Stress-induced increases in avoidance responding: an animal model of post-traumatic stress disorder behavior?

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Abstract: One prominent symptom of post-traumatic stress disorder (PTSD) is avoidance of stimuli reminiscent of the traumatic event. We attempted to study this aspect of PTSD in two experiments. Groups of rats received forty 3-s tailshocks, or served as home cage controls (HCC). Twenty-four hours later, all subjects received a 4-h session of leverpress escape/avoidance conditioning. In Experiment 1, shock periods in the absence of a response were 1 s; in Experiment 2 they were 30 s. No group differences were observed in Experiment 1. In Experiment 2, previously shocked animals made more avoidance responses and had a higher percent avoidance during the fourth hour of the session than controls. Further, previously shocked animals had a higher efficiency ratio (the percent of responses that were avoidances). No group differences were observed in leverpresses during the safety period (an index of anxiety) in either study. Results are discussed in terms of the effects of stress on avoidance behavior as a potential model for this important feature of PTSD.

Keywords: stress, escape/avoidance, post-traumatic stress disorder, PTSD

Introduction

Post-traumatic stress disorder (PTSD) is an anxiety disorder that often appears after exposure to a severe stressful event (see Sullivan and Gorman 2002, for a review). There are a number of symptoms that characterize the disorder, including flashbacks and intrusive thoughts, physiological hyperarousal, as well as behavioral avoidance of situations or stimuli that are reminiscent of the traumatic event (APA 1994; Bower and Sivers 1998).

The most frequently used animal model of PTSD is exposure to inescapable electric shock (eg, Maier 2001). Exposure to inescapable shock in rats leads to a variety of consequences including increases in basal plasma corticosterone (Ottenweller et al 1992), urinary corticosterone (Brennan et al 2000), and persistent increases in acoustic startle responding (Servatius et al 1995) and sleep disturbances (Sanford et al 2001). These symptoms collectively resemble the symptoms seen in PTSD patients.

The inescapable shock model has been criticized for not being a good model of the behavioral aspects of PTSD (Yehuda and Antelman 1993). Inescapable shock typically produces decreases in responding, while the avoidance aspect of PTSD could be a phenotypically active process. One prior study did find increases in shuttlebox avoidance after exposure to inescapable shock in rats (Koba et al 2001). We have studied escape/avoidance (E/A) conditioning using a leverpress paradigm for a number of years (Brennan et al 1992, 2003b). The purpose of the present experiments was to assess the effect of prior stress on the development of escape and

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avoidance responding in a discrete trial leverpress procedure and to assess its possible utility as an animal model of this feature of PTSD.

Methods

Subjects

Subjects were 48 male, Sprague-Dawley (SD) rats obtained from Charles River, Kingston, NY, USA. They were approximately 60-days-old and 300–350 g at the time of testing. They were maintained on ad lib food and water except during the stress and escape/avoidance sessions. Subjects were maintained on a 12:12 light/dark cycle, with lights on at 0700.

Apparatus and general procedures

All animals were randomly assigned to groups, and all procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the East Orange VA Medical Center. The 24 animals that received shock were restrained in plastic tubes (Harvard Apparatus, Inc, Holliston, MA, USA) and had tail electrodes attached. The single shock session consisted of forty 3-s, 2-mA tailshocks, presented on a variable time (VT) 3-min schedule, making the shock session approximately 2 h in duration.

Escape/avoidance conditioning. E/A sessions were conducted in 4 operant chambers (Coulbourn, Inc, Allentown, PA, USA). The chambers were 30.5 cm wide × 25.4 cm deep × 30.5 cm high and had a lever mounted on one wall. There was a houselight mounted on the upper portion of the chamber in the wall directly across from the lever. Subjects were allowed approximately 1 min to explore the chamber before the session began. The first trial commenced with the onset of the warning signal (WS) and houselight. The WS was a 1000-Hz tone emitted from a speaker mounted in the chamber and clearly audible to the animal. If the animal had not made a leverpress after 60 s of the WS, they began to receive 1.0-mA footshock through the grid floor. The shock, WS, and houselight were all terminated by a leverpress. After a leverpress, the animal was given a 6-min period of safety (Brennan et al 2003a). A flashing light located on the wall above the lever was a discrete safety signal. Due to the 6-min safety period, there was a maximum of 10 trials per hour.

A leverpress after the shock had begun was classified as an "escape" (even if it occurred in the absence of shock), while a response that occurred during the initial 60 s of the

WS before the shock came on was classified an "avoidance" (as in Berger and Brush 1975). A trial thus ended with a leverpress. A new trial began with the safety signal terminating and the reintroduction of the houselight and WS. In the absence of a leverpress, the shocks were presented on a VT 60 s schedule. Subjects were given a "free" escape by the experimenter (the lever was manually depressed from the outside of the box) if no response had occurred in 20 min.

We analyzed the number of escape and avoidance responses by hour across the session, percent avoidance, and the number of leverpresses during the safety period (a putative measure of anxiety) (Berger and Starzec 1988). Finally, we calculated an efficiency ratio (Steinmetz et al 1993). The efficiency ratio was the total number of avoidance responses divided by the total number of responses (escapes + avoidances + leverpresses during safety). Occasionally an animal would make one or more "pseudo-avoidances" while exploring the chamber during the very first warning period(s). These responses were not counted in any dependent measure. Data were analyzed via repeated measure or one-way ANOVA (analysis of variance) models, with Tukey-Kramer post hoc tests to detect specific differences.

Experiment 1

Twenty-four male SD rats were randomly assigned to a stress (n=12) or control (n=12) condition. Twenty-four hours later, all subjects received a 4-h E/A conditioning session. Two stressed and two control animals were always run contemporaneously to control for circadian effects. In the
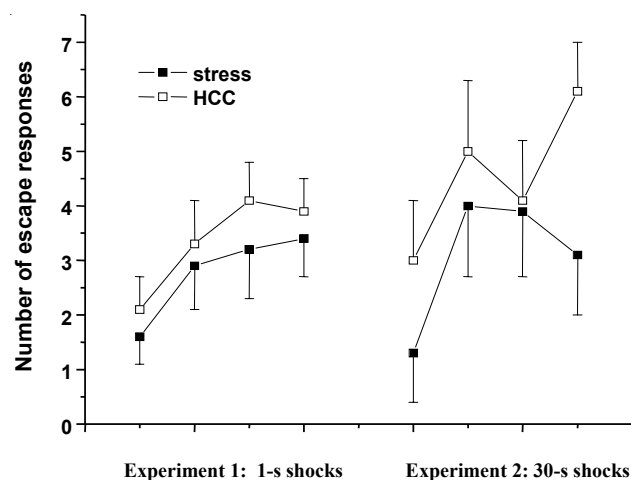

Figure 1 Number of escape responses by hour across the 4-h session. Left: Experiment 1. Right: Experiment 2.

absence of a leverpress response, the shock periods were 1 s in duration.

Results

The number of escape responses across the session is presented in Figure 1 (left). Subjects increased the number of escape responses across the session as they acquired the response, $F(3, 96)=4.93$, $p<0.01$. Neither the main effect of stress, $F(1,96)<1.0$, nor the interaction, $F(3, 96)<1.0$, were significant. The number of avoidance responses across the session is presented in Figure 2 (left). Subjects also increased the number of avoidance responses across the session, $F(3, 96)=10.32$, $p<0.0001$. There was neither a main effect of stress, $F(1,96)<1.0$, nor an interaction, $F(3, 96)=1.16$, $p>0.05$. Percent avoidance also increased across the session, $F(3, 96)=9.1$, $p<0.001$. Again, there was neither a main effect of stress, $F(1,96)<1.0$, nor an interaction, $F(3, 96)<1.0$. There was no difference between stress and control animals in leverpresses during the safety period, $F(1, 24)<1.0$. The grand mean was 69.4 leverpresses during safety. Finally, there was also no difference in efficiency ratios between groups, $F(1, 24)<1.0$. The grand mean was 4.4.

Experiment 2

In Experiment 2, we attempted to make the task more stressful by increasing the shock length in the absence of a leverpress. Data from human subjects has indicated that PTSD patients can show normal behavior in baseline conditions, but show altered responsiveness when challenged with a stressor (Grillon and Morgan 1999). Twenty-four naive male SD rats were randomly assigned to a stress (n=12) or control (n=12) condition. Twenty-four hours later, all subjects received a 4-h E/A conditioning session. In the absence of a response, the shock periods were 30 s in duration. Four stressed animals and two controls made no responses for the entire 4-h session and were thus excluded from all analyses.

Results

The number of escape responses across the session is presented in Figure 1 (right). Animals increased the number of escape responses across the session as they acquired the response, $F(3, 72)=4.12$, $p=0.01$. There was neither a main effect of stress, $F(1,72)=1.77$, $p>0.05$, nor an interaction, $F(3, 72)=1.23$, $p>0.05$. The number of avoidance responses
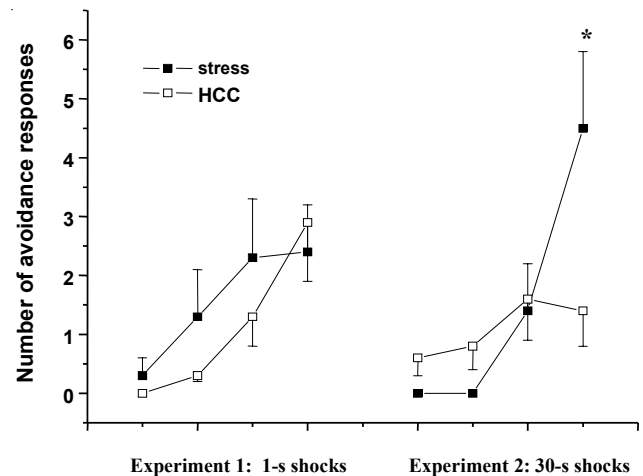

Figure 2 Number of avoidance responses by hour across the 4-h session. Left: Experiment 1. Right: Experiment 2.

across the session is presented in Figure 2 (right). Animals also increased the number of avoidance responses across the session as they acquired the response, $F(3, 72)=11.93$, $p<0.001$. The main effect of stress was not significant, $F(3, 72)<1.0$. However, the stress × hour interaction was significant, $F(3, 72)=6.61$, $p<0.001$. Tukey-Kramer follow-up tests indicated that stressed animals made more avoidance responses than controls during the fourth hour of the session, $p<0.05$. The percent avoidance data mirrored the raw number of avoidances. There was again no overall effect of stress, $F(1, 72)<1.0$. The significant effect of hour, $F(3, 72)=11.22$, $p<0.001$, was superseded by the significant stress × hour interaction, $F(3, 72)=6.88$, $p<0.001$. Tukey-Kramer follow-up tests indicated that stressed animals had a higher percent avoidance than controls during the fourth hour of the session, $p<0.05$. There was no difference in leverpresses during safety between stress and control animals, $F(1, 18)=1.26$, $p>0.05$. The grand mean was 31.7 leverpresses during the safety period. Finally, stressed animals had a higher efficiency ratio than controls, $F(1, 18)=4.26$, $p=0.05$.

Discussion

The results demonstrated that rats previously exposed to tailshock had a higher percent avoidance than home cage controls during the final hour of a 4-h session. Also, a higher percentage of responses performed by shocked animals were avoidance responses. These data appear to indicate superior learning in the previously stressed group. No group differences were observed in escape responding or leverpresses during safety. The lack of effect on leverpresses

during safety is interesting since that has traditionally been viewed as an index of anxiety (Berger and Starzec 1988). These data are consistent with prior findings (Koba et al 2001) demonstrating increased shuttlebox avoidance after exposure to shock stress.

Interestingly, group differences were only apparent in animals trained with 30-s shock periods in the absence of a response. An obvious interpretation would be that the 30-s shock periods were more stressful than the 1-s shock periods. Classic animal learning experiments indicate that more continuous shock periods tend to produce escape performance in a leverpress paradigm (D'Amato et al 1964). Perhaps it is this increase in the stressfulness of the training situation that allows the PTSD-like symptoms to emerge. Human PTSD patients often respond normally in non-threatening environments (Grillon and Morgan 1999).

Individuals with PTSD display a myriad of symptoms, including alterations in stress hormones (Yehuda 2002) and increased startle response (Grillon and Morgan 1999). These symptoms have been modeled in animal subjects (Servatius et al 1995; Brennan et al 2000). However, it may be that avoidance behavior is the most debilitating of all PTSD symptoms. In an extreme form, avoidance behavior may be indistinguishable from agoraphobia. Prior work looking at the suppressive effect of inescapable shock on escape responding (eg, Maier 2001) does not appear to model the behavioral avoidance characteristic of PTSD (Yehuda and Antelman 1993). The current leverpress E/A task may be a better model of the behavioral aspects of PTSD.

In summary, we found that prior exposure to tailshock led to an increase in the number of avoidance responses 24 h later. This procedure may model the avoidance behavior observed in PTSD patients. Although corticosterone levels were not measured in these experiments, they would have been very informative and may have confirmed how closely the animal model mimics PTSD in humans. Studies underway are attempting to analyze the physiological mechanisms behind the stress-induced facilitation of conditioning. Further studies are planned to examine other dependent measures such as disruptions in REM sleep and their relevance to PTSD (eg, Pawlyk et al 2004).

Acknowledgments

References

APA. 1994. Diagnostic and statistical manual of mental disorders – Fourth edition (DSM-IV). Washington: APA Pr.

Berger DF, Brush FR. 1975. Rapid acquisition of discrete-trial lever-press avoidance: effects of signal-shock interval. *J Exp Anal Behav*, 24: 227–39.

Berger DF, Starzec JJ. 1988. Contrasting leverpress avoidance performance of spontaneously hypertensive and normotensive rats (Rattus norvegicus). *J Comp Psych*, 102:279–86.

Bower GH, Sivers H. 1998. Cognitive impact of traumatic events. *Dev Psychopathol*, 10:625–53.

Brennan FX, Beck KD, Servatius RJ. 2003a. Leverpress escape/avoidance in performance in rats: safety signal length and avoidance performance. *Integr Physiol Behav Sci*, 38:36–44.

Brennan FX, Beck KD, Servatius RJ. 2003b. Low doses of interleukin-1β improve the avoidance performance of Sprague-Dawley rats. *Neurobiol Learn Mem*, 80:172–5.

Brennan FX, Berger DF, Starzec JJ, et al. 1992. Plasma glucose levels and leverpress avoidance versus escape behaviors in rats. *Physiol Behav*, 51:723–7.

Brennan FX, Ottenweller JE, Seifu Y, et al. 2000. Persistent stress-induced elevations of urinary corticosterone in rats. *Physiol Behav*, 71:441–6.

D'Amato MR, Keller D, DiCara L. 1964. Facilitation of discriminated avoidance learning by discontinuous shock. *J Comp Physiol Psych*, 58:344–9.

Green B. 2003. Post-traumatic stress disorder: symptom profiles in men and women. *Curr Med Res Opin*, 19:200–4.

Grillon C, Morgan CA. 1999. Fear-potentiated startle conditioning to explicit and contextual cues in Gulf War veterans with posttraumatic stress disorder. *J Abnorm Psych*, 108:134–42.

Koba T, Kodama Y, Shimizu K, et al. 2001. Persistent behavioural changes in rats following inescapable shock stress: a potential model of posttraumatic stress disorder. *World J Biol Psychiatry*, 2:34–7.

Maier SF. 2001. Exposure to the stressor environment prevents the temporal dissipation of behavioral depression/learned helplessness. *Biol Psychiatry*, 49:763–73.

Ottenweller JE, Servatius RJ, Tapp WN, et al. 1992. A chronic stress state in rats: effects of repeated stress on basal corticosterone and behavior. *Physiol Behav*, 51:689–98.

Pawlyk AC, Jha SK, Brennan FX, et al. 2004. Neutral and conditioned aversive contexts differentially affect sleep architecture following fear conditioning. *Biol Psychiatry*. In press.

Sanford LD, Silvestri AJ, Ross RJ, et al. 2001. Influence of fear conditioning on elicited ponto-geniculo-occipital waves and rapid eye movement sleep. *Arch Ital Biol*, 139:169–83.

Servatius RJ, Ottenweller JE, Natelson BH. 1995. Delayed startle sensitization distinguishes rats exposed to one or three stress sessions: further evidence toward an animal model of PTSD. *Biol Psychiatry*, 38:539–46.

Steinmetz JE, Logue SF, Miller D. 1993. Using signaled barpressing tasks to study the neural substrates of appetitive and aversive learning in rats: behavioral manipulations and cerebellar lesions. *Behav Neurosci*, 107:941–54.

Sullivan GM, Gorman JM. 2002. Finding a home for post-traumatic stress disorder in biological psychiatry. Is it a disorder of anxiety, mood, stress, or memory? *Psychiatr Clin North Am*, 25:463–8.

Yehuda R. 2002. Current status of cortisol findings in post-traumatic stress disorder. *Psychiatr Clin North Am*, 25:341–68.

Yehuda R, Antelman SM. 1993. Criteria for rationally evaluating animal models of posttraumatic stress disorder. *Biol Psychiatry*, 33:479–86.