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Acoustic startle response in rats predicts inter-individual variation in fear extinction

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ABSTRACT

Although a large portion of the population is exposed to a traumatic event at some point, only a small percentage of the population develops post-traumatic stress disorder (PTSD), suggesting the presence of predisposing factors. Abnormal acoustic startle response (ASR) has been shown to be associated with PTSD, implicating it as a potential predictor of the development of PTSD-like behavior. Since poor extinction and retention of extinction learning are characteristic of PTSD patients, it is of interest to determine if abnormal ASR is predictive of development of such deficits. To determine whether baseline ASR has utility in predicting the development of PTSD-like behavior, the relationship between baseline ASR and freezing behavior following Pavlovian fear conditioning was examined in a group of adult, male Sprague-Dawley rats. Baseline acoustic startle response (ASR) was assessed preceding exposure to a Pavlovian fear conditioning paradigm where freezing behavior was measured during fear conditioning, extinction training, and extinction testing. Although there was no relationship between baseline ASR and fear memory following conditioning, rats with low baseline ASR had significantly lower magnitude of retention of the extinction memory than rats with high baseline ASR. The results suggest that baseline ASR has value as a predictive index of the development of a PTSD-like phenotype.

1. Introduction

Although the majority of the population is exposed to a traumatic event at some point throughout the lifetime, prevalence of posttraumatic stress disorder (PTSD) is relatively low (Breslau et al., 1998; Kessler, Sonnega, Bromet, Hughes, & Nelson, 1995). The disparity between the number of individuals exposed to trauma and the number of individuals who develop PTSD suggests the presence of predisposing factors that render some individuals more susceptible to the development of PTSD than others. Identification of such predisposing factors is crucial to the advancement of the understanding of the development of PTSD and the development of preventative and interventional treatments for susceptible individuals. Because PTSD is characterized by a failure to recover from a normal fear response (Rothbaum & Davis, 2003), animal studies utilizing Pavlovian fear conditioning paradigms can be used to gain insight into the development and maintenance of PTSD-like symptoms. Such animal models have facilitated a greater understanding of the neurobiological mechanisms underlying the pathological fear response that characterizes PTSD (Johansen, Cain, Ostroff, & LeDoux, 2011; Maren, 2001; Parsons & Ressler, 2013), allowing for the development of pharmacological treatments intended to reduce symptoms following trauma (Cain, Maynard, & Kehne, 2012; Steckler & Risbrough, 2012). However, little progress has been made in prospective identification of the individuals most likely to develop a pathological fear response following exposure to trauma (Yehuda & LeDoux, 2007).

The acoustic startle response (ASR) is a reflex that occurs in response to an abrupt acoustic stimulus and results in the rapid contraction of the facial and skeletal muscles. The startle response has attracted much attention because exaggerated ASR is often observed in the aftermath of trauma in patients with PTSD (Butler et al., 1990; Grillon, Morgan, Davis, & Southwick, 1998; Morgan, Grillon, Southwick, Davis, & Charney, 1996; Shalev, Peri, Orr, Bonne, & Pitman, 1997). Although an exaggerated startle response is most frequently associated with PTSD, several studies have reported normal startle responses in PTSD patients (Grillon, Morgan, Southwick, Davis, & Charney, 1996; Orr, Solomon, Peri, Pitman, & Shalev, 1997; Shalev, Orr, Peri, Schreiber, & Pitman, 1992), while a few studies have even reported blunted ASR in a small sample of children (Ornitz & Pynoos, 1989) and a sample of trauma-exposed women (Medina, Mejia, Schell, Dawson, &

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Margolin, 2001). Some authors have proposed that elevated startle in PTSD patients, when it is observed, reflects a conditioned emotional response that generalizes to the experimental context (Morgan, Grillon, Southwick, Davis, & Charney, 1995; Grillon et al., 1998). Whether or not ASR is a primary symptom of PTSD or a phenomena linked to certain experimental situations and/or subject populations does not address the possibility of whether or not pre-trauma differences in ASR exist, because all of these studies have assessed ASR in patients with PTSD following trauma. Such retrospective designs do not yield conclusions about whether the altered ASR is present prior to the traumatic event or is a symptom of PTSD that develops in the aftermath of the trauma. A limited number of studies have assessed the relationship between pre-trauma baseline ASR and PTSD symptoms, finding that pre-trauma skin conductance in response to acoustic startle stimuli was predictive of posttraumatic stress severity in firefighters (Guthrie & Bryant, 2005), greater pre-trauma skin conductance during presentation of an acoustic startle stimulus under a high threat condition was predictive of severity of PTSD symptoms in police cadets (Pole et al., 2009), and that pre-trauma startle response is not predictive of post-deployment PTSD symptoms in active duty marines (Glenn et al., 2016). Similarly, studies utilizing animal models to prospectively identify susceptibility to development of a PTSD-like phenotype are limited. One study that has investigated the relationship between baseline ASR and PTSD-like behavior in rats found that elevated startle response following exposure to a mild stressor is predictive of long-lasting elevated startle response following fear conditioning and impaired rate and magnitude of extinction (Nalloor, Bunting, & Vazdarjanova, 2011). Another study found that rats which had a high baseline ASR prior to shock exposure displayed significantly further exaggerated startle following shock and elevated basal plasma corticosterone levels relative to a control group (Rasmussen, Crites, & Burke, 2008). Although the results linking baseline ASR and development of PTSD or PTSD-like behavior are inconsistent, the possibility that baseline ASR has predictive value has not been adequately addressed.

In an effort to elucidate the relationship between baseline ASR and fear memory formation and expression, we examined whether or not baseline ASR predicts freezing behavior following Pavlovian fear conditioning and extinction in rats. Because deficits in extinction are known to be characteristic of patients with PTSD (Milad et al., 2008; Milad et al., 2009) and both humans and rodents display heterogeneity in the expression of fear responses following traumatic stress (Bush, Sotres-Bayon, & LeDoux, 2007; Holmes & Singewald, 2013), assessment of extinction following fear conditioning provides a good translational measure of PTSD-like behavior in rodents. We investigated whether or not baseline ASR can be used to predict acquisition of fear conditioning, rate and magnitude of extinction learning, and retention of the extinction memory following fear conditioning. Because PTSD is thought to involve a failure to extinguish a fear memory (Rothbaum & Davis, 2003), we hypothesized that baseline ASR would be related to magnitude and rate of extinction learning and retention of the extinction memory, but not fear memory prior to extinction.

2. Materials and methods

2.1. Subjects

Forty-eight, adult, male Sprague-Dawley rats (Charles River Laboratories, Raleigh, NC), weighing 300–325 g upon arrival, were housed in pairs with food and water freely available. A 12 h light/-dark cycle was maintained (lights on at 7 am). Behavioral testing began two weeks after the animals' arrival, and all procedures were carried out during the light portion of the cycle. Rats were handled daily for the seven days preceding behavioral testing. Three rats were excluded from analysis for either failure to learn due to shocker malfunction, or very low freezing behavior during extinction training and testing. Forty-five rats were included in data analyses. All procedures were conducted with approval from the Stony Brook University Institutional Animal Care and Use Committee.

2.2. Apparatus

2.2.1. Baseline acoustic startle response

Startle amplitude was assessed using a *Startle Monitor II* system (Kinder Scientific, Poway, CA; Version 8.15). Rats were tested in a 17.5 cm × 9.2 cm × 7.5 cm restrainer which sat atop a load cell sensor within a 40.64 cm × 40 cm × 49.53 cm sound attenuating chamber. Startle responses were evoked by the presentation of white noise bursts (50 msec, 95 db) delivered through speakers mounted in the ceiling of the chambers. Background noise was delivered through the same speakers as the startle stimuli. Acoustic startle response was measured by the displacement of the restrainer detected by the load cell as the maximal force (Newtons) that occurred during the first 500 ms after the onset of the white noise burst.

2.2.2. Freezing

Fear conditioning occurred in 32 cm × 26 cm × 21 cm conditioning chambers (Clever Sys. Inc., Reston, VA; CSI-CHM-FRM). The chambers were made of stainless steel and Plexiglas with a shock grid floor and were placed within sound attenuating 45.7 cm × 43.2 cm × 43.2 cm isolation boxes (Clever Sys. Inc., Reston, VA; CSI-BOX-STD). On extinction training and extinction testing days, the context was altered to resemble a context different from the one the rats were conditioned in. During fear conditioning (Context A) the boxes were illuminated with 28 V, incandescent, house light bulbs (Chicago Miniature Lighting, Suffolk, United Kingdom). During extinction training and retention testing (Context B), the boxes were illuminated with infrared, LED lights (Univivi IR Illuminator, Shenzhen, China; U48R). Furthermore, in Context B, the apparatus was altered by placing painted metal inserts over the shock grid floor and the back and side walls, the size and shape of the chamber were altered with a bent 33.5 × 21.3 cm metal insert, the chambers were wiped down with 5% acetic acid, rats were handled by a different experimenter, and rats were carried into the freezing room in buckets rather than brought in their home cages. Overhead cameras recorded all conditioning and testing sessions, and freezing behavior was scored by FreezeScan 2.00 Software (Clever Sys. Inc., Reston, VA) as the percent of each 30 s bin a rat spent freezing.

2.3. Behavioral procedures

Forty-five rats were assessed for startle amplitude in the described apparatus on two consecutive days, followed by assessment of freezing behavior during fear conditioning, extinction training, and extinction testing on the following three days, respectively. Rats were presented with 30 trials of a 95 dB, 50 ms, white noise burst (30 s inter-trial intervals) following a five minute acclimation period. Sound measurements were taken using a Digital Sound Level Meter (RadioShack, Fort Worth, Texas; 33-2055). Following two days of startle assessment, rats were exposed to a Pavlovian fear conditioning paradigm in the freezing apparatus described above (Context A). The conditioned stimulus (CS) was a 4 kHz, 76 dB, 30 s tone. Sound measurements were taken using a Sound Meter (Sper Scientific, Scottsdale, Arizona; 840005), and calibrated using an amplifier (Behringer, Willich, Germany; HA400).