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Research report

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.

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

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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 12 h/12 h 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 5 min 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 cm × 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 cm × 25 cm × 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 cm × 16 cm × 25 cm) consisted of two compartments of equal size, separated by a door (11 cm × 9 cm) that remained open throughout the session. The chamber was enclosed in a sound-attenuated box and illuminated by a white light (10 lx). 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 min. 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 vs. 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 s. 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 s, after which the shock was terminated if the subject had not escaped. Pseudorandom inter-trial intervals consisted of durations ranging from 10 to 110 s. 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 h and vaginal epithelial cells were imaged using an Olympus light microscope at 10× 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.