated between average escape latency and open-field parameters, to determine if the prior behavior of each subject would be predictive of helpless behavior.

3. Results

3.1. Strain differences

3.1.1. Strain differences in open-field behavior—The three strains were tested in an open-field apparatus across two consecutive days (novel and familiar sessions) prior to exposure to any stressor. The locomotor activity was scored in terms of ambulation, rearing, and time spent in the center of the field. Then two types of scores were computed: a general-activity score, calculated by summing the activity scores from both novel and familiar sessions, and a novelty-reactivity score, calculated by dividing activity measures in the novel session by the same measures in the familiar session. The means and standard errors for general activity and the novelty-specific index are presented in the top and bottom halves, respectively, of Table 1. Only one general activity parameter was significant: average rearing duration, $F(2,26) = 7.19$ ($p < 0.01$), which Tukey's post-hoc test showed to be significantly greater in the Long-Evans subjects as compared to the Sprague-Dawley subjects ($p < 0.01$).

3.1.2. Strain differences in escape latency—A repeated measures ANOVA (3×2), with strain (Holtzman, Sprague-Dawley, and Long-Evans) as a between-subject variable and response contingency (average of FR1 vs. average of FR2 trials) as a within-subject variable, tested whether strain differences would give rise to differences in escape behavior. There was a significant interaction between strain and response contingency, $F(2,26) = 19.556$ ($p < 0.001$), indicating that Sprague-Dawley subjects maintained low escape latencies across both FR1 and FR2 trials while the other two strains showed an increase in escape latencies during the FR2 trials (Fig. 1). There was also a significant main effect of strain, $F(2,26) = 9.12$ ($p < 0.01$), with the Holtzman (18 ± 2 s) and Long-Evans (13 ± 2 s) strains showing significantly greater escape latencies than the Sprague-Dawley (4 ± 1 s) strain ($p < 0.01$). However, there was no significant difference between the Holtzman and Long-Evans strains.

The average latency to perform the first crossing of the fixed-ratio-two (FR2) response also showed a significant group difference, $F(2,26) = 12.4$ ($p < 0.001$). The Holtzman (14 ± 3 s) subjects took longer to make this first crossing than did either Long-Evans (7 ± 1 s) or Sprague-Dawley (2 ± 0.3 s) subjects ($p < 0.05$), but the Long-Evans and Sprague-Dawley strains were not significantly different from each other. Another ANOVA showed a significant group difference in the number of failures to terminate the shock in less than 30 seconds, $F(2,26) = 6.78$ ($p < 0.01$). The Holtzman (34% failed trials) and Long-Evans (27% failed trials) rats had

significantly more failures than Sprague-Dawley (2% failed trials) subjects ($p < 0.05$), but were not significantly different from each other.

3.2. Effect of prior shock exposure in Holtzman rats

The next analysis assessed the effects of prior shock exposure for both sexes, testing whether twenty Holtzman subjects which did not undergo inescapable shock (“naïve group”) showed escape latencies that were significantly different from the sixty-two Holtzman rats which did undergo inescapable shock (“prior shock group”). A repeated measures ANOVA ($2 \times 2 \times 2$) used group and sex as between-subject variables, and response contingency (FR1 vs. FR2) as a within-subject variable. A significant interaction between group and response contingency was found, $F(1,78) = 11.0$, $p < 0.01$. Simple-effects tests showed that the FR1 contingency was sensitive to the effect of prior shock exposure while the FR2 contingency was not (Fig. 2).

3.3. Sex effects

3.3.1. Sex differences in escape latency—The repeated measures ANOVA ($2 \times 2 \times 2$), which was used to analyze prior shock exposure above, found no interaction between sex and inescapable shock, but it did find a significant interaction between sex and response contingency, $F(1,78) = 7.32$, $p < 0.01$. Simple-effects tests of the interaction showed sex differences with the FR2 contingency but not with the FR1 contingency, with females performing better than males in the more difficult FR2 task (Fig. 3). Average latency to make the first crossing of the FR2 response, as well as average failure rate, showed similar trends in sex differences, but neither effect was significant, $F_s(1,60) = 2.14$ and 1.85 , respectively.

3.3.2. Estrus cycle and escape latency—To test whether a subject’s estrus cycle had an effect on the development of helplessness, the 31 female subjects in the prior shock group were given vaginal smears on both the day of inescapable shock presentation and the subsequent day of shuttle box helplessness testing. On the day of inescapable shock training, there were 13 rats in estrus, 11 rats in diestrus, and 7 rats in proestrus. On the day of escapable shock testing, there were 15 rats in estrus, 11 rats in diestrus, and 5 rats in proestrus. One-way ANOVAs tested whether the estrus phase had a significant effect on escape latencies. The estrus phase during inescapable shock had no significant effect on either FR1 or FR2 escape latencies, $F_s(2,28) = 0.150$ and 0.103 , respectively. The estrus phase during helplessness testing also had no effect on either FR1 or FR2 latencies, $F_s(2,28) = 1.01$ and 0.488 , respectively. Average first crossing latency and average failure rate were similarly unaffected by estrus cycle.

3.3.3. Sex differences in open-field activity—A series of ANOVAs analyzed the open-field activity of the Holtzman subjects ($n = 41$ males, 41 females) for sex differences in the same locomotor parameters used for the analysis of strain differences. The means and standard errors for these parameters for male and female Holtzman rats are shown in Table 2. The ANOVAs on the novel-plus-familiar index of general activity found that the females reared more than males, $F(1,80) = 14.5$, $p < 0.01$. The ANOVAs on novelty-specific activity (novel-to-familiar ratio) found no significant sex differences.

3.4. Prior behavior predicting LH response

3.4.1. Individual differences within the Holtzman strain—Previous work investigating individual differences in stress vulnerability has consistently demonstrated bimodal distributions, with most subjects either very resistant or very vulnerable and few subjects in between [5,22]. We calculated a frequency distribution using the percentage of failed trials for the 82 Holtzman subjects which underwent helplessness testing (Fig. 4). As

predicted, two peaks are seen, with most subjects performing either very well or very poorly on the escape task.

3.4.2. Regression with open-field behavior—To determine if any locomotive behaviors during open-field testing were predictive of the phenotype eventually exhibited by these subjects, a series of linear regressions was computed with each locomotive parameter as the independent variable and the FR1 and FR2 average escape latencies as the dependent variables (Table 3). In general, the regression analyses indicated that subjects with higher general activity had shorter escape latencies when tested in the escape task. Multiple linear regression analyses indicated that ambulation, velocity, and rearing collectively explained 11% of the variance in FR1 escape latencies and 18% of the variance in FR2 escape latencies for animals which received inescapable shock. However, the opposite relationship was observed for novelty-specific measures, with higher novelty-specific scores predicting longer FR1 escape latencies. Specifically, ambulation, rearing, and center zone time collectively explained 12% of the variance in FR1 escape latencies. The fact that this relationship is observed only in subjects which received inescapable shock (prior shock but not naïve) and only with the response schedule which showed an effect of inescapable shock (FR1 but not FR2) suggests that the novelty-specific index is associated with helplessness vulnerability and not merely the baseline ability to perform the task.

3.4.3. Sex differences in regression with open-field activity—To take into account the possibility that sex could mediate the relationship between open-field and escape behavior, the above regression analyses were repeated separately for males and females (for only Holtzman subjects receiving inescapable shock). As before, beta coefficients for both general activity and novelty-specific activity were calculated (Table 4). Although the specific measures reaching statistical significance vary by sex and response contingency, the overall pattern of results was unchanged, with general activity predicting better escape performance and novelty-specific activity predicting worse escape performance for both males and females. However, eliminating the variance associated with sex improves the predictability of male behavior. For example, a multivariate function which combines measures of ambulation, velocity, rearing, and center-zone exploration shows that general activity accounts for 28% of the variance in the FR1 escape latencies of males, but only 12% for that of females. Novelty-specific activity is an even better predictor of FR1 activity, accounting for 33% of the variance of males and 20% of the variance of females.

3.4.4. Analysis of covariance—Since the regression analyses suggested that baseline differences in activity level can explain some of the variance in escape latency, we were interested to see if the mean differences in escape latency attributed to strain and sex could be due to baseline activity differences. That is, there was a general trend for reduced activity in the Holtzman strain and increased activity in females, which might explain the greater and lesser vulnerability to helplessness exhibited by Holtzman rats and females, respectively. In order to address this possibility, analyses of covariance (ANCOVAs) were performed on the FR2 trials, using total ambulatory counts as a covariate, to see if controlling for individual differences in open-field ambulation would eliminate strain and sex differences in escape latency. There was still a highly significant effect of strain on escape latency, $F(2,25) = 15.0$, $p < 0.001$, with Sprague-Dawley rats performing better than either Long-Evans or Holtzman strains. However, the effect of sex on escape latency was no longer significant, $F(1,77) = 1.16$. Therefore, the observed strain differences in escape behavior cannot be attributed to strain differences in activity level, but the apparent resilience of females in the escape test may be attributed to their hyperactivity relative to males.

4. Discussion

4.1. Holtzman rats appear most predisposed to helplessness

We were able to replicate one of Wieland et al.'s [41] main findings: namely, Holtzman rats showed greater escape latencies compared to Sprague-Dawley rats. In addition, we found that Long-Evans rats, which had been previously untested in the learned helplessness paradigm, were more similar to Holtzman rats in showing poor escape performance. However, of the three strains, Holtzman rats had the longest escape latencies and the highest percentage of failed trials. Although there was a general trend for reduced activity of Holtzman rats, none of the individual measures of general activity were significantly different between all strains. Long-Evans rats showed longer rearing durations compared to Sprague-Dawley rats, but not Holtzman rats. In general, however, strain differences in open-field behavior were minimal and could not account for the strain differences in escape latencies. This suggests that strain differences in escape latencies are due to an inherent susceptibility or resistance to helpless behavior that is not related to baseline locomotor behavior.

4.2. Holtzman rats appear helpless even in FR1 trials

Surprisingly, naïve Holtzman rats showed poor escape performance equal to that of inescapably shocked rats when the operant response required them to run out of the start compartment and back into it: a fixed ratio of 2 (FR2) schedule, which is generally considered necessary to demonstrate learned helplessness in rats [9,12,18]. However, prior shock did significantly increase escape latencies for the FR1 trials. Indeed, 100% of naïve Holtzman rats were successful with the FR1 contingency, whereas a full one-third of inescapably shocked Holtzman rats failed this task, which is generally considered too easy for demonstrating learned helplessness in rats [12]. This finding cannot be due to an anomaly with our apparatus or task requirements since Sprague-Dawley rats performed both FR1 and FR2 schedules with great ease. Rather, Holtzman rats appear to have such a strong predisposition to become immobile in response to stress that even naïve animals give up trying to escape under the added burden of an FR2 contingency.

Although we cannot rule out strain differences in pain processing as a contributor to the strain differences in escape behavior, this seems an unlikely explanation for several reasons. First, there is nothing in the literature to suggest that Holtzman rats have altered pain sensitivity. For example, LaCroix-Fralish et al. reported no significant differences in painful responses to stimuli among Holtzman, Sprague Dawley, and Long Evans male rats after lumbar root injury [21]. Second, as indicated in our methods section, we tried several different shock intensities up to 1 mA, which did not alter escape performance, while 0.5 mA was sufficient to motivate FR1 escape performance in naïve rats. Finally, all rats consistently vocalized in response to shock, indicating significant emotional distress. Therefore, a lack of aversive motivation cannot explain the poor escape behavior of Holtzman rats.

We also cannot rule out that a general learning deficit associated with the Holtzman strain contributed to poor escape learning. However, naïve Holtzman rats are capable of achieving 100% efficiency when trained to press a lever to escape shock [7] and they have superior acquisition of an avoidance response when compared to two other albino strains (Wistar and Sprague Dawleys) [19]. Therefore, there is no evidence in the existing literature to suggest deficits in the Holtzman strain's pain perception or general learning ability. A more likely explanation for their poor escape performance is that the Holtzman strain is particularly predisposed to show hypoactivity as a consequence of stress [37]. Indeed, we observed that many of the inescapably shocked rats reacted to the very first trial of escape learning with complete immobility. The operant response cannot be discovered if the rats stop moving when shocked.

4.3. Superior escape performance by females is explained by open-field activity

Like Dalla et al. [4], we found that females appeared less helpless than males in terms of FR2 escape performance and that this sex difference appears independent of reproductive hormones, in that estrus cycle phase was unrelated to escape performance. However, the superior performance by females appears more related to differences in baseline escape ability than to differences in learned-helplessness susceptibility. This is because there was no sex difference in FR1 escape latency, which was the schedule which showed an effect of learned helplessness training. Moreover, a difference in baseline activity level appears to underlie the sex difference. Females were more active than males in the open-field, and controlling for this activity difference eliminated the sex difference in FR2 escape performance. If increased locomotion in the open-field translates to increased locomotion in the shuttle box, this should give females a better chance to acquire the operant response and may explain their shorter escape latencies.

4.4. General activity and novelty reactivity are opposite predictors of helpless behavior

Based on activity differences observed in rats selectively bred for helpless behavior [32], we hypothesized that novelty reactivity (hyperactivity evoked by novel environments) would also confer greater risk for developing learned helplessness in a randomly bred population of rats. For the current study, we developed two classes of activity measures—general activity and novelty reactivity—and evaluated these measures as predictors of escape latency using linear regression. From Tables 3 and 4, one notices that the regression coefficients for general activity are universally negative while the regression coefficients for novelty-specific activity are, with one exception, positive. All but one general activity measure predicted significantly superior FR2 escape performance for females, explaining why controlling for general activity level eliminated the observed sex difference in FR2 escape behavior. Overall, open-field behavior accounted for 12%–33% of the variance in FR1 escape latency (the contingency which showed an effect of inescapable shock), offering some support for generalized hyperactivity as a protective factor and novelty reactivity as a risk factor for the development of helplessness. For males, a full third of the variance in escape behavior was explained by novelty reactivity. Such predictive trends in a general population could be magnified by selective breeding and thus give rise to the more dramatic novelty-reactivity differences previously observed in congenitally helpless rats [32].

It should be noted, however, that the literature is mixed with regard to whether novelty seeking is a marker of resilience or vulnerability to stress. In terms of the human literature, a study of Israeli undergraduates [8] found that decreased novelty-seeking (as determined by Cloninger's Tridimensional Personality Questionnaire, or TPQ; Cloninger) [3] predicted the development of PTSD after later exposure to a terrorist explosion. However, other studies using the TPQ found that combat veterans with PTSD showed greater novelty seeking [39]. High novelty seeking was also the strongest correlate of PTSD symptom severity in combat veterans [30].

In terms of the animal literature, rats vulnerable to helplessness show an increased drive to explore novel environments [32,38]. However, Minor et al. [23] also tested open-field behavior before helplessness training, and reported that neophobia or low novelty-seeking predicted vulnerability to helplessness. There are several differences between our study and that of Minor et al., including age of subjects, voluntary vs. involuntary access to the open field, and most importantly, the rat strain used. It is possible that the predictive relationship between novelty seeking and stress vulnerability may be reversed between “normal” and “extreme” populations, such as Sprague-Dawley and Holtzman, respectively. This may also help explain the conflict in the human literature. For example, novelty seeking scores in combat veterans with PTSD [39] were roughly twice those of Israeli undergraduates with PTSD [8]. Therefore the predictive relationship with novelty seeking may be different depending on the population, with combat veterans representing an extreme, similar to the congenitally helpless and Holtzman rats.

Although we have framed our novelty-specific index as reflecting excitement to novel environments, it should be acknowledged that this index could also reflect a faster habituation process, which would seem to be less linked to temperament and more linked to learning. Examining strain differences in other learning paradigms could help inform the question of whether some brain processes may be common to both habituation and learned helplessness, including those responsible for suppression and/or inhibition of behavioral responses.

4.5. Potential biological mechanisms

Both the increased novelty seeking and the predisposition to developing helplessness seen in the Holtzman strain may be linked to abnormalities in glucocorticoid regulation. Congenitally helpless rats show elevated activity in the paraventricular nucleus of the hypothalamus [34], as do high novelty-responding rats [14]. Both congenitally helpless rats and high novelty responders also have reduced glucocorticoid receptor mRNA expression in the hippocampus [15,20], and low novelty responders can be converted into high responders with injection of a glucocorticoid antagonist into the hippocampus [15] – a manipulation that also turns otherwise resilient rats into helpless ones [27]. In humans, lower cortisol levels predict higher novelty seeking scores in combat veterans with PTSD [39].

Additionally, based on our metabolic brain mapping work with congenitally helpless and nonhelpless rats (reviewed by Shumake and Gonzalez-Lima [35]), we may speculate that Holtzman and Sprague-Dawley strains will show similar brain differences as these lines. Namely, Holtzman rats may show a hyperactive habenula coupled with hypoactive mesocorticolimbic dopamine system [35]. Additionally, there may be a functional disconnection between forebrain and brainstem regions [33], leading to disinhibition and dysregulation of the stress response.

4.6. Conclusion

In summary, the Holtzman strain appears to offer a commercially available model for studying susceptibility to helplessness and, translationally, to stress-related psychopathology. Their strong predisposition toward passive coping should further allow investigators to utilize less shock at lower intensities along with simpler escape testing procedures (e.g. FR1 instead of FR2) to demonstrate learned helplessness, which would reduce experimental time and animal distress. Additionally, our results support Dalla et al.'s [4] conclusion that the learned helplessness paradigm may be inadequate for modeling emotional disorders in females, and they offer a possible explanation for why this is the case. Namely, the baseline hyperactivity of some females might override or mask a state of helplessness, and escape behavior may not accurately reflect emotional disturbance in such hyperactive animals. In addition to the apparent protective effect of generalized hyperactivity in females, our results also offer some support that novelty-evoked hyperactivity confers greater risk for developing helpless behavior.

Acknowledgments

We thank Christina Sheridan, Michelle Machie, and Bailey Kermath for their technical assistance. This work was supported by NIH grants R01 MH076847 to FGL and T32 MH65728 fellowships to EP, DB, JS.

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Figure 1.

Mean $\pm$ standard error escape latencies (in seconds) during helplessness testing of Holtzman (HO, $n = 9$), Long-Evans (LE, $n = 10$), and Sprague-Dawley (SD, $n = 10$) subjects. The first five trials represent a fixed-ratio-one (FR1) contingency, and the last 25 trials represent a fixed-ratio-two (FR2) contingency.

Figure 2.

Mean $\pm$ standard error escape latencies (in seconds) during the 5 FR1 trials and 25 FR2 trials, for Holtzman subjects assigned to either the naïve group ($n = 20$), which did not receive inescapable footshock exposure prior to helplessness testing in the shuttle box, or the prior shock group ($n = 62$), which did receive inescapable shock prior to testing. * $p < 0.01$.

Figure 3.

Mean $\pm$ standard error escape latencies (in seconds) during the 5 FR1 trials and 25 FR2 trials, for both male ($n = 31$) and female ($n = 31$) Holtzman subjects, all of which received inescapable footshock prior to helplessness testing in the shuttle box. * $p < 0.05$.

Figure 4.

Frequency distributions of the total number of Holtzman subjects that failed a given percentage of FR2 trials in the shuttle box. Subjects were grouped into bins of 20% increments, from 0% to 100% failure rate. The black line indicates number of male subjects (n = 41); the dotted line indicates number of female subjects (n = 41).

Table 1
Mean open-field activity by rat strain.

	Holtzman (n=9)	Long-Evans (n=10)	Sprague-Dawley (n=10)
Open Field Activity Parameter	Mean $\pm$ S.E.	Mean $\pm$ S.E.	Mean $\pm$ S.E.
GENERAL ACTIVITY (Sum of Novel and Familiar Sessions)			
Total Ambulatory Counts	1835 $\pm$ 239	1907 $\pm$ 291	2649 $\pm$ 245
Average Velocity (cm/sec)	2.08 $\pm$ 0.16	2.11 $\pm$ 0.23	2.21 $\pm$ 0.12
Total Rearing Counts	222 $\pm$ 34.8	224 $\pm$ 29.1	309 $\pm$ 28.1
Average Rearing Duration (sec)	1.14 $\pm$ 0.08	1.36 $\pm$ 0.13 *	0.84 $\pm$ 0.06
Center Zone Time (sec)	129 $\pm$ 20.3	111 $\pm$ 21.3	104 $\pm$ 15.2
NOVELTY-SPECIFIC INDEX (Ratio of Novel: Familiar Sessions)			
Total Ambulatory Counts	0.93 $\pm$ 0.08	1.13 $\pm$ 0.22	1.43 $\pm$ 0.20
Average Velocity (cm/sec)	0.99 $\pm$ 0.13	0.99 $\pm$ 0.12	1.27 $\pm$ 0.26
Total Rearing Counts	0.98 $\pm$ 0.08	0.98 $\pm$ 0.17	1.63 $\pm$ 0.35
Average Rearing Duration (sec)	0.92 $\pm$ 0.16	0.96 $\pm$ 0.09	0.98 $\pm$ 0.22
Center Zone Time (sec)	0.65 $\pm$ 0.12	1.73 $\pm$ 0.53	2.05 $\pm$ 0.73

* LE greater than SD, $p < 0.05$

Table 2
Mean open-field activity of male and female Holtzman rats.

Open Field Activity Parameter	Males (n = 41)	Females (n = 41)
	Mean $\pm$ S.E.	Mean $\pm$ S.E.
GENERAL ACTIVITY (Sum of Novel and Familiar Sessions)		
Total Ambulatory Counts	1893 $\pm$ 100	2210 $\pm$ 128
Average Velocity (cm/sec)	2.06 $\pm$ 0.07	2.19 $\pm$ 0.07
Total Rearing Counts	232 $\pm$ 14.1	320 $\pm$ 18.2 ^{**}
Average Rearing Duration (sec)	1.01 $\pm$ 0.04	1.04 $\pm$ 0.05
Center Zone Time (sec)	126 $\pm$ 9.8	125 $\pm$ 8.6
NOVELTY-SPECIFIC INDEX (Ratio of Novel: Familiar Sessions)		
Total Ambulatory Counts	1.26 $\pm$ 0.09	1.47 $\pm$ 0.09
Average Velocity (cm/sec)	1.08 $\pm$ 0.06	1.25 $\pm$ 0.10
Total Rearing Counts	1.28 $\pm$ 0.08	1.47 $\pm$ 0.13
Average Rearing Duration (sec)	0.95 $\pm$ 0.06	0.98 $\pm$ 0.06
Center Zone Time (sec)	1.50 $\pm$ 0.22	1.72 $\pm$ 0.29

^{**}
p < 0.01

Table 3
Standardized beta (R) coefficients for open-field variables predicting escape latencies in prior shock versus naïve Holtzman subjects.

Open Field Activity Parameter	Prior Shock (n = 62)		Naïve (n = 20)	
	FR 1 Trials	FR 2 Trials	FR 1 Trials	FR 2 Trials
GENERAL ACTIVITY (Sum of Novel and Familiar Sessions)				
Total Ambulatory Counts	-0.30 [*]	-0.40 ^{**}	-0.12	-0.48 [*]
Average Velocity (cm/sec)	-0.30 [*]	-0.34 ^{**}	-0.07	-0.23
Total Rearing Counts	-0.27 [*]	-0.37 ^{**}	-0.23	-0.45 [*]
Average Rearing Duration (sec)	-0.20	-0.12	-0.25	-0.40
Center Zone Time (sec)	-0.19	-0.15	-0.18	-0.27
NOVELTY-SPECIFIC INDEX (Ratio of Novel: Familiar Session)				
Total Ambulatory Counts	0.31 [*]	0.07	0.02	-0.06
Average Velocity (cm/sec)	0.23	0.11	0.33	0.26
Total Rearing Counts	0.30 [*]	0.18	0.03	0.04
Average Rearing Duration (sec)	0.07	0.11	0.17	0.18
Center Zone Time (sec)	0.33 ^{**}	0.16	-0.12	0.06

^{**} p<0.01;

^{*} p<0.05

Table 4
Standardized beta (β) coefficients for open-field variables predicting escape latencies in male versus female Holtzman subjects.

Open Field Activity Parameter	Males (n = 31)		Females (n = 31)	
	FR 1 Trials	FR 2 Trials	FR 1 Trials	FR 2 Trials
GENERAL ACTIVITY (Sum of Novel and Familiar Sessions)				
Total Ambulatory Counts	-0.26	-0.27	-0.32	-0.47 ^{**}
Average Velocity (cm/sec)	-0.42 [*]	-0.34	-0.20	-0.36 [*]
Total Rearing Counts	-0.24	-0.11	-0.29	-0.41 [*]
Average Rearing Duration (sec)	-0.25	-0.07	-0.18	-0.23
Center Zone Time (sec)	-0.11	-0.04	-0.30	-0.36 [*]
NOVELTY-SPECIFIC INDEX (Ratio of Novel: Familiar Sessions)				
Total Ambulatory Counts	0.22	0.15	0.43 [*]	0.21
Average Velocity (cm/sec)	0.01	0.04	0.36 [*]	0.26
Total Rearing Counts	0.36 [*]	0.39 [*]	0.32	0.23
Average Rearing Duration (sec)	0.23	0.29	-0.03	0.11
Center Zone Time (sec)	0.47 ^{**}	0.24	0.34	0.29

^{**}
p<0.01;

^{*}
p<0.05