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# Acoustic Startle and Fear-Potentiated Startle in Alcohol-Preferring (P) and -Nonpreferring (NP) Lines of Rats

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MCKINZIE, D. L., T. J. SAJDYK, W. J. MCBRIDE, J. M. MURPHY, L. LUMENG, T.-K. LI AND A. SHEKHAR. *Acoustic startle and fear-potentiated startle in alcohol-preferring (P) and -nonpreferring (NP) lines of rats.* PHARMACOL BIOCHEM BEHAV **65**(4) 691–696, 2000.—The objective of the present study was to determine whether alcohol-preferring P and -nonpreferring NP rats differ in their acoustic startle response and in fear-potentiated startle. In Experiment 1, male P and NP rats were tested on the startle response to acoustic stimuli ranging from 90–115 dB. Experiments 2 and 3 examined fear-potentiated startle and extinction of the response. In Experiment 2, rats received two light foot shock training sessions separated by 3–4 h. Testing consisted of ten acoustic startle (115 dB) and fear-potentiated startle (light preceding the acoustic startle) presentations administered every 24 h for 9 consecutive days. To test potentiated startle learning under reduced training conditions, a single training session was administered in Experiment 3, and a single within-session extinction test of 50 startle and 50 potentiated startle trials occurred the following day. Results of Experiment 1 indicated that P and NP rats did not differ in startle at any of the acoustic intensities tested. Following fear-potentiated startle conditioning in Experiment 2, however, both acoustic startle and potentiated startle responding were consistently greater in P than NP rats over most of the first 6 test days with P rats having approximately a 100% greater acoustic startle and 50–100% greater potentiated startle response. Moreover, following a single training session in Experiment 3, only P rats showed significant fear-conditioned startle. Additionally, P rats exhibited a 50–100% elevated acoustic startle response over that observed in NP rats. Taken together, the data indicate that, although experimentally naive male P and NP rats show similar acoustic startle responses, P rats become more responsive to both startle-alone and potentiated startle stimuli following fear conditioning. The change in general startle reactivity of the P rat following aversive conditioning, along with facilitated light foot shock learning, suggests that stress exposure may be an important variable in examining associations between anxiety and alcohol drinking behavior. © 2000 Elsevier Science Inc.

Acoustic startle  P and NP lines of rats  Fear conditioning

ALTHOUGH a relatively high degree of comorbidity exists between alcohol abuse and certain anxiety disorders, a "tension-reduction" theory of alcoholism has received mixed empirical support. Clinical studies clearly indicate that alcohol-dependent individuals are more likely than the general population to exhibit symptoms of anxiety, but it remains unclear whether anxiety disorders precede and, therefore, precipitate alcohol abuse, or whether anxiety symptoms are a consequence of alcohol dependence and protracted withdrawal states (42). Similarly, studies examining selectively-bred rat lines for divergent alcohol preference or emotional reactivity have found both positive (6,16,20,40,41,43) and neg-

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ative (2,33,44,45) associations between alcohol preference and measures of innate anxiety levels.

Many possible factors may contribute to the discrepant findings in the literature, such as choice of model, type of measure or procedural parameters used. One potential factor that is often overlooked is the role of previous stress and later anxiety levels. A number of studies indicate that exposure to stress can produce later increases in ethanol intake levels (1,28,32,36,39). Therefore, it is possible that stress may differentially produce long-lasting effects in people or in animal lines disposed for high alcohol drinking behavior. For example, alcohol-preferring (P) rats exhibit significantly lower concentrations of corticotropin releasing factor (CRF) in several brain regions compared to alcohol-nonpreferring (NP) rats, but show enhanced reactivity to ICV administration of CRF, suggesting an upregulation of CRF receptors in P rats (17). The differences in basal CRF concentrations and subsequent reactivity to exogenous CRF challenge in some regions of the brain suggest that P and NP lines of rats may differ in their response to a stressful event.

The objective of the present study was to determine whether selectively bred P and NP rats differ in a measure of anxiety both under basal conditions and following aversive conditioning. It was hypothesized that line differences in aversive responses between the P and the alcohol-nonpreferring (NP) rats may be accentuated following a stressor. We used the fear-potentiated startle paradigm to test this hypothesis. The potentiated startle paradigm has been used extensively as a behavioral model of anxiety (7,8,12,13), and has the advantage of assessing unconditioned (acoustic startle alone), as well as conditioned contributions of fear (potentiated startle). Experiment 1 examined startle responses of experimentally naive male P and NP rats to a range of acoustic stimuli. This experiment served as a baseline measure of startle reactivity. Experiment 2 examined the effects of previous fear conditioning on later startle in these lines of rats and its enduring consequences over 9 consecutive days of testing. Experiment 3 determined whether line differences in fear-potentiated startle would be evident following a reduced fear conditioning procedure (i.e., a single training session).

## METHOD

### *Subjects*

Subjects were ethanol-naive, adult male rats from the S-39–40 generations of alcohol-preferring (P) and -nonpreferring (NP) rats. Separate groups of P and NP rats were used for each experiment with bodyweights ranging between 325–400 g. Animals were housed two per plastic tubs (18 × 24 × 45 cm) with wire grid tops in a temperature (21°C)-and humidity (50%)-controlled vivarium maintained on a 12 L:12 D cycle (lights on 0700 h). All experiments were conducted between 0900–1200 h, during the light portion of the light cycle. Harlan rat chow (Teklad Diet #7001, Harlan Industries, Indianapolis, IN) and tap water were available ad lib in the home cages throughout the experiment.

### *Apparatus*

Training and testing occurred in an automated SR-Lab startle reflex system (San Diego Instruments). The system consisted of a transparent acrylic rodent cylinder with a grid floor for shock delivery (programmable grid floor shocker, Coulbourn Instruments) situated on top of a pressure-sensitive base. The reflex system was contained within a sound-attenuated chamber equipped with an internal light and sound source. A desktop computer controlled stimulus presentations and recorded all chamber movement information. Each experimental session began by placing the rat into the acrylic cylinder within the chamber for a 5-min acclimation period. During this time and throughout the session, a 70-dB background noise was present. Startle responses were transduced by a piezoelectric accelerometer mounted below the cylinder and converted into arbitrary units based on calculations from latency and force of startle.

### *Startle Threshold Protocol (Experiment 1)*

Experimentally naive P and NP rats ($n$ = 10/group) were placed individually in the acrylic cylinder and exposed to a 5-min acclimation period and a background noise of 70 dB. Following the acclimation period, rats received 10 samples each of seven different decibel levels of a 750 ms white noise burst (70, 90, 95, 100, 105, 110, and 115 dB) presented in a random order with an average intertrial interval (ITI) of 75 s (range 30–120 s).

### *Conditioned Fear Training (Experiments 2 and 3)*

Experimentally naive P and NP rats ($n$ = 9–10/group) received 10 trials of light (25 W) followed by a foot shock (0.2 or 0.4 mA for 750 ms). Each light/foot shock pairing began with the light illuminated for 3700 ms prior to shock onset, then the light remained on together with the shock for an additional 750 ms. All training occurred in one or two sessions on a single day. When two conditioning sessions occurred (Experiment 2), a 0.2-mA foot shock was delivered in the morning session (0900–1100 h) and a 0.4-mA foot shock was used in the afternoon conditioning session (1300–1500 h). A foot shock intensity of 0.4 mA was delivered when a single training session occurred (Experiment 3). Light foot shock trials were presented on a variable-time 180-s ITI, with a range of 90–270 s.

### *Startle Testing: Between-Day Extinction (Experiment 2)*

Test sessions took place 24 h after training, and consisted of two types of startle conditions: one being an auditory startle alone (a 750 ms, 115 dB white noise), and the other being the same acoustic stimulus preceded by a light cue (fear-potentiated startle). On fear-potentiated startle trials, the light was illuminated for 3700 ms and remained on during the 750 ms acoustic stimulus. The test began with a 5-min acclimation period to a background noise level of 70 dB. Nine introductory acoustic startle stimuli then followed with the first three intensities at 90 dB, the second three at 95 dB, and the last three at 115 dB. These nine initial acoustic startles were used to further acclimate the rats to the test conditions, and were not included in the calculations. After the initial acoustic startles, 12 each of the acoustic and fear-potentiated startles were presented in random order with a fixed-ITI of 30 s. This testing procedure was conducted for 9 consecutive days.

### *Startle Testing: Within-Session Extinction (Experiment 3)*

All rats received a single fear-potentiated startle session, with a 0.4-mA shock level. All other training parameters were identical to those of Experiment 2. Testing for startle-alone and fear-potentiated startle responses took place 24 h later. The test session consisted of 50 acoustic startle (115 dB) and 50 fear-potentiated startle trials presented in random order with a variable-ITI of 30 s.

RESULTS

*Startle Threshold in Experimentally-Naïve P and NP Rats (Experiment 1)*

A mixed-factor ANOVA was conducted with line (P vs. NP) and decibel (70, 90, 95, 100, 105, 110, and 115 dB) serving as independent variables. Only a main effect of decibel was found, indicating that startle amplitude increased with higher acoustic stimuli, $F(6, 78) = 71.39$, $p < 0.001$. Post hoc analysis using the protected least significant difference test (25) determined that significant startle amplitudes began at 95 dB (Fig. 1). Startle amplitudes increased at 105 and 110 dB intensities, and the highest amplitudes occurred at 115 dB. Neither the effect of line nor the line × decibel interaction approached significance (all $p$-values > 0.75).

*Startle Testing: Between-Day Extinction (Experiment 2)*

A line (P vs. NP) × trial (startle-alone vs. potentiated startle) × day (test days 1–9) mixed ANOVA indicated main effects of line, trial, and day, $F(1, 14) = 6.97$, $p < 0.02$, $F(1, 14) = 49.69$, $p < 0.001$, and $F(8, 112) = 14.49$, $p < 0.001$, respectively. In addition, a trial × day, $F(8, 112) = 20.01$, $p < 0.001$, and trial × day × line interaction, $F(8, 112) = 2.55$, $p < 0.01$, were observed. Follow-up analyses of the three-way interaction revealed that P rats exhibited greater startle amplitudes than NP rats on test days 1–6 (top panel; Fig. 2) and greater potentiated startle on days 3, 5, 6, and 9 of testing (bottom panel; Fig. 2). When startle versus potentiated, startle trials were compared within each line, P rats exhibited higher potentiated startle amplitudes on days 1, 2, and 5 of testing. NP rats expressed significant potentiated startle on the first 4 days of testing.

*Startle Testing: Within-Session Extinction (Experiment 3)*

A line (P vs. NP) × trial (startle alone vs. potentiated startle) × block [1–5] mixed ANOVA determined main effects of line, trial, and block, $F(1, 15) = 8.85$, $p < 0.01$, $F(1, 15) = 12.16$, $p < 0.003$, and $F(4, 60) = 14.12$, $p < 0.001$, respectively. Line × trial, $F(1, 15) = 6.44$, $p < 0.02$, and line × trial × block interactions, $F(4, 60) = 2.74$, $p < 0.04$, were also found. Analysis of the three-way interaction determined that P rats had higher startle amplitudes than NP rats on blocks 1, 2, 4, and 5 (top panel; Fig. 3). Moreover, P rats exhibited greater potentiated startle responses on all five blocks compared to NP rats (bottom panel; Fig. 3). As shown in Fig. 4, when mean startle and potentiated startle trials were collapsed over the entire 50 trials (line × trial interaction), only P rats expressed significant potentiated startle.

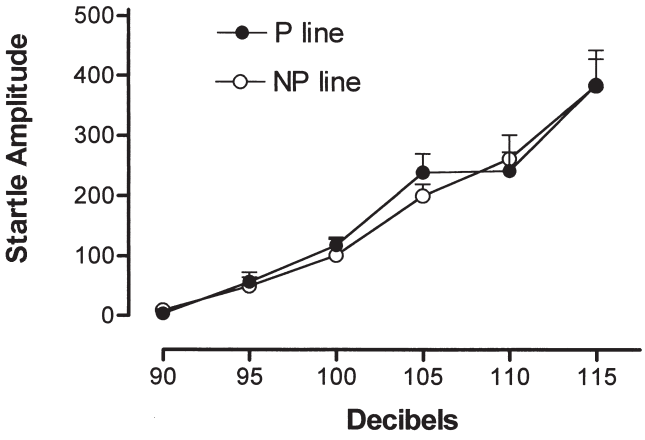


FIG. 1. Mean startle amplitudes in male P and NP rats as a function of acoustic stimulus intensity. Rats were experimentally naive, and received 10 presentations of each intensity (90, 95, 100, 105, 110, and 115 dB) given in a random order. Although no differences in startle amplitude existed between lines at any of the decibels tested, progressively louder acoustic stimuli produced greater startle responding. Data are mean startle amplitudes of the 10 trials for each acoustic intensity (±SEMs).

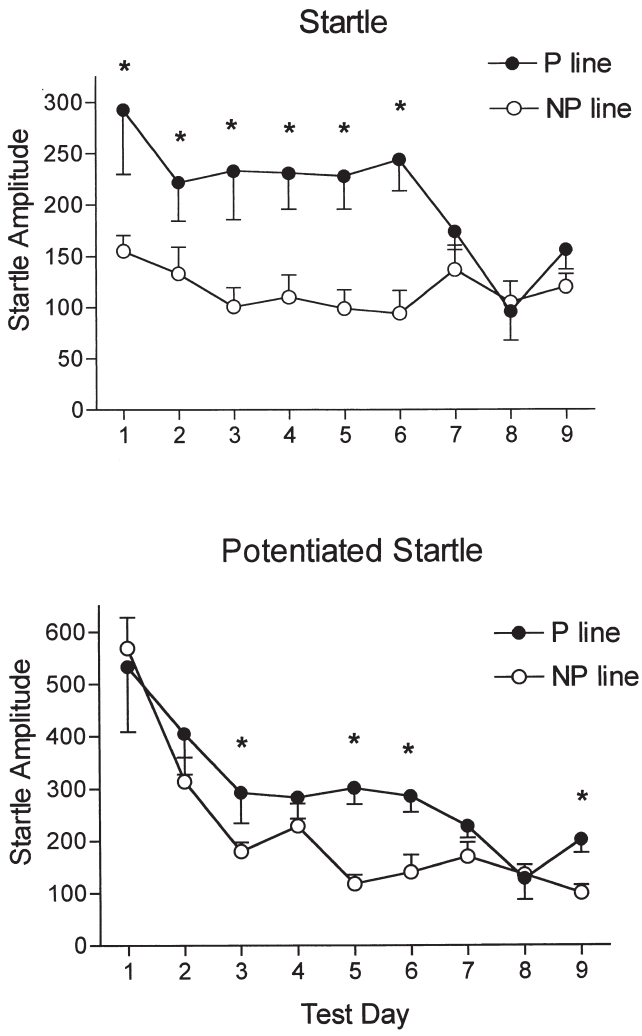


FIG. 2. Mean startle amplitudes in male P and NP rats on 9 consecutive days. Rats received two light foot shock training sessions 24 h previously. The top panel represents acoustic startle alone (115 dB), and the bottom panel shows fear-potentiated startle (light preceding the 115 dB noise). Relative to NP rats, P rats maintained elevated startle-alone and potentiated startle amplitudes for up to 6 days following training (*$p < 0.001$). Data are mean startle amplitudes for each test day (±SEMs).

DISCUSSION

When experimentally naive P and NP male rats were tested for their responsiveness to a range of acoustic intensi-

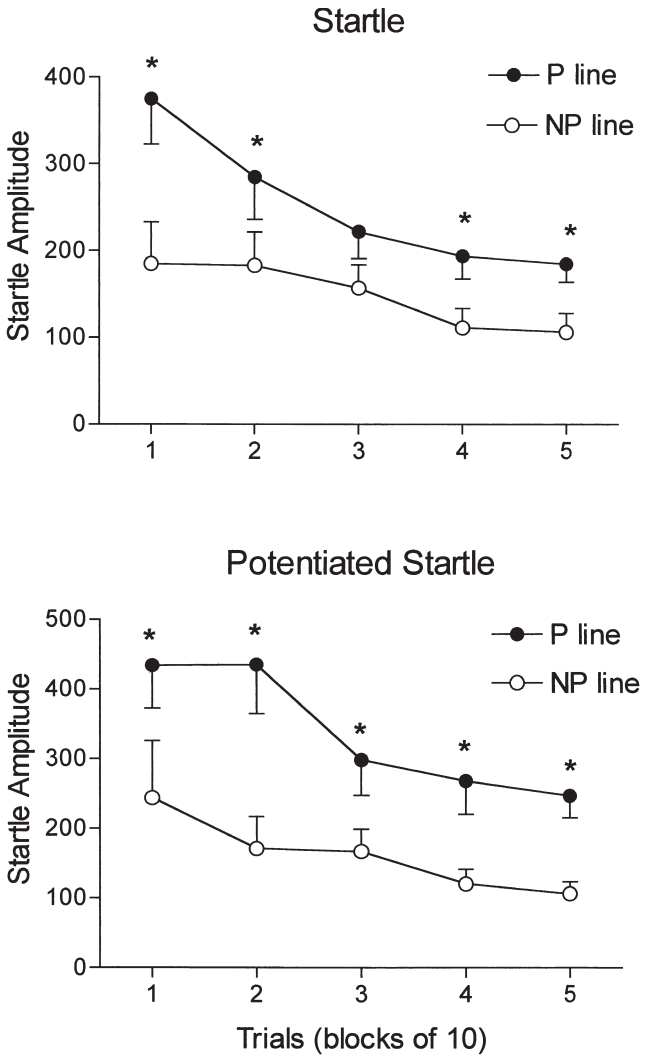


FIG. 3. Mean startle amplitudes in male P and NP rats 24 h after a single light foot shock training session. The top panel represents acoustic startle alone (115 dB) and the bottom panel shows fear-potentiated startle (light preceding the 115 dB noise) over the single test period. P rats exhibited consistently higher startle alone and fear-potentiated startle responses throughout the test period than did NP rats (*$p < 0.04$). Data are mean startle amplitudes (±SEMs).

ties, there were no differences between the lines in startle amplitude at any of the decibels tested (Fig. 1). Interestingly, when the rats were subjected to classical conditioning of light and foot shock on day 1, on the following day of testing with light and startle alone, consistent differences between the lines emerged (Fig. 2). Compared to NP rats, P rats showed enhanced startle responses to both acoustic stimuli alone and light-potentiated trials. Although differences in baseline startle make interpretation of potentiated startle difficult, it appears that the P line of rats expressed greater fear conditioning to the light conditioned stimulus. For example, under the reduced training conditions used in Experiment 3, only the P line exhibited significant light-potentiated startle at testing (Figs. 3 and 4). Thus, prior fear conditioning elicited greater startle-alone and potentiated startle responses in the P, compared to the NP, line of rats.

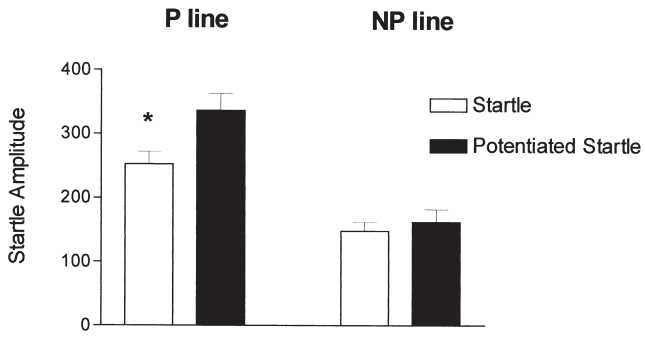


FIG. 4. Startle amplitudes in male P and NP rats averaged over the 50 startle and fear-potentiated startle presentations. Rats had received a single training session 24 h earlier. P rats had higher startle and potentiated startle responses than did NP rats. Moreover, whereas the P line of rats demonstrated significant fear-potentiated startle responding, relative to startle-alone trials, no differences existed between startle and potentiated startle presentations in NP rats (*$p < 0.02$ for startle vs. potentiated startle in P rats).

The potentiated startle paradigm has been used extensively as an animal model of anxiety (8). In particular, the difference between the startle response alone and the enhanced startle following cued-fear conditioning has been used to assess anxiety and therapeutic effects of pharmacological agents. Many clinically efficacious anxiolytics have been shown to reduce fear-potentiated startle without affecting baseline startle (9,13). Careful anatomical and pharmacological analyses have determined that the amygdala is a critical site for fear-potentiated startle learning (10,14). Besides being a critical neural substrate for fear conditioning, the amygdala has also been implicated as an important site mediating alcohol drinking behavior (26,27).

Although P rats exhibited potentiated startle under conditions in which the NP rats did not, the most robust effects in the current study were the line differences observed in startle baseline following light foot shock training (Figs. 2–4). Because experimentally naive P and NP rats did not exhibit differences in the acoustic startle response (Fig. 1), it appears that a prior aversive experience in the startle apparatus produced an enduring enhancement of startle reactivity in P compared to NP rats. This finding further suggests that the startle response alone may be a sensitive measure of changes in emotional reactivity.

The notion that startle-alone vs. fear-potentiated startle may reflect different neuroadaptive mechanisms has been proposed by Davis and colleagues (11,15). They suggest that responses to the startle stimulus alone may reflect innate levels of anxiety, whereas potentiated startle trials involve cue-specific fear learning (46). Evidence exists for such a neuroanatomical dissociation, in that, while lesions or pharmacological blockade of the central nucleus of the amygdala eliminates fear-potentiated startle, such unconditioned manipulations as light-enhanced or CRH-enhanced startle remain unaffected (29,46). Conversely, light- or CRH-enhanced startle is disrupted by lesions in the bed nucleus of the stria terminalis, whereas fear-potentiated startle is preserved (29,46). In the context of the present findings, perhaps P rats are more affected by a stressor, such as light foot shock training, and consequently exhibit enduring changes in emotional reactivity when tested later. This is an interesting possibility, as a previ-

ous report indicated that P rats have an enhanced EEG response to ICV CRF administration, relative to NP rats (17). Moreover, neurochemical assessments of the amygdala in P and NP rats has revealed differences between the lines in neuropeptide Y, mu-opioid, 5-$HT_3$, 5-$HT_{1B}$, and 5-$HT_{2C}$ functioning (5,18,19,23,30,31,34). Although the bed nucleus of the stria terminalis has not been well-characterized in the P and NP rats, a recent report found that Preprotachykinin-A mRNA levels in the bed nucleus were 50% lower in alcohol-preferring (sP) vs. alcohol-nonpreferring (sNP) lines of rats (38). Whether any of these neurobiological differences between the disparate alcohol drinking lines of rats are associated with differences observed between the lines in the present study in the startle and potentiated startle responses requires additional studies.

The present study is also consistent with reports of a positive association between anxiety and alcohol preference. Although P rats scored higher on various measures of anxiety (40,43), other investigators have not confirmed such a relationship (2,45). Moreover, studies with other rat lines selectively bred for divergent alcohol preference have also yielded inconsistent results (33,44). These inconsistencies lead one to conclude that, at best, only a modest correlation exists between alcohol preference and innate anxiety. Alternatively, perhaps the organism's reactivity to stress may be a more sensitive indicator of alcohol preference. Stress consistently induces higher levels of ethanol drinking behavior in both humans and animals (3,4,24,35–37). Moreover, individuals with a positive family history of alcoholism are more reactive to both an avoidable and unavoidable shock (21,22).

These data suggest that exposure to stress may interact with underlying anxiety states to produce a high reactive disposition that, in turn, may lead to a propensity for high alcohol drinking behavior. It is possible that previous inconsistencies in the literature regarding an anxiety–ethanol association may be clarified somewhat if external variables such as stress are incorporated into the experimental design. In summary, strong line differences emerged between P and NP rats in both startle and potentiated startle following exposure to an aversive conditioning. Given the important therapeutic implications for determining whether an association for anxiety and alcoholism exist, stress exposure should be considered as a variable in future studies.

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