The Role of Norepinephrine in Differential Response to Stress in an Animal Model of Posttraumatic Stress Disorder

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Background: Posttraumatic stress disorder (PTSD) is a prevalent psychiatric disorder precipitated by exposure to extreme traumatic stress. Yet, most individuals exposed to traumatic stress do not develop PTSD and may be considered psychologically resilient. The neural circuits involved in susceptibility or resiliency to PTSD remain unclear, but clinical evidence implicates changes in the noradrenergic system.

Methods: An animal model of PTSD called Traumatic Experience with Reminders of Stress (TERS) was developed by exposing C57BL/6 mice to a single shock (2 mA, 10 sec) followed by exposure to six contextual 1-minute reminders of the shock over a 25-day period. Acoustic startle response (ASR) testing before the shock and after the last reminder allowed experimenters to separate the shocked mice into two cohorts: mice that developed a greatly increased ASR (TERS-susceptible mice) and mice that did not (TERS-resilient mice).

Results: Aggressive and social behavioral correlates of PTSD increased in TERS-susceptible mice but not in TERS-resilient mice or control mice. Characterization of c-Fos expression in stress-related brain regions revealed that TERS-susceptible and TERS-resilient mice displayed divergent brain activation following swim stress compared with control mice. Pharmacological activation of noradrenergic inhibitory autoreceptors or blockade of postsynaptic α_1-adrenoreceptors normalized ASR, aggression, and social interaction in TERS-susceptible mice. The TERS-resilient, but not TERS-susceptible, mice showed a trend toward decreased behavioral responsiveness to noradrenergic autoreceptor blockade compared with control mice.

Conclusions: These data implicate the noradrenergic system as a possible site of pathological and perhaps also adaptive plasticity in response to traumatic stress.

Key Words: Amygdala, bed nucleus of stria terminalis, clonidine, locus coeruleus, norepinephrine, posttraumatic stress disorder, prazosin, resilience, stress, ventral tegmental area

The extreme psychological stress of severe trauma leads to posttraumatic stress disorder (PTSD) in susceptible persons. Others are resilient despite exposure to qualitatively and quantitatively similar trauma. Understanding the neurobiology of susceptibility and resiliency could lead to improved interventions to treat and even prevent PTSD (1–3). Although characteristics of the neural circuitry that determines PTSD susceptibility or resilience are unclear, increasing clinical evidence suggests brain noradrenergic system involvement in PTSD neurobiology (4–6). In persons with PTSD, pharmacological reduction of brain noradrenergic outflow (7–9) or responsiveness at postsynaptic α_1-adrenoreceptors (ARs) (10–12) reduces some PTSD symptoms. In contrast, pharmacological enhancement of brain noradrenergic outflow precipitates PTSD symptoms in individuals with the disorder (6,13).

A better understanding of the neurobiology of individual differences in response to traumatic stress can be gained by examining animal models of PTSD (14). Pynoos *et al.* (15) developed a model of PTSD in which mice were exposed to a single footshock followed by contextual reminders of the shock. These weekly, brief situational reminders (SRs) phenomenologically mimicked the episodes of reexperiencing, which are characteristic of PTSD in people, and perpetuated the adverse effects of the shock (15). Our laboratory has refined and expanded this model by adding a baseline acoustic startle response (ASR) test along with posttests that measure behavioral correlates of PTSD symptoms. We refer to this model as traumatic experience with reminders of stress (TERS). Here, we demonstrate that susceptible and resilient mice from an inbred strain can be identified by measuring ASR before and after exposure to the TERS paradigm. In addition to an increase in ASR, TERS-susceptible mice develop other behavioral correlates of PTSD symptoms, whereas TERS-resilient mice behave similar to a no-shock control group on these tests. The TERS-susceptible and TERS-resilient mice may undergo different neurobiological adaptations following the shock, as they show different neural activation patterns in response to stress. We also report that the behavioral sequelae of PTSD we observe in TERS-susceptible mice can be normalized by pharmacologically blocking noradrenergic outflow or postsynaptic α_1-ARs, suggesting that increased noradrenergic receptor activation may contribute to the behavioral correlates of PTSD symptoms we observe in TERS-susceptible mice.

Methods and Materials

Animals

A total of 615 adult male C57BL/6CRL mice (Charles River Laboratories, Hollister, California) were used for experiments. Mice were maintained under a 12-hour light/dark cycle with ad libitum access to food and water. Six-week-old mice were group-housed for at least 1 week after arrival. To avoid the confounding effects of aggressive behavior previously reported by researchers using a similar protocol (15), mice were single-housed at least 1 week before the first ASR test and remained so for the duration of experiments. All

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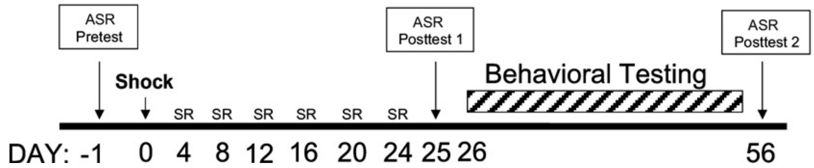

Figure 1. Time line of the traumatic experience with reminders of stress protocol. Mice undergo baseline acoustic startle response (ASR) testing and shock and no-shock groups are balanced so the average baseline ASR of the two groups does not differ. The following day, mice are exposed to the shock box. On the fourth day after the shock and every following fourth day for a total of six exposures, mice were exposed to the situational reminder, during which each mouse was restricted to the light chamber of the box for 1 minute. The day after the last situational reminder or 25 days after the shock, mice were subjected to a second ASR test (posttest 1). For about a month after posttest 1, mice in behavioral experiments underwent behavioral testing. In c-Fos experiments, mice were euthanized 3 days after ASR posttest 1. To demonstrate persistence of behavioral changes, some mice underwent a third ASR test (posttest 2) 56 days or 2 months after the shock. SR, situational reminder.

experiments were conducted in accordance with the guidelines of the Veterans Administration Puget Sound Health Care System Institutional Animal Care and Use Committee.

The TERS Paradigm

Mice were subjected to a single brief shock followed by six SRs (Figure 1). The day before TERS conditioning, mice were given an ASR pretest to determine baseline ASR. Control and shocked groups were balanced so that the average baseline ASRs of each group were similar at the start of the experiment. The day after the ASR pretest, mice were exposed to the shock apparatus, which consisted of two chambers separated by a remotely moveable door (Gemini Avoidance System, San Diego Instruments, San Diego, California). One chamber was brightly lit with an odorant that served as an additional contextual cue. The other chamber was dark with no odorant. Each mouse in the shocked group was placed in the well-lit chamber and allowed to acclimate to the environment (1.5 min). The door to the dark chamber was then opened remotely. When the mouse entered the dark chamber, the door was shut and the mouse was administered a footshock (2 mA, 10 sec) and then returned to its home cage. On the fourth day after the shock, mice were exposed to the first SR, during which each mouse was restricted to the light chamber for 1 minute. Situational reminders were repeated five times every following fourth day for six total SRs. The day after the last SR, mice were given a second ASR test (posttest 1). Mice in behavioral experiments then underwent behavioral testing over the course of about a month. To demonstrate persistence of behavioral changes, some mice underwent a third ASR test (posttest 2) 2 months after the shock. Control mice received the same treatment as shocked mice, including SRs, but were not shocked during their initial exposure to the box.

Differentiation of TERS-Resilient and TERS-Susceptible Mice. The TERS-exposed mice were separated into cohorts: TERS-susceptible individuals (or mice that develop a PTSD-like syndrome) and TERS-resilient individuals (or mice that do not develop a PTSD-like syndrome). The TERS-susceptible mice were defined as those whose percentage increase in ASR between the ASR pretest and posttest 1 was greater than 70% (about 45.7% of shocked mice). The TERS-resilient mice were defined as those whose percentage change in ASR between the ASR pretest and posttest 1 was less than 30% (about 40.3% of shocked mice). Mice whose percentage change in ASR was between 30% and 70% (about 14% of shocked mice) displayed a mixed behavioral phenotype and were excluded from the study. The proportion of susceptible, resilient, and excluded mice varied across experiments.

Behavioral Protocols

Acoustic Startle Response. Acoustic startle response was measured using four startle chambers (SR-Lab System, San Diego Instruments). Each chamber included a ventilated, sound-attenuated cabinet with a speaker and contained a cylindrical Plexiglas enclosure with a piezoelectric accelerometer to detect movement. Chambers were regularly calibrated for both sensitivity to movement and sound level to ensure consistency between chambers and experiments. Testing took place 3 to 7 hours following the onset of light cycle. Mice were acclimated to the testing room for a minimum of 1 hour before ASR testing. Each ASR session began with a 5-minute acclimation period, during which 65 dBB white noise was played. Trials consisted of 10 consecutive presentations of a 40-millisecond, 100 dB white noise stimulus. Startle stimuli were presented for an average of 15 seconds at intervals of 21, 7, 20, 9, 14, 21, 11, 8, and 23 seconds. Interstimulus intervals were varied in this way to avoid anticipatory compensation by the mouse. Acoustic startle response data were calculated by averaging responses over the 10 trials. Between tests, each chamber was cleaned thoroughly. Mice with pretest ASR scores over 405 mV were not included because a high ASR pretest made it difficult to accurately classify them into susceptible and resilient groups by the percentage change in startle. For pharmacological experiments, mice underwent posttest 1, as described in the TERS protocol. After 3 days of rest, mice received an injection of saline, prazosin, or clonidine and were then given another ASR test. The ASR protocol was modified from methods previously described (16).

Locomotion during Sixth SR. Locomotor behavior during the last SR was quantified by dividing the chamber into quadrants. Movement from one quadrant to another was considered one locomotor count.

Light-Dark Box Test. Locomotor behavior during the light-dark box (LD) test was scored by a blinded observer as previously described (17). Mice were placed in the light side of a two-chambered box with one light chamber and one dark chamber. Mice were observed for 5 minutes, and the time spent in either chamber was recorded.

Resident Intruder. Resident intruder (RI) testing was conducted as previously described (18). Mice underwent three RI trials (up to 5 min, data are from third trial) over 3 days, during which a stress-naïve group-housed intruder mouse of equal or slightly lesser weight was introduced into the resident's home cage. The trial was terminated if a mouse vocalized or if wrestling continued for more than 5 seconds, as these were indicators of attack biting that could cause injury.

Social Interaction. Mice were acclimated to the social interaction (SI) arena (circular, 1-m diameter) for 10 minutes a day over 2 days. On the third day, mice were placed in the arena with a stress-naïve group-housed companion mouse of equal or slightly lesser size that was marked to distinguish it from the experimental mouse. Mice were videotaped for 7 minutes. Total interaction time was recorded by SMART Videotrack system (San Diego Instruments) and