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Stress-induced attenuation of acoustic startle in low-saccharin-consuming rats

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ABSTRACT

Exposure to stress can lead to either increased stress vulnerability or enhanced resiliency. Laboratory rats are a key tool in the exploration of basic biobehavioral processes underlying individual differences in the effect of stress on subsequent stressors' impact. The Occidental low (LoS) and high (HiS) saccharin-consuming rats, which differ in emotional reactivity, are useful in this effort. In the present study, footshock affected acoustic startle amplitude 4 h later among LoS but not HiS rats. Surprisingly, shock attenuated startle rather than sensitizing it, a finding not previously reported for male rats exposed to shock. Attenuation was blocked by administering the anxiolytic drug alprazolam prior to stress, implicating anxiety in the effect. Preliminary tests provided no evidence of mediation by adenosine or corticosterone. This novel result encourages further study of the stressor and dispositional variables that modulate the timecourse of effects of stress on startle and identification of its mechanisms.

Stressful events can affect reactivity to subsequent stressors, either increasing stress vulnerability or enhancing resilience (Heim et al., 2003; Levine, 2005; McEwen, 2004). In humans, alterations in stress vulnerability have been implicated in depression (Gutman and Nemeroff, 2003; Nolen-Hoeksema, 2001), post-traumatic stress disorder (PTSD; Silva et al., 2000; Stam, 2007), substance abuse (Dawes et al., 1999; Koob, 2006), and schizophrenia (Mueser et al., 2002). Both heightened and attenuated stress reactivity from prior stress also have been documented in other mammals (Koolhaas et al., 2006). Milder stressors tend to attenuate later stress responses, whereas trauma tends to enhance them. However, the same stressor can affect various stress response systems differently (e.g. sympathetic versus hypothalamic-pituitary-adrenal activity, Schommer et al., 2003). Futhermore, situational and dispositional variables moderate the impact of prior stress on subsequent vulnerability (e.g., Gunnar and Vasquez, 2006; Moore et al., 2006).

A useful paradigm for studying sequential stress effects in laboratory rats centers on modulation of acoustic startle amplitude. Startle is a defensive reflex, and startle testing after earlier stress constitutes a mild stressor reexposure (Commissaris et al., 2004; Cranney, 1988). Prior experimental stress, such as inescapable shock, usually sensitizes startle. Sensitization has been observed seconds (Pilz et al., 1999), minutes (Davis, 1989), and days (Servatius et al., 1995) after shock. As stressor severity increases, sensitization emerges later and lasts longer. Sensitization emerges a few minutes after ten 0.5-s, 0.6-mA footshocks and is gone within 40 min; at 1.0 or 1.4 mA, sensitization emerges in 20–30 min and is robust at 40 min (Davis, 1989). Sensitization is detectable a week after one session of forty 3-s, 2-mA tailshocks, but not until 10 days after three sessions (Servatius et al., 1995; see also Matuszewich et al., 2007).

The degree to which shock sensitizes startle also varies among rats. Sprague–Dawley rats sensitize more than Wistars (Pilz et al., 1999; also see, Faraday, 2002). Using startle nonhabituation after shock as a PTSD model, Garrick et al. (2001) observed nonhabituation only in rats with low baseline startle amplitudes. Milde et al. (2003) also found sensitization after shock in a subset of rats, those with low plasma corticosterone (CORT).

Compared to sensitization, stress-induced startle attenuation seems rarer and more circumscribed. Beck and Servatius (2005, 2006) observed attenuation 2 h after shock but not after restraint, and only in intact females, concluding that attenuation depends on nociception and ovarian hormones. However, Conti and Printz (2003) observed attenuated startle 24 h after restraint in males of some rat strains. Stress-attenuated startle appears to be a potentially important phenomenon in need of further study.

Accounting for individual differences in stress-induced changes in startle requires approaches in which dispositional variables are well characterized. Selective breeding is such an approach: Lines are developed on a clearly operationalized phenotype, after which phenotypic correlates and functional relationships are examined. For example, Roman low (RLA) and high (RHA) avoidance rats are

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selectively bred on an avoidance phenotype, and excessive anxiety has been implicated in RLA rats' poor avoidance (Driscoll et al., 1998; Steimer and Driscoll, 2003; see also Brush, 2003). The line difference in anxiety manifests as greater baseline startle and greater shock-induced startle sensitization among RLA rats (Schwegler et al., 1997).

In the present study, Occidental high (HiS) and low (LoS) saccharin-consuming rats were used to further explore the role of dispositional emotionality in sequential stress effects. Selectively breeding on a taste phenotype has yielded lines that differ in emotional reactivity. Relative to HiS rats, LoS rats hyperstartle, defecate more in a novel open field, show stronger stress-induced hypoalgesia and anorexia, and are more affected by food deprivation and glucoprivation (Dess et al., 2000; Dess and Minor, 1996; VanderWeele et al., 2002); similar relationships exist in humans (Craig et al., 2003; Dess and Edelheit, 1998). Stress usually sensitizes startle, more so in the anxiety-prone RLA rats (Schwegler et al., 1997), so the most straightforward prediction regarding startle after stress is greater sensitization among LoS rats. On the other hand, sensitization is greater among rats with lower baseline startle (Garrick et al., 2001) or lower CORT (Milde et al., 2003), both of which characterize HiS rats (Dess et al., 2000; VanderWeele et al., 2002). Thus, contrary to other measures of stressor reactivity to date, HiS rats could show greater sensitization. Finally, because startle attenuation has been reported for some male rats (Conti and Printz, 2003), differential attenuation was yet another possible outcome.

1. Experiment 1

Experiment 1 assessed the effect of stress on startle in LoS and HiS rats and the mediating role of anxiety in any effect. No standard protocol exists for studying stress-induced changes in startle. Diverse stressors, parameters, and rat strains are used. In light of our prior research, the relationship between stressor severity and onset of altered startle, and ethical and practical considerations, we selected twenty 5-s 0.6-mA footshocks as a stressor and a 4-h stress–startle interval. This shock regime is more severe than that after which Davis (1989) observed sensitization for at least 40 min and is less severe than stressors that delay sensitization for days (Servatius et al., 1994, 1995). The pre-stressor anxiolytic treatment was alprazolam, which is short acting and should have minimal direct effects more than 4 h later (Lau and Heatherington, 1997). Alprazolam functions much like other benzodiazepines in anxiety paradigms (e.g. Griebel et al., 1996; Isogawa et al., 2005; Lobarinas and Falk, 2000). It reduces startle sensitization (Commissaris et al., 2004; Hijzen et al., 1995) via anxiety reduction (Gifkins et al., 2002; Joordens et al., 1998).

This and the following experiments employed nested designs (Winer et al., 1991). Complete factorial designs often provide superfluous information and thus are not always the most efficient or ethical choice. Our designs provided sufficient power for key comparisons while minimizing rat numbers and distress. In Experiment 1, because this stressor was not expected to affect HiS rats, only LoS rats received alprazolam pretreatment. If anxiety mediates any effect of stress on startle, alprazolam should block that effect, yielding startle in drug-pretreated stressed LoS rats comparable to that of non-stressed LoS controls. Together, those groups should startle differently from stressed LoS rats given no anxiolytic.

1.1. Method

1.1.1. Rats

Experimentally naïve male Occidental HiS ($N = 20$) and LoS ($N = 25$) rats aged 60–80 days from, respectively, eight and nine litters in Generations 27–28 were tested. Body weight averaged 412 ± 5 g (mean ± S.E.M.) and did not differ between groups. Rats were housed individually on a 12:12 light:dark cycle (lights off at 7 p.m.) with Purina 5001 chow and water freely available. Rats' care and use conformed to Public Health Service and institutional policies for humane treatment.

1.1.2. Apparatus and materials

The footshock apparatus was a 16 cm × 32.5 cm × 19.5 cm ($w \times l \times h$) clear acrylic box. Two stainless steel plates lined the sides of the box and angled toward the bottom to create a 1.5-cm gap that the rat had to straddle, thus completing the circuit when current was applied to the plates. The box was placed in an illuminated sound-attenuating chamber. Footshocks were generated by constant current generators (Model 82400, Lafayette Instruments, Lafayette, IN). Shock delivery was computer controlled.

Acoustic startle testing was conducted in a commercial startle chamber (Model SR-Pilot, San Diego Instruments, San Diego, CA) with dimensions of 14 cm × 21 cm × 23 cm ($w \times l \times h$). The startle stimulus was a 40-ms, 95-dB white noise burst. Startle amplitude was detected with a piezoelectric sensor and was displayed digitally in arbitrary units (20–2000). Trial initiation and data recording were manual. The startle apparatus was housed in a sound-attenuating box with a 7-W light bulb and 65-dB ambient masking white noise, located in the same large room as the footshock apparatus.

Alprazolam (Sigma–Aldrich Inc., St. Louis, MO) was prepared with glycol/ethanol/saline vehicle. A dose of 2 mg/kg or vehicle was injected i.p. at a volume of 1 ml/kg.

1.1.3. Experimental design

A five-group nested design was used. Line (HiS versus LoS) and stress condition (Stress versus No Stress) were completely crossed. Drug treatment was nested within the LoS/Stress condition: Eight LoS/Stress rats were injected with alprazolam (LoS/Stress/APZ), and nine were injected with vehicle (LoS/Stress). All HiS/Stress rats ($n = 10$) were injected with vehicle. Remaining rats (LoS/No Stress, $n = 8$; HiS/No Stress, $n = 10$) were not stressed. Within a line, rats were assigned randomly to groups.

1.1.4. Procedure

Rats received brief gentle handling for 4 days before testing. Beginning at 9:30 a.m. on the test day, LoS/Stress/APZ were injected with alprazolam and LoS/Stress and HiS/Stress rats were injected with vehicle. Twenty-five min later, these groups were exposed to twenty 5-s 0.6-mA footshocks on a variable time 60-s schedule. Afterwards, they were returned to their home cages. LoS/No Stress and HiS/No Stress rats remained in their home cages.

Four hours after the stress session ended, startle testing began. Rats were individually placed in the startle chamber. After a 3-min adaptation period, 30 startle trials occurred on a fixed-time 10-s schedule. The rat then was returned to his home cage, and the inside of the chamber was cleaned with 5% ammonium hydroxide solution.

Squads of rats were staggered to hold injection-stress and stressor-startle intervals roughly constant at, respectively, 25 min and 4 h. Groups were balanced in the testing order.

1.2. Results and discussion

Startle amplitude in 10 three-trial blocks is shown in Fig. 1. To minimize undue influence of outlying values, the median value in each block of three trials for each rat was used in the present experiments, as elsewhere (Dess et al., 2000). Startle among LoS rats given alprazolam before footshock was comparable to that of non-stressed LoS controls. Startle among stressed LoS rats given