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A Novel AX+/BX– Paradigm to Assess Fear Learning and Safety-Signal Processing with Repeated-Measure Designs

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Abstract

One of the core symptoms of anxiety disorders, such as Post-Traumatic Stress Disorder is the failure to overcome feelings of danger despite being in a safe environment. This inability likely stems from an inability to fully process safety signals, which are cues in the environment, that enable healthy individuals to over-ride fear in aversive situations. Studies examining safety signal learning in rodents, humans, and non-human primates currently rely on between-groups designs. Because repeated-measures designs reduce the number of subjects required, and facilitate a broader range of safety signal studies, the current project sought to develop a repeated-measures safety-signal learning paradigm in non-human primates. Twelve healthy rhesus macaques of both sexes received three rounds of auditory fear-potentiated startle training and testing using an AX+/BX– design with all visual cues. Cue AX was paired with an aversive blast of air, whereas the same X cue in compound with another B cue (BX) signaled the absence of an air blast. Hence, cue B served as a safety signal. Once animals consistently discriminated between the aversive (AX+) and safe (BX–) cues, measured by greater startle amplitude in the presence of AX vs. BX, they were tested for conditioned inhibition by eliciting startle in the presence of a novel ambiguous combined cue (AB). Similar to previous AX+/BX– studies, healthy animals rapidly learned to discriminate between the AX+ and BX– cues as well as demonstrate conditioned inhibition in the presence of the combined AB cue (i.e. lower startle amplitude in the presence of AB vs AX). Additionally, animals performed consistently across three rounds of testing using three new cues each time. The results validate this novel method that will serve as a useful tool for better understanding the mechanisms for the regulation of fear and anxiety.

1. Introduction

Humans, monkeys, and rodents have the ability to associate cues in their environment to dangerous/aversive situations using Pavlovian learning. This adaptive ability allows individuals to predict potentially dangerous situations and to respond appropriately. Additionally, these associations can be generalized to other potentially threatening situations. However, after experiencing a particularly traumatic event, some predisposed humans go on to develop anxiety disorders, such as Post-Traumatic Stress Disorder (PTSD) (for review, see Pitman et al., 2012). These individuals are unable to appropriately modulate their fear response, particularly when faced with cues that have previously predicted danger,

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despite the fact that all other cues in their environment indicate a condition of safety (Jovanovic et al., 2012, Pitman et al., 2012). For instance, a PTSD combat veteran may suffer from debilitating panic attacks when viewing a fireworks demonstration, despite the presence of family members. Thus, a clinically relevant research area needs to focus on how these learned fears could be diminished or inhibited. Animal models of fear conditioning and, more importantly, fear inhibition offer useful tools not only to study the neural bases of these learning processes but also to develop pharmacological agents to treat or alleviate these clinical disorders.

In the laboratory, fear-potentiated startle paradigms have become an important tool for studying the potential mechanisms of fear and safety-signal learning (Christianson et al., 2012). Fear-potentiated startle measures increased startle amplitude elicited in the presence vs. the absence of a cue previously paired with an aversive stimulus and is commonly used to assess conditioned fear (Davis, 2006). The paradigm that has yielded the most consistent results across species is a type of conditioned inhibition paradigm termed AX+/BX–, developed in rats (Myers and Davis, 2004), monkeys (Winslow et al., 2008, Kazama et al., 2012) and humans (Jovanovic et al., 2005, 2012). Two cues (A and X) presented together are paired with an aversive event (shock in rats, air blast in monkeys and humans) and two cues (B and X) signal that no aversive event will occur. As training progresses, Cue A develops the highest level of fear because it is a consistent signal for an aversive event, cue B develops the lowest level of fear because it predicts the lack of an aversive event. Cue X is somewhat fearful because it has been associated with cue A and an aversive event but also with the safety cue B and no aversive event. Transfer of fear inhibition by cue B is tested when it is presented together with the aversive cue A (AB test trial) for the first time, leading to lower startle amplitude in the presence of cue AB vs. AX.

Currently, rodent and monkey AX+/BX– paradigms use visual (light), auditory (tone), and tactile (fan) stimuli as cues. However, the use of these stimuli limits the types of studies that can be carried out in a single individual. Once a stimulus has been associated with a fearful situation, that stimulus cannot be re-used for future investigations within the same animal. In addition, changes in the parameters of these cues (amplitude, intensity, duration etc...) are not appropriate because they may lead to generalization. Thus, this kind of AX+/BX– paradigm is not conducive to experiments requiring repeated measures on the same animals, such as within-subject measures across developmental time points, repeated cortical inactivation experiments, and pharmacological drug assessments. To broaden the study of fear regulation, we capitalized on the exquisite visual abilities of monkeys to discriminate a large number of pictures presented via a computer screen, even when the pictures contain overlapping features (Zeamer et al., 2011; Murray et al., 2007). Thus, the traditional light, tone and fan cues were replaced by various picture cues. In this way, novel images can be substituted as novel cues for each additional experiment on the same subjects and the paradigm becomes similar to that developed to study fear conditioning and inhibition in humans (Jovanovic et al., 2012), making it extremely useful for translational studies of fear inhibition.

The overall goals of this study were to test the validity of this novel, strictly visual, AX+/BX– paradigm in non-human primates as a model of fear and safety signal learning. It also sought to determine whether modifying the stimulus set would allow the paradigm to be used in a repeated-measure design without substantial generalization. A preliminary report of these data has been presented in abstract form (Kazama et al., 2011).

2. Materials and methods

2.1. Subjects

Twelve adult rhesus macaques (*Macaca mulatta*) of both sexes (7 females, 5 males), ranging from 6 to 11 years of age participated in this study. All were born at the Yerkes National Primate Research Center Field Station (Lawrenceville, GA), and were reared with their mothers in large social groups. As adults, all animals were transferred to the Yerkes Main Station (Atlanta, GA), and were housed either singly or in pairs in cages allowing visual contacts to other monkeys and humans. Behavioral training/testing began approximately one month after the animals had been acclimated to their new location. Animals were fed monkey chow (Purina Mills LCC, St. Louis) and fresh fruits and vegetables daily, and received water *ad libitum* while in their home cages. Toys were also provided to enrich their environment. Animals weighed between ~6.5–11 kg at the start of the study, and were weighed weekly. All procedures were approved by the Institutional Animal Care and Use Committees (IACUC) of Emory University.

2.2. Apparatus

Subjects were first trained to sit in a non-human primate restraint chair (Crist Instrument Co., Hagerstown, MA) using a combination of successive approximation shaping techniques and pole and collar training with positive reinforcement (food treats). After animals reliably sat in the restraint chair, they were then placed in a primate startle box (Industrial Acoustics Company, New York, NY). The startle box was a ventilated, sound-attenuated steel chamber with a piece of plexi-glass on one wall to allow visual access to a video monitor positioned approximately 40 cm from the animal's face. Visual cues were displayed on the video monitor via a Powerpoint Presentation (Microsoft, Redmond, WA). The chair was secured to a platform containing a load cell that translated startle-induced movement of the animal into an electrical signal. Measurement took place for 0.6 sec beginning immediately with the onset of either the US, as well as 30 sec after the onset of the US for the purposes of measuring the animal's movement between trials. This signal was then amplified, converted to a digital format, and stored on a computer for later analysis (Version 2.1, Med Associates, St. Albans, VT).

2.3. Stimuli

The aversive stimulus (US) was 500-msec jet of compressed air (100 PSI) generated by an air compressor located outside the chamber and projected at the face of the monkey via four air jet nozzles. The startle stimulus was a 50-msec burst of white noise (5-msec rise-decay time) of varying intensities (95 and 105 dB), emitted by speakers (Non-Human Primate Startle Reflex System, Med Associates Inc.; St. Albans, VT) mounted above the animal. The speakers also delivered a constant 65 dB background white noise. Each round of testing used a set of three unique pictures of simple shapes that differed as much as possible from each other and from shapes used across successive rounds to avoid or reduce generalized learning. Thus, for each round of testing, three visual cues served as either an aversive conditioned stimulus (A), a safety conditioned stimulus (B) or a third stimulus (X) as illustrated at the top of each round on Figure 2. These ~10 × 10 cm visual conditioned stimuli (CS) were presented on a black background either alone at the center or in pairs side by side with a gap of ~8 cm displayed on the computer monitor for 10 seconds prior to the onset of the US. The CS assignments as cues A, B or X were pseudo-random and counter-balanced across animals.

2.4. Behavioral procedures

All 12 animals underwent three unique rounds of training separated by a 1-month rest period, and each round consisted of four phases: Habituation, Pre-Training, AX+/BX– Training, and Probe Test. Length of each daily session depended upon the stage of training ranging from approximately 20 min to 1 hour, and inter-session intervals were 72 hrs. Table 1 provides the trial types included in each phase. When two cues were simultaneously presented on the screen, their left/right positions and their locations on the screen remained constant through all phases. Details for the presentation of each trial type are provided on Figure 1.

2.4.1. Habituation phase (CS only)—The purpose of this phase was to acclimate the animals to both the box and the visual stimuli. The animals were first habituated to sit in the box while being exposed to the three visual stimuli selected for each particular round (ex. Round 1 – Blue Circle, Green Star, and Red Square). Animals received two sessions (separated by 72 hours) of 30 trials each during which the to-be-conditioned visual cues (e.g. circle, star, and square) and their combinations (circle/square, star/square, circle/star, etc.) were presented in the absence of the startle noise. No stimuli were presented in the first 5 min of the session, then the stimuli appeared for 10 sec at 30-sec inter-trial intervals (ITI) but were not accompanied by the startle stimuli or air puff. As no acoustic startle noises were delivered, no data were collected during this phase.

2.4.2. Pre-training phase—After 72 hours, startle was elicited by a 95-dB startle noise in the presence of various single or compound cues to assess any unconditioned effects of the cues on the startle response prior to conditioning. Animals were given a minimum of two sessions of 60 trials each, consisting of 30 trials with the startle noise alone (95 dB) and 30 trials in which startle was elicited in the presence of one of the to-be-conditioned cues (A, B and X) or their combinations (AX, BX, AB) for 5 trials each pseudo-randomly ordered. Within each cue-startle trial, the startle stimulus was presented 10 sec after the onset of the CS. These pre-training sessions were repeated for each monkey until presentation of the cue that was assigned to serve as the safety signal (cue B) for that animal produced less than a 100% increase in startle amplitude compared to noise alone presentations, calculated as: $[(\text{startle amplitude with picture}) - (\text{startle amplitude alone}) / \text{startle amplitude alone}] \times 100$. Due to the fact that errant movements by the animal during the window of measurement could potentially skew the results as measured by the mean of the trials, a median average was used to quantify startle amplitudes across individual sessions.

2.4.3. AX+/BX– training phase—The purpose of this phase was to train the animal to associate the cue combination AX with the aversive air blast (+), AX+, and to discriminate this CS/US pairing with that of the safety cue combination BX associated with no air blast (–), BX–. Each session lasted approximately 50 min and consisted of: 10 min of initial habituation in which no stimuli or noises were presented, a 95-dB noise alone trial, a 1-min intertrial interval (ITI), an AX+ cue/air puff pairing, followed by a 1-min ITI. Sessions then proceeded with presentations of pseudo-randomly ordered trial types separated by 1-min ITIs consisting of: two AX+ cue/airpuff pairings; AX cue/startle noise pairings (6 trials at 95 dB and 6 at 105 dB); BX cue/startle noise pairings (6 trials at 95 dB and 6 at 105 dB); and startle noise alone (6 trials at 95 dB and 6 at 105 dB). Each session ended with a final AX+/air puff pairing (making 4 AX+/air puff presentations total per session). Fear potentiated startle was computed as: $[(\text{startle amplitude with picture}) - (\text{startle amplitude alone}) / \text{startle amplitude alone}] \times 100$.

Animals received the AX+/BX- Training for a minimum of two sessions, or until there was a difference of 100% fear-potentiated startle between the two compound cues (% fear-potentiated startle to AX minus fear-potentiated startle BX = 100 or more).

2.4.4. AB testing/transfer test—Animals were tested for conditioned inhibition (i.e. transfer) in a single session within 72 hrs after the last AX+/BX- training session to examine the potential inhibitory effects of B on A. This 48-trial probe session consisted of all trial types, including two A+ air-blast pairings intermixed within (a) 95-dB and 105-dB Noise Alone trials (6 trials each), (b) 95-dB and 105-dB cue pairings (A, B, AX, BX, 5 trials each per noise intensity), and (c) 95-dB and 105-dB compound cue (AB, 5 trials per noise intensity). All trials were presented pseudo-randomly. When trained in this way transfer of fear on the AB test trial could not be accounted for by configural learning (Myers and Davis, 2004).

2.5. Training in successive rounds

After testing of the first round was completed, the animals were left on rest for one month before the next round started. Testing in the second and third rounds was given in exactly the same way, but the visual shapes (A, B, X) were changed to novel shapes (See Fig. 2, top).

2.6. Data analysis

Throughout the entire paradigm, startle amplitudes were recorded via the Med Associates software and amplified via the load cell. To verify that the raw acoustic startle amplitudes were consistent across the three rounds of testing, acoustic startle (millivolts) on noise alone trials was compared to that of trials where the startle noise was preceded by the visual cues, across the three rounds of probe tests using a three way ANOVA with Decibel (2) X Trial type (2) X Round (3) as repeated measures. Next, to assess whether animals' ability to discriminate between the aversive and safety cues remained constant across rounds, the number of sessions required to reach criterion across each of the three rounds was compared using a one-way ANOVA with repeated measures. Finally, we assessed whether animals could use the safety signal to modulate their fear response in the presence of the aversive cue (conditioned inhibition) during the single probe session they received for each round.

Because previous reports (Winslow et al., 2007; Kazama et al., 2012) examining acoustic startle in monkeys have revealed clear individual differences in optimal startles at the 95 dB or 105 dB, the decibel level for which each animal showed the greatest discrimination between the AX and BX (e.g. greatest difference between startle at AX versus BX) was first determined. Startle values obtained at this specific decibel level for each trial type during the probe session were used for the statistical analyses. In addition, because percent fear potentiated startle values are not normally distributed (see Table 2) the data were transformed using a logarithmic base 10 transformation (Winslow et al., 2008). Comparisons of startle amplitudes between cues AX, BX, and AB for each round were analyzed using repeated measures ANOVAs (Trial Type X Round). Planned comparison tests were used to analyze startle values between Trial type within each separate round.

Given that a previous study in rodents had revealed an effect of estrogen on safety-signal learning, it was important to test for potential sex differences (Toufexis et al., 2007). Thus, ANOVA statistics were used to examine potential sex differences across the five males and seven females on the previously mentioned behavioral metrics. No significant sex differences were detected, and therefore data for both males and females were pooled for the following analyses.

3. Results

3.1 Fear/safety signal discrimination learning (AX+/BX– training)

The purpose of these analyses was to determine whether animals showed good discrimination between the AX and BX cues, and whether or not training in successive rounds would have resulted in generalized learning, thus making each new set of stimuli more difficult to learn. Overall, the average number of sessions each animal took to reach criterion across all three rounds of testing was 5.3 (SEM: 0.59; see Figure 2). Repeated-measures ANOVA comparing number of sessions across the three rounds revealed no significant differences in the number of sessions animals took to reach discrimination criterion [$F(2,22) = .761, p > .05$].

Based on data from the probe session, a repeated-measure ANOVA (Decibels X Rounds) revealed that baseline startle was consistent across the three rounds [$F(2,22) = .688, p > .05$] as well as across the two decibel levels [$F(1,11) = .878, p > .05$]; and no significant interaction was found between Round and Decibel level [$F(2, 22) = .072, p > .05$].

To analyze whether the acoustic startle elicited in the presence of cues AX and BX remained constant across Decibels and Rounds, a repeated-measures ANOVA with 3 factors (Cues X Decibels X Rounds) was used. There was no significant main effect of Decibel level [$F(2, 11) = .034, p > .05$], or Rounds [$F(2, 22) = 1.21, p > .05$], but acoustic startle amplitude was greater in the presence of the aversive AX cue as compared to the BX cue [$F(1, 11) = 13.576, p < .005$]. The interaction between the three factors did not reach significance [$F(2, 22) = 1.05, p > .05$], indicating that acoustic startle for cues AX and BX remained stable across rounds of testing and was a good indicator of cue discrimination.

3.2 Modulation of fear in the presence of the safety signal (probe test)

Finally, to determine whether or not the animals modulated their fear when the aversive cue was presented together with the safety cue (AB), startle amplitude elicited in the presence of cues AX, BX, and AB in the probe test across the three rounds (Figure 3) was analyzed using repeated-measures ANOVA (Stimulus type X Rounds). There was no effect of Rounds [$F(2,22) = .93, p > .05$] but a significant effect of Stimulus type [$F(2,22) = 34.79, p < .001$]. Post-Hoc Analyses revealed that fear potentiated startle in the presence of the AX cue (aversive) was significantly greater than in presence of either the BX (safety) [$F(1,11) = 27.03, p < .001$] or AB (combined) cues [$F(1,11) = 96.27, p < .001$] across all three rounds. Although the interactions did not reach significance [$F(4,44) = .791, p > .05$], planned comparisons between the three stimulus types within each round (e.g. AX cue (aversive) versus BX cue (safety) or AB cue (aversive/safety) were performed. For the first and second rounds, fear-potentiated startle was greater for the AX cue vs. both the BX and AB cues ($ps < .03$). For the third round, there was a significant difference between the AX cue and the AB cue ($p < .006$), but the difference between the AX and BX cues fell just short of significance ($p = .086$).

4. Discussion

This report describes a novel AX+/BX– paradigm to allow repeated-designs study of fear conditioning and conditioned inhibition in monkeys. This paradigm used highly discriminable visual stimuli presented on a computer monitor that could serve as aversive, safety or neutral cues, and could be changed for each round of testing. The data revealed consistent baseline acoustic startle across three rounds of testing, as well as a steady rate of Pavlovian conditioning to the aversive and safety cues. Finally, conditioned inhibition in the presence of the combined aversive/safety signal (AB cue) was consistent and significant across three rounds of testing. Taken together, this novel paradigm provides an effective

repeated-measure method for examining the flexible modulation of fear in non-human primates, and for assessing the efficacy of potential drug therapy for anxiety disorders in animal models as well as in the clinical setting.

4.1 Baseline acoustic startle

The raw baseline acoustic startle remained steady across multiple rounds of testing. This finding is consistent with previous acoustic startle studies in both non-human (Winslow et al., 2007) and human primates (Swarzkopf et al., 1993; Cadenhead et al., 1999). As described by Davis and colleagues (Davis et al., 1982; Lee et al., 1996), the basic acoustic startle circuit is fairly straightforward beginning with inputs to cochlear root neurons in the cochlear nerve, proceeding down to the nucleus reticularis pontis caudalis, and on to spinal motor neurons. Subsequent studies have shown that this basic acoustic startle can be modulated by other structures including the amygdala, hippocampus, and bed nucleus of the stria terminalis in the presence of an emotion-eliciting stimulus (cf. Davis, 2006). As discussed below, cues that have been conditioned with aversive stimuli result in heightened startle in the presence of that cue, despite a relatively stable baseline acoustic startle.

4.2 Pavlovian conditioning

The current paradigm using only visual cues differs from previous AX+/BX− studies that used visual, auditory, and tactile cues (Winslow et al., 2008, Kazama et al., 2012) not only in the type of cues selected (all visual) but also in the stages of training given. Thus, the training was reduced from three stages (A+ Training, A+/B− Training, AX+/BX− Training) to a single AX+/BX− stage.

The selection of visual cues seems to be a critical factor to avoid generalization, a phenomenon known to occur when features of several conditioned stimuli overlap and result in a blurring of discrimination among similar stimuli (c.f. Pearce, 1987). Indeed, a pilot study that was carried out prior to this one indicated that the use of complex visual stimuli (intricate shapes and multiple colors that could overlap between stimuli) resulted in poor discriminability between the conditioning cues (Kazama et al., 2011 SFN abstract). The use of simpler cues (e.g. star, square, and circle of differing colors), however, provided strong learning such that the number of sessions needed to reach a 100% difference in fear-potentiated startle in the presence of the aversive and fear cue remained consistent across all three rounds. Additionally, results from the probe test given at the end of each round revealed consistent discrimination between the aversive (AX+) and safety (BX−) cues, demonstrating that the safety signal (B) inhibited fear to the danger cue (A) on its very first presentation. Nevertheless, it is still possible that the use of these simple cues might limit the number of rounds an animal can learn before features between stimuli begins to overlap and produces generalization, although this remains to be tested.

The reduction of the number of training stages did not alter the performance on fear conditioning or conditioned inhibition. To the contrary, it is possible that the shortened training actually resulted in stronger conditioned inhibition. This is because prior A+ alone and A+/B− training would be expected to lead to high levels of fear to A. Now, when A is presented in compound with X, followed by the aversive air blast, it would block the development of associative strength to X (Kamin, 1968). Because inhibitory learning to B, presented with X followed by no aversive airblast, is directly related to the excitatory strength of X (Wagner, and Rescorla, 1972), this would lead to less inhibitory learning to B. In contrast, with only AX+/BX− training, X would be expected to become more excitatory and hence B more inhibitory.

4.3 Conditioned inhibition

The ability to modulate anxiety to the aversive cue in the presence of the safety signal (conditioned inhibition) was also fairly consistent across the three rounds of testing. One puzzle that remains is why the startle to the AB cue is on average slightly, but not significantly, less than the startle in the presence of the safety cue (BX). Although this has been mostly ruled out in both rodent (Toufexis et al., 2007) and human (Jovanovic et al., 2005) studies, one possibility that cannot fully be dismissed is that the decreased fear-potentiated startle in the presence of the ambiguous AB test trial may be due, at least partially, to external inhibition. External inhibition can occur when a novel configuration of cues results in a reduction of the conditioned response to the cue (Pavlov, 1927).

4.4 Conclusions

The current paradigm should provide a method to study safety signal learning for experimental designs that include several manipulations on the same animals. For example, it could be used in non-human primate studies using temporary deactivation of selective brain structures to help identify the neural circuitry associated with various aspects of safety-signal processing in the same subjects. It could be used to assess the effects of pharmacological treatments to treat impaired safety signal learning clinically. This non-human primate version of the paradigm can easily be adapted for human safety-signal testing and thus provide an excellent translational tool for the testing of drug therapies. Finally, it could be given to animals at different time points throughout development and provide important findings on the emergence of safety-signal processing and on the ontogenetic origin of certain anxiety disorders in humans, including PTSD.

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Highlights

1. Very few methods exist for examining conditioned inhibition in non-human primates.
2. A repeated measures version of the AX+/BX- Paradigm was developed and validated.
3. The novel AX+/BX- paradigm is a valuable tool for studying fear regulation.

Figure 1.

Example trial types from Round 1

A. Startle noise alone. B. Conditioned aversive cue with air puff. C. Conditioned aversive cue with startle noise. D. Conditioned safety cue with startle noise and E. Probe transfer cue with startle noise.

Figure 2.**AX+/BX- Discrimination Learning Across Rounds**

Scores are mean ($\pm$ SEM) number of daily sessions needed to reach a criterion of 100% Fear-Potentiated Startle discrimination in the presence of the AX+ (aversive) cue versus the BX- (safety) cue for Round 1 to Round 3. The colored stimuli presented in each round are depicted in grayscale above each round.

Figure 3.

Conditioned Inhibition Probe Across Rounds

Scores are log-transformed mean ($\pm$ SEM) percent fear-potentiated startle for the optimal decibel level per animal for each cue. Startle amplitudes elicited in the presence of the aversive cues (AX+) were significantly higher than in the presence of the transfer cue (AB) in all Rounds ($p < .05$). The colored stimuli presented in each round are depicted in grayscale above each round.

Table 1**AX+/BX– Task Description**

<i>Training Stages per Round</i>	<i>Stimuli</i>	<i>Startle Noise (dB)</i>
Pretraining without startle	All Cues (A, B, X, AX, BX, AB)	None
Pretraining with startle	All Cues, NA	95
AX+/BX– Training	AX/Airpuff, AX/Noise, BX/Noise, NA	95, 105
Transfer Test	A/Noise, B/Noise, AX/Airpuff, AX/Noise, BX/Noise, AB/Noise, NA	95, 105
One Month Rest		

Chronological list of training stages as they occurred during training (top to bottom). For each stage, the types of visual cues (A, B, X) presented, and the Startle Noise (decibel of the 0.05 sec startle noise) used are given.

Table 2

AX+/BX− Criterion Performance (%FPS)

Case	Round 1		Round 2		Round 3	
	AX	BX	AX	BX	AX	BX
C-1	226	78	658	160	378	226
C-2	2088	908	2638	2216	1965	213
C-3	77	−53	199	88	221	69
C-4	570	206	248	−28	117	−7
C-5	1376	366	674	294	1118	771
C-6	1465	903	312	189	647	414
C-7	601	164	1106	743	829	179
C-8	589	328	218	105	671	184
C-9	167	36	176	26	222	99
C-10	1432	497	495	110	263	14
C-11	249	119	423	197	90	−28
C-12	205	94	295	100	479	53
<i>Avg.</i>	<i>753.75</i>	<i>303.83</i>	<i>620.17</i>	<i>350.00</i>	<i>593.33</i>	<i>182.25</i>
<i>S.E.M.</i>	<i>191.77</i>	<i>92.24</i>	<i>199.28</i>	<i>178.83</i>	<i>154.36</i>	<i>64.43</i>

Percent fear-potentiated startle (%FPS) during the session just prior to the probe test for the aversive AX cue and the safe BX cue. Avg. and S.E.M. are the mean and standard error of the mean across animals per condition, respectively.