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Research Article

EFFECTS OF BUSPIRONE AND ALPRAZOLAM TREATMENT ON THE STARTLE-POTENTIATED STARTLE RESPONSE

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The startle potentiated startle (SPS) paradigm has been reported to be an effective procedure for studying the conditioned enhancement of acoustic startle in the absence of electric shocks or extinction. This study examines the effects of two anxiolytic treatments, buspirone and alprazolam, on this SPS effect. Subjects were tested in the SPS paradigm 2 days a week (Monday and Thursday) for 10 weeks. Each startle test session consisted of 10 Noise Alone trials (115 dB acoustic noise burst presented for 40 ms) and 10 Light+Noise trials (115 dB acoustic stimuli presented during the latter 40 ms of a 3,540 ms period in which a 15-watt light was illuminated). Although there was no difference in startle amplitude on Noise Alone trials when compared to Light+Noise trials initially, by the end of the first test session and continuing throughout the duration of the experiment, startle amplitude on Light+Noise trials was significantly (approximately 50–75%) greater than on Noise Alone trials. After five control (i.e., no injection) SPS test sessions, once-weekly drug challenges were conducted over the course of 7 weeks. In these weekly drug challenges, subjects received acute treatment with various doses of the benzodiazepine anxiolytic alprazolam (0.25, 0.5, 1.0 mg/kg) or the novel anxiolytic buspirone (1.0, 2.0, 4.0 mg/kg); subjects also received vehicle treatment (0.5% methylcellulose) on one treatment day. All treatments were administered intraperitoneally (IP), 15 min before the start of startle testing. Consistent with previous reports, buspirone increased and alprazolam decreased startle amplitude on the Noise Alone trials; these effects were dose-related. Both agents reduced the magnitude of the SPS effect when it was expressed as the Light+Noise startle amplitude minus the Noise Alone startle amplitude. These findings are similar to the effects of these treatments in the traditional shock-based fear-potentiated startle paradigm. Depression and Anxiety 19:146–151, 2004. © *2004 Wiley-Liss, Inc.*

Key words: *extinction; buspirone; acoustic startle; classical conditioning; alprazolam; fear potentiated startle; startle potentiated startle*

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INTRODUCTION

The Fear-Potentiated Startle (FPS) paradigm is a widely used procedure in behavioral pharmacology that has been used to study both fear/anxiety-like behavior as well as classical conditioning [Berg and Davis, 1985; Davis, 1991; Davis et al., 1993, 1997; McAllister and McAllister, 1971]. In this paradigm, subjects (typically rats) are conditioned through repeated pairings of an otherwise neutral stimulus (typically a light) with an aversive stimulus (typically an electric foot shock). After these Light+Shock pairings, acoustic startle is sampled under conditions of either no light (Noise Alone trials) or after brief presentation of the light previously paired with the electric shock (Light+Noise trials). Startle amplitude on Light+Noise trials is greater than that on Noise Alone trials after this conditioning; animals do not exhibit a difference in Light+Noise versus Noise Alone trials in the absence of conditioning. The increase in startle amplitude on Light+Noise trials is referred to as the FPS effect and has been argued to reflect the influence of conditioned fear/anxiety [Brown et al., 1951; Davis, 1991; McAllister and McAllister, 1971]. Consistent with this interpretation, the FPS effect is blocked by acute treatment with a variety of clinically effective anti-anxiety (anxiolytic) treatments, including benzodiazepines and the novel anxiolytic agent buspirone [Davis, 1991; Davis et al., 1993].

A potential drawback with the FPS paradigm is that testing typically is conducted under conditions of extinction (i.e., during FPS testing, the shock is no longer paired with the presentation of the light). Because of this, FPS testing is typically a single-test paradigm. This is in contrast to many repeated measures paradigms in behavioral pharmacology, where dose-response curves or time-course information can be determined using repeated testing and within-subject designs. As a result, the examination of various doses or pretreatment times typically requires additional test groups. Moreover, because the "light predicts shock" conditioned effect is not maintained during the course of typical FPS testing, the influence of a particular treatment on the FPS effect could be confounded by extinction of the conditioned behavior over the course of many trials during an FPS test session.

We have reported on a modification of the traditional FPS paradigm that does not utilize light+shock training and does not exhibit extinction. This startle potentiated startle (SPS) paradigm is based in part on earlier reports by Leaton and Cranney [1990]; these investigators demonstrated that anticipation of the startle stimulus increased the response to the noise burst. In this SPS paradigm, rats are tested repeatedly, with each startle session consisting of 10 Noise Alone trials and 10 Light+Noise trials [McQueen et al., 2001; Winston et al., 2001]. Initially there is no difference in startle amplitude for the two trial types. Within the course of a single 20-trial test session, and continuing throughout many weeks of repeated startle test sessions, startle amplitude on the Light+Noise trials is consistently higher than on the Noise Alone trials. It should be noted that with this procedure, the conditioned effect is measured without testing under conditions of extinction. In this regard, the SPS paradigm is analogous to the Estes-Skinner conditioned emotional response (CER) operant conflict paradigm [Estes and Skinner, 1941]. This SPS paradigm might be useful for repeated measures designs, such as the determination of within-subjects dose-response curves or time course parameters.

There have been only limited studies on the effects of anxiolytic treatments on the SPS paradigm. The only published study examined the effects of buspirone (BUS) and the 5-hydroxytryptamine-1A agonist 9-hydroxy-2-(di-*n*-propylamino)tetralin (8-OHDPAT) in two strains of rats selectively bred for differences in the hypothermic effects of 8-OHDPAT. Consistent with its effect to reduce conditioned enhancement of startle in the FPS paradigm, buspirone treatment reduced the magnitude of the SPS effect in both rat strains [McQueen et al., 2001]. The effects of buspirone on the SPS effect in more ordinary rats (e.g., outbred SD) have not been reported. Moreover, there are no reports on the effects of benzodiazepine anxiolytics in this SPS paradigm.

The purpose of this study was to determine the effects of acute treatment with the traditional benzodiazepine anxiolytic alprazolam (ALP) and the atypical anxiolytic buspirone (BUS) on SPS behavior in Sprague-Dawley rats.

METHODS

ANIMALS

The subjects were naïve Sprague-Dawley male rats, purchased from Charles River Farms (Cambridge, MA). The rats weighed 225–250 g at the time of arrival at Wayne State University (WSU). All animals were housed in the WSU vivarium for at least 10 days before the initiation of the present studies. Throughout the present studies, the animals were housed 2/box or 3/box in the American Association of Laboratory Animal Care (AALAC) approved animal facility maintained by the WSU Department of Lab Animal Research (DLAR). In the animal quarters the lights were on 0700–1900 h; the temperature was 21–23°C; the relative humidity was 40–50%. Food and water were continuously available in the home cage. All procedures involving experimental animals were reviewed and approved by the WSU Animal Investigation Committee and followed all applicable NIH and USDA guidelines.

APPARATUS

The apparatus used for startle and potentiated startle (SPS) testing and training was the SR-LAB Startle Response System-R, purchased from San Diego Instruments Inc. (San Diego, CA) [McQueen et al., 2001; Winston et al., 2001]. Test chambers were clear Plexiglas® cylinders, 15 cm long and 9 cm inside diameter, each resting on a solid Plexiglas® base. An accelerometer was attached to the base of the startle test cage cylinder and served as the transducer. Cage movement resulted in a change in voltage within the accelerometer; the resultant voltage was converted to a startle amplitude. The test cage and Plexiglas® support were enclosed in a sound-attenuating cubicle. This cubicle was equipped with 1) a house light which was not used in the present experiment, 2) a 15-watt lamp that was used for potentiated startle training and testing, and 3) a speaker (Radio Shack Super Tweeter®) for background noise and for presentation of the acoustic startle stimuli. All parameters of the acoustic startle test sessions (acoustic stimulus intensity, inter-stimulus interval [ISI], presence or absence of the conditioned stimuli, sampling interval, etc.) were controlled by a microprocessor (IBM-386) using PSR2 software purchased from San Diego Instruments (SDI). The startle test chambers were standardized at the beginning of each day of startle testing using the SR-Lab Standardization Unit purchased from SDI.

PROCEDURE

For each SPS test session, subjects were placed individually in the Plexiglas® test cylinder. All test sessions were preceded by a 5-min acclimation period (background noise only = 70 dB). Each startle test session consisted of 20 startle stimuli (115 dB noise bursts; 40 ms in duration). Half of these startle stimuli were presented in darkness and half were presented during the final 40 ms of a 3,540 ms presentation of a 15-watt incandescent light. The order of presentation of the two trial types was constant across all startle test sessions and was based on a randomization scheme determined in earlier experiments [Winston et al., 2001]. The startle test chamber was dark during the inter-trial interval (ITI). ITI values over the course of the 20 stimulus presentations ranged from 26–34 s in 2-s increments. It should be noted that half of the trials in each session were of the "Light+Noise" type and half of the trials were of the "Noise Alone" type. These daily startle sessions served two purposes. First, for the experimental subjects, these test sessions established and maintained the conditioned association between the light and the startle stimulus. At the same time, these test sessions provided information regarding the effects of prior conditioning (startle amplitude on the Noise Alone versus Light+Noise trials). Thus, as with the Estes-Skinner fear-conditioned emotional response (CER) operant conflict paradigm [Estes and Skinner, 1941], subjects in this SPS paradigm were tested repeatedly for the effects of classical conditioning on the acoustic startle response, but they were never tested under conditions of extinction.

CONTROL SPS BEHAVIOR AND DRUG TREATMENTS PROCEDURE

All subjects were tested for the Startle-Potentiated Startle (SPS) effect on 20 occasions over the course of 10 weeks. These test sessions were approximately 15 min in duration. Two test sessions were conducted each week, typically on Monday and Thursday; these test sessions were separated by a period of 2–4 days. For the first five sessions (SPS acquisition and baseline determinations), no drug or vehicle treatments were administered. For the last 15 sessions, i.e., over the course of the last 7 weeks of SPS testing (one treatment per week), the effects of acute challenges with alprazolam and buspirone were determined. All subjects received ALP (0.25, 0.5, 1.0 mg/kg), BUS (1.0, 2.0, 4.0 mg/kg) and vehicle (Veh; 0.5% methylcellulose) treatments over the course of 7 weeks. Alprazolam free base was obtained from Pfizer-Pharmacea-Upjohn (Kalamazoo, MI) and was prepared in a 0.5% methylcellulose suspension; buspirone HCl was purchased from Research Biochemicals Inc. (Natick, MA) and was dissolved in deionized water. All treatments were administered intraperitoneally (IP) in a volume of 1 mg/kg body weight, 10 min before the start of the startle test session. Thus, the effects of the drugs were determined during the interval approximately 15–25 min. after injection. The drug doses were selected because they produce reductions in the FPS effect and other anxiety-like behaviors at the indicated dose ranges [Fontana et al., 1999; Jung et al., 2000; Kehne et al., 1988; McQueen et al., 2001; Schefke et al., 1989; Wichmann et al., 2000]. For each subject, the order of the seven treatments received was randomized across the 7 weeks of SPS testing. In addition to the drug treatment sessions, subjects were tested in additional control (i.e., non-drug) SPS sessions each week.

DEPENDENT VARIABLE AND STATISTICAL ANALYSES

The primary dependent variable in this experiment was the maximum startle response and was measured as the maximum change in velocity of cage movement as measured by the accelerometer during the 100 ms interval beginning at the onset of the 40 ms noise burst. Data were analyzed using factorial analysis of variance (ANOVA) with repeated measures, with post hoc comparisons made using the Student-Newman-Kuels (SNK) test. At the end of every acoustic startle test session, the number of fecal boli in the test chamber was recorded and the test chamber was cleaned. Data on the number of fecal boli remaining at the end of each test session were analyzed by factorial ANOVA with repeated measures. In all statistical comparisons,

$P<.05$ was used as the criterion for statistical significance [Steele and Torrie, 1985].

RESULTS

ACQUISITION OF THE SPS RESPONSE

Figure 1 depicts the acquisition of the SPS response. Data from Day 1 (before conditioning) are depicted on the top two panels, and data from Day 5 (after 4 days of conditioning) are depicted on the lower two panels. These data are presented in two ways; the left panels display the startle amplitude by Trial Number within the SPS test session, irrespective of Trial Type. Data in the right panels are re-plotted according to both Trial Type and Trial Number (i.e., in a 2×10 array). There was little difference between Noise Alone and Light+Noise trials on Test Day 1, but on Test Day 5 startle amplitude was dramatically higher on Light+Noise trials. Analysis of the last 18 startle trials showed that there was no significant effect of Trial Number on Test Day 1 ($F[17,170] = 1.34$, *ns*), but this same effect was significant on Test Day 5 ($F[17,70] = 2.57$, $P<.05$). Analysis of the data with a 2×10 factorial ANOVA (using both Trial Type and Trial Number as factors) showed a significant effect of Trial Type on Test Day 5 ($F[1,9] = 8.91$, $P<0.05$) but not Test Day 1 ($F[1,9] = 1.05$, *ns*).

STABILITY OF SPS EFFECT ACROSS CONTROL TEST SESSIONS

Figure 2 illustrates that this SPS effect, once acquired, is relatively stable across test days of repeated SPS testing. As can be seen, the SPS effect is present for all test days after Test Day 1. Statistically, the main effects for Test Day ($F[4,40] = 5.68$, $P < .05$) and Trial Type ($F[1,10] = 13.17$, $P < 0.05$) were significant; the Test Day × Trial Type interaction ($F[4,40] = 2.16$, $P < 0.05$, one-tailed) was significant using a one-tailed comparison. Post hoc SNK tests showed that startle amplitude on Light+Noise trials was significantly greater than on Noise Alone trials for Test Days 2–5. Also depicted on Figure 2 are the values for Noise Alone and Light+Noise startle trials on the vehicle treatment day during the drug challenges (Test Sessions 6–20). Although there was a tendency for the SPS effect to be greater on the Vehicle Challenge

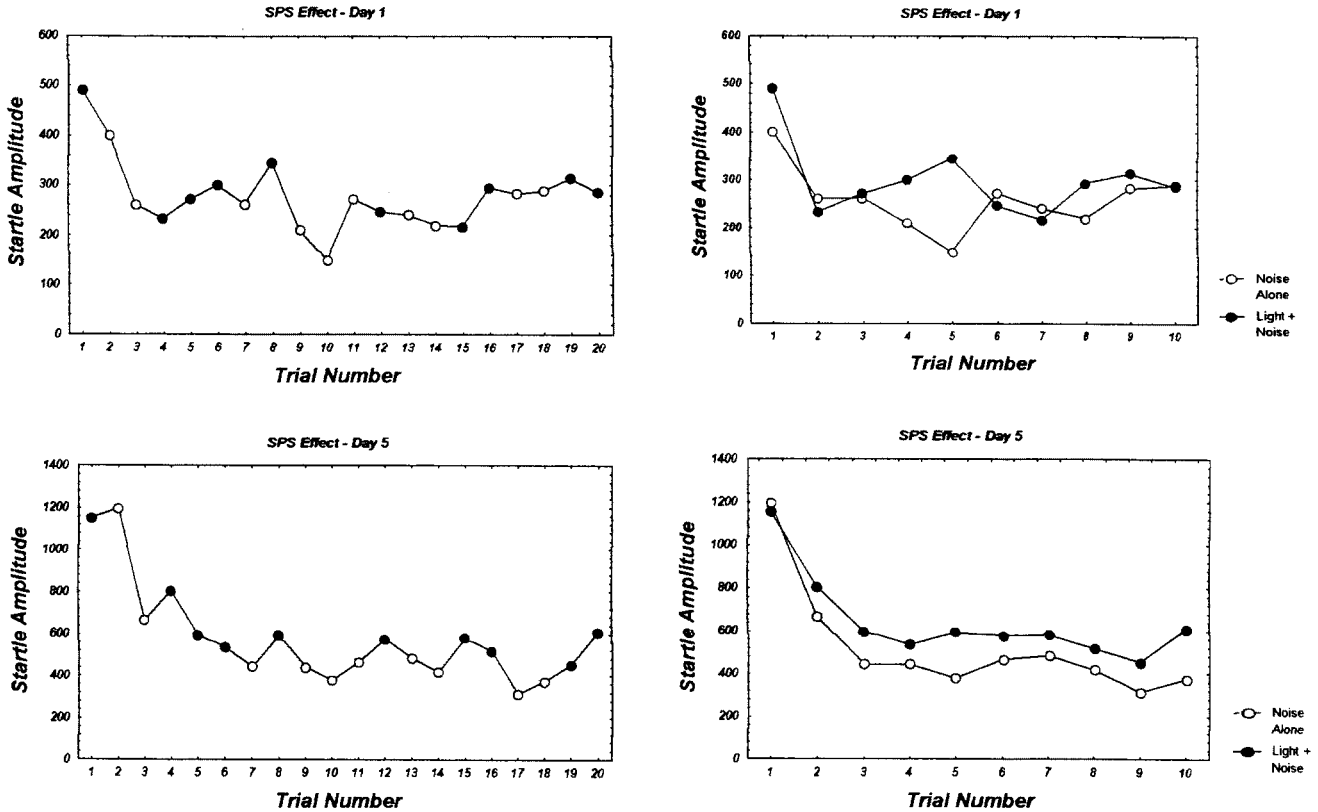

Fig. 1. Acquisition of the Startle Potentiated Startle (SPS) response. Data plotted in the left panels are the Mean values ($n = 12$) for acoustic startle over the course of 20 startle trials on Test Day 1 (top panel) and Test Day 5 (bottom panel). Open circles represent Noise Alone startle trials and filled circles represent Light+Noise startle trials. Data plotted in the right panels are the same Mean values, but plotted separately for each Trial Type (i.e., Noise Alone versus Light+Noise). ANOVA showed a significant Main Effect for Trial Type on Test Day 5, but not Test Day 1.

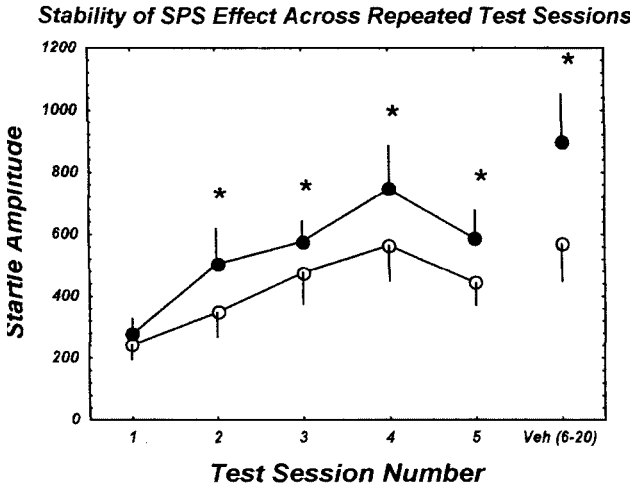

Fig. 2. SPS effect across Test Days 1–5. Plotted are the Mean+SEM values ($n = 12$) for startle amplitude on Noise Alone trials (open circles) and Light+Noise trials (filled circles) across the first five SPS test sessions and also for the acute vehicle treatment SPS session. *Acoustic startle amplitude for Light+Noise trials is significantly different from Noise Alone trials on the indicated Test Day, post hoc SNK after ANOVA.

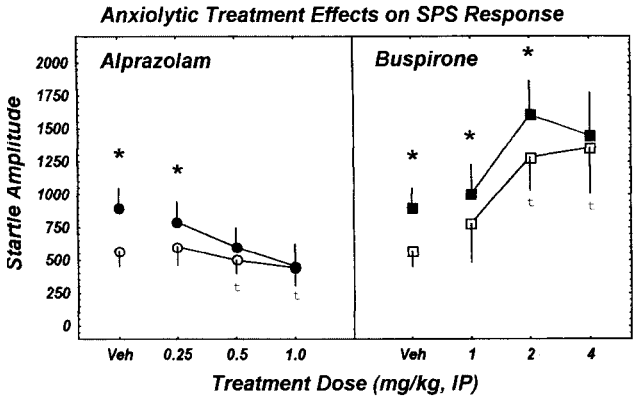

Fig. 3. Alprazolam and buspirone effects on the SPS response. Plotted are the Mean+*sem* values ($n = 12$) for startle amplitude on Noise Alone trials (open symbols) and Light+Noise trials (filled symbols) after acute treatment with Vehicle (Veh), alprazolam (0.25–1.0 mg/kg) or buspirone (1.0–4.0 mg/kg). *Acoustic startle amplitude for Light+Noise trials is significantly different from Noise Alone trials for the indicated treatment, post hoc SNK test after factorial ANOVA. *t*, Noise Alone startle for that treatment is significantly different from Noise Alone startle amplitude after Vehicle treatment, post hoc SNK test after factorial ANOVA.

sessions (Sessions 6–20) when compared to Test Days 4 and 5, this difference was not statistically significant (Interaction: $F[2,29] = 1.21$, *ns*). After Test Day 1, the SPS effect persisted throughout the period of drug challenges.

The number of defecations emitted during the 10-min startle test sessions was highest on Test Day 1 and diminished to near-zero levels over the course of 5 days of SPS testing (Day 1, 2.0 ± 0.54; Day 2, 0.75 ± 0.41; Day 3, 0.75 ± 0.35; Day 4, 0.5 ± 0.29; Day 5, 0.17 ± 0.17. There was a significant effect of Test Days for this measure ($F[4,50] = 3.58$, $P < .05$).

ANXIOLYTIC TREATMENT EFFECTS ON THE SPS RESPONSE

Figure 3 depicts the effects of acute treatment with alprazolam or buspirone on the SPS response. As can be seen, treatment with alprazolam (circles) reduced baseline (i.e., Noise Alone; open circles) startle amplitude in a dose-related manner. These same doses of alprazolam also reduced the SPS effect in a dose-related manner. Statistically, the main effect for Alprazolam Dose was significant ($F[3,30] = 2.37$, $P < .05$, one-tailed) and the main effect for Trial Type was significant ($F[1,10] = 13.62$, $P < .05$). The Alprazolam Dose × Trial Type interaction also was significant ($F[3,30] = 3.73$, $P < .05$). Post hoc SNK tests showed that doses of 0.5 and 1.0 mg/kg alprazolam significantly blocked the SPS response.

In contrast to the effects of alprazolam, treatment with buspirone (squares) increased baseline (i.e., Noise Alone; open squares) startle amplitude in a dose-related manner. These same doses of buspirone also reduced the magnitude of the SPS response. Statistically, the main effects for Buspirone Dose ($F[3,30] = 7.81$, $P < .05$) and Trial Type ($F[1,10] = 10.80$, $P < .05$) were significant, although the Buspirone Dose × Trial Type interaction was not ($F[3,30] = 1.31$, *ns*). Post hoc SNK tests showed that the dose of 4 mg/kg buspirone significantly blocked the SPS response.

DISCUSSION

In the present study, the Startle-Potentiated Startle (SPS) effect developed over the course of a single startle test session and, once acquired, was relatively stable over the course of several weeks of startle test sessions. This finding is consistent with earlier reports using with the SPS paradigm [McQueen et al., 2001; Winston et al., 2001]. This SPS paradigm may be a useful procedure for longitudinal repeated measures studies on the conditioned enhancement of acoustic startle. A major purpose of the present study was to determine the effects of the traditional benzodiazepine anxiolytic alprazolam and an atypical anxiolytic buspirone on this SPS effect.

The effects of acute pre-test treatment with alprazolam and buspirone produced opposite effects on baseline (i.e., Noise Alone) startle amplitude, with alprazolam reducing Noise Alone startle amplitude in a dose-related manner and buspirone treatment increasing Noise Alone startle amplitude. These findings are consistent with the effects of these agents on acoustic

startle amplitude in other studies [Davis, 1991; Davis et al., 1993; Kehne et al., 1988; Wichmann et al., 2000].

Treatment with alprazolam or buspirone reduced the magnitude of the SPS response, defined as the difference in startle amplitude on Light+Noise and Noise Alone trials. As mentioned above, both treatments also affected Noise Alone startle amplitude. The increase in Noise Alone startle amplitude per se produced by buspirone treatment likely did not prevent the SPS effect, as other startle-increasing treatments, e.g., amphetamine and apomorphine, have been found to increase Noise Alone startle amplitude and not reduce the SPS effect (Commissaris et al., personal communication). Similarly, the reduction in the SPS effect produced by alprazolam treatment likely was not secondary to a reduction in Noise Alone startle amplitude because the dose of 0.5 mg/kg alprazolam significantly reduced the SPS effect but did not significantly reduce Noise Alone startle amplitude. The ability to reduce the SPS effect likely is not related to floor (alprazolam) or ceiling (buspirone) effects. In this respect, the effects of these agents on the SPS conditioned enhancement of startle resemble the effects of these agents on the FPS paradigm [Davis, 1991; Davis et al., 1993; Kehne et al., 1988; Wichmann et al., 2000].

The present findings do not allow for a resolution of the question of whether the SPS effect is a model for conditioned fear or aversion. The similarity between the effects of alprazolam and buspirone on FPS and SPS responses is consistent with the interpretation that the SPS paradigm is a conditioned fear paradigm, a suggestion proposed initially by Leaton and Cranney [1990]. On the other hand, data showing a clear decline across days in the number of defecations emitted during startle testing, without a similar decline in the magnitude of the SPS effect, are not consistent with the idea that SPS test sessions are stressful or fear-provoking. It is also possible, however, that differences in the SPS and fecal boli measures across repeated startle test sessions reflect differences in the sensitivity of these two behaviors as measures of fear or anxiety. Ultimately, determination of the fear/anxiety/aversion nature of the SPS response will have to await the results of further studies. To this end, studies using the conditioned aversion place preference/aversion task are planned.

In summary, the SPS paradigm seems to be a valid repeated measures, non-shock, non-extinction paradigm for studying the conditioned enhancement of the acoustic startle response. The demonstration that the SPS response is reduced by treatment with the anxiolytics alprazolam and buspirone suggests that this paradigm may represent the effects of conditioned aversion or fear, although this tentative conclusion remains to be empirically tested.

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