Vulnerability factors in anxiety: Strain and sex differences in the use of signals associated with non-threat during the acquisition and extinction of active-avoidance behavior

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ABSTRACT

Rats that exhibit a behaviorally inhibited temperament acquire active-avoidance behaviors quicker, and extinguish them slower, than normal outbred rats. Here we explored the contribution of stimuli that signal periods of non-threat (i.e. safety signals) in the process of acquiring active-avoidance behavior. Utilizing a discrete lever-press escape–avoidance protocol, outbred Sprague–Dawley (SD) rats and inbred, behaviorally inhibited, Wistar–Kyoto (WKY) rats were tested under conditions where a flashing light was either presented or not during periods of non-threat (the inter-trial interval, ITI). For males, we found the absence of the ITI-signal slowed the acquisition of avoidance behavior selectively in WKY rats. However, extinction of the avoidance behavior was not influenced by training with or without the ITI-signal; WKY males extinguished slower than SD males. For females, the presence of the ITI-signal did not affect acquisition in either strain. However, after training with the ITI-signal, females of both strains extinguished quicker in its absence than in its presence. In order to determine if facilitated acquisition of avoidance learning in male WKY rats was due to a paradigm-independent influence of the visual stimulus used as ITI-signal upon associative learning, we conducted eyeblink conditioning in the presence or absence of a similar visual stimulus. No differences in acquisition, as a function of this visual stimulus, were observed within the male WKY rats, but, as was observed in avoidance learning, male WKY rats extinguished slower than male SD rats. Thus, avoidance susceptibility for male WKY rats may be tied both to the presence of non-threat signals as well as a resistance to extinguish Pavlovian-conditioned associations. Female susceptibility to resist extinguishing avoidant behavior is discussed with respect to the possible role of stimuli serving as occasion setters for threat contexts.

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1. Introduction

Anxiety disorders are quite prevalent in western society, but prevalence is not equally distributed across the population. For example, a behaviorally inhibited temperament is associated with a higher prevalence of anxiety disorders (Biederman et al., 2001; Hirshfeld et al., 1992; Hirshfeld-Becker et al., 2007; Rosenbaum et al., 1991a, 1991b, 1993) as is female sex (Breslau et al., 1998; Kessler et al., 1994, 1995; Wittchen et al., 1994, 1999). Despite identification of

vulnerable populations, the mechanisms underlying these vulnerability factors are only beginning to be identified. Further, as we identify the biological sources of anxiety vulnerability, we will be able to prospectively determine whether vulnerability is a function of exceeding a singular threshold or if multiple factors can have an additive effect in increasing anxiety vulnerability.

Our lab has focused efforts upon determining how two vulnerability factors, behaviorally inhibited temperament and female sex, may influence the rate by which avoidant behavior is adopted and subsequently extinguished, since avoidance is a symptom represented in all anxiety disorders (American Psychiatric Association, 2000). The animal used to model behavioral inhibition, the inbred Wistar–Kyoto (WKY) rat, acquires active avoidance behaviors quicker than outbred Sprague Dawley (SD) rats and is slower to extinguish the response once acquired (Beck et al., 2010; Jiao et al., 2011; Servatius et al., 2008). This behavior is somewhat paradoxical and unexpected when one considers that, in many other situations, the predominant response emitted by WKY rats is to become immobile or retract

from novel stimuli (Baum et al., 2006; Ferguson and Cada, 2004; Malkesman et al., 2005; Pare, 2000), which is the behavioral inhibition phenotype.

The other factor, female sex, is associated with fast acquisition of active avoidance behavior in SD rats; however, it has not yet been shown in the generally quicker WKY rats, most likely because of a ceiling effect (Beck et al., 2010). Still female SD rats are similar to WKY rats in their non-reinforced responses. Despite not showing quite the same level of asymptotic performance as WKY rats, female SD rats exhibit a similar pattern of non-reinforced responses (as WKY rats) when the number of responses emitted during the inter-trial-interval (ITI) are examined. Female SD and both sexes of WKY rats exhibit greater responding during the ITI following successful escape responses than following avoidance responses; a pattern not observed in male SD rats (Beck et al., 2010). This suggests that one common feature in the susceptibility to acquire active avoidant behaviors may lie in the behavioral response patterns during the ITI.

Unlike many other learning procedures conducted in the laboratory, the perception of the ITIs may be particularly critical to the acquisition of avoidance behaviors in animals. When others have introduced explicit stimuli during the ITI, a period of non-threat, avoidance learning is facilitated (Dillow et al., 1972). As subjects acquire the requisite avoidance responses, the onset of these non-threat-associated cues occurs more quickly following the emitting of the behavior (Berger and Brush, 1975; Berger and Starzec, 1988; Berger et al., 1981; Brennan et al., 2003; Dillow et al., 1972). Because they facilitate avoidance learning, these ITI-signals have been commonly termed "safety signals" (Berger and Brush, 1975; Berger and Starzec, 1988; Best, 1975; Brennan et al., 2003, 2004; Dillow et al., 1972; Servatius et al., 2008). Our previous research utilized a discrete visual signal throughout the entire 3 min ITI in order to ensure acquisition would occur in SD rats (Beck et al., 2010; Servatius et al., 2008), while also minimizing the number of responses emitted during the ITI (Brennan et al., 2003); yet, we do not know if this signal is *required* for either strain to acquire the response. Distinguishing the contribution non-threat stimuli may have in modulating the acquisition of avoidance behavior is potentially very important for understanding anxiety vulnerability. If avoidance susceptibility can be traced to acquiring additional knowledge about periods of perceived safety, then, solely targeting warning signal-threat association may not reliably aid in helping those people with trait anxiety vulnerabilities — as is the case for exposure therapies, highly regarded as a treatment for post-traumatic stress disorder (Ballenger et al., 2000). Therefore, the potential contribution of these additional non-threatening stimuli to avoidance behavior susceptibility needs to be tested in the vulnerability models under current study: behavioral inhibition and female sex.

Thus, the main focus of this study is to determine if the ITI-signal has a greater effect upon the animals more susceptible to acquire active avoidance behavior (the WKY strain and/or female sex). As stated above, strain and sex differences in the acquisition of lever-press avoidant behavior, coupled with the differences in non-reinforced responding during the ITI, led us to question whether the ITI-signal was contributing differently to the avoidance acquisition process across these 2 vulnerability factors. Therefore, we conducted avoidance learning in the presence or absence of a flashing light presented during each ITI during acquisition. Presumably, those animals less susceptible to avoidance learning may show less difference in acquisition across ITI-signal presence and absence conditions.

Acquisition of avoidance responding was assessed both between and within-sessions. Both analyses were conducted in order to assess general acquisition of avoidance responding, as well as to ascertain whether strain-dependent difference in avoidance learning warm-up is linked to the stimuli utilized in the paradigm. Warm-up refers to the process by which an animal exhibits poorer performance at the beginning of a session when compared to performance levels at the end of the previous session (Hoffman et al., 1961); in short, the

behavior appears to require some re-acquisition of the association in the beginning trials of the subsequent training session. Commonly observed in avoidance learning, this characteristic learning pattern is not well expressed in WKY rats, as it is in SD rats (Beck et al., 2010; Servatius et al., 2008). However, we do not know if warm-up decrements previously observed in WKY rats during avoidance learning are in any way tied to the signals used during the sessions. Therefore, a within-session trial analysis was conducted for that purpose.

A counter-argument to a safety-signal role for the ITI-signal is that the stimulus increases arousal during the non-threat periods, thereby, maintaining arousal consistently throughout the training session. Based on this theory, we introduced the same flashing light stimulus into another learning paradigm — eyeblink conditioning. Again, we hypothesized that those sensitive to the presence/absence of the flashing light could show a stimulus-dependent facilitation of simple reflexive learning, if their sensitivity to the stimulus causes a paradigm-independent increase in arousal.

Finally, we ended both avoidance learning and classical conditioning training with several sessions (avoidance) or blocks (eyeblink conditioning) of extinction training. In both cases, the unconditional stimulus (US) was removed, footshock in avoidance and eyelid stimulation in eyeblink conditioning. Extinction was assessed in both paradigms for two reasons. First, it is not clear if the presence of the ITI-signal during extinction causes slower extinction in WKY rats, although previously data suggest it may maintain some level of responding in the absence of the US (Servatius et al., 2008). Second, WKY rats are slower to extinguish lever-press avoidance behavior (Beck et al., 2010; Jiao et al., 2011; Servatius et al., 2008), which could be due to strain differences in how Pavlovian associations are retained (i.e. the predictive component to avoidance learning). Therefore, an assessment of extinction in eyeblink conditioning provides important information regarding WKY rats' general propensity to retain Pavlovian associations more strongly than SD rats. Given that WKY rats are resistant to proactive interference effects in eyeblink conditioning (Ricart et al., 2011a), one could hypothesize that they should extinguish quicker than SD rats. Moreover, if the flashing light is encoded as a contextual cue, removal of the light for extinction blocks in eyeblink condition or avoidance learning could cause more rapid extinction of the response (compared to those trained without the additional visual stimulus). Conversely, if WKY rats acquire and retain fully paired Pavlovian associations more strongly than SD rats regardless of any external cues, WKY rats may be slower to extinguish the emission of conditioned eyeblink responses as well. Slower extinction of Pavlovian conditioned responses in WKY rats would further model behavior observed in trait-anxious humans (Sehlmeyer et al., 2011).

2. Methods

2.1. Animals

All rats (8–10 weeks old) were obtained from both Charles River Labs (Kingston, NY) and Harlan Sprague Dawley (Indianapolis, IN). Each strain was obtained from both vendors. Rats were housed individually in polycarbonate tubs (12:12 light cycle, lights on 0700). Upon arrival, all rats had at least 2 weeks to acclimate to their living conditions prior to the start of testing or surgery. Rats had free access to water and food in their home cages. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) in accordance with AAALAC standards. For Experiment 1, 24 SD and 23 WKY male rats were trained in active avoidance behavior. For Experiment 2, 16 SD and 16 WKY female rats were trained in active avoidance behavior. For Experiment 3, 19 SD and 19 WKY male rats were trained to emit classically conditioned eyeblinks.

2.2. Apparatus

The operant chambers used for lever-press escape–avoidance training are described in detail elsewhere (Beck et al., 2010; Servatius et al., 2008). Briefly, Coulbourn Instruments operant chambers were used that had a grid floor for delivering 1 mA shocks (0.5 s in duration every 3 s) and were fitted with a white cue light (80 lx) 10 cm directly above a lever that was 10.5 cm above the floor. A speaker 26 cm above the floor delivered the 1 kHz tone (75 dB) that was used to signal the oncoming shock (warning signal). The opposing wall of the chamber had a mounted house-light that provided a baseline luminance of approximately 40–50 lx (depending on the exact location in the chamber).

For eyeblink conditioning, the rats were placed in a sound-attenuating testing chamber (Med Assoc., St. Albans, VT) and EMG recording electrodes are connected to a differential AC amplifier with a 300–500 Hz band-pass filter (A-M Systems model 1700, Everett, Washington) and were amplified by a factor of 10 K. A computer equipped with an A/D board (E-serves multifunction I/O card, National Instruments) acquired the EMG signals. The timing of the stimulus presentation and EMG data collection were controlled using a program written in LabView (National Instruments). The conditional stimulus (CS) was a shaped 82 dBA generated white noise stimulus (500 ms duration) with a 10 ms rise/fall (Coulbourne Instruments, Allentown, Pennsylvania). A Bioelectric Stimulus Isolator (Coulbourne Instruments, Allentown, Pennsylvania) produced the unconditional stimulus (US). The US was a 10-ms square-wave stimulus of 10 V, which is sufficient to elicit an unconditional eyeblink (Servatius, 2000). For half of the subjects, a strobe light was attached to the window of the test chamber. Opaque plastic shields were stacked in-between the glass and the bulb until the light measured 80 lx on the inside of the window (ambient light was 30–40 lx depending on specific location). The control conditions had opaque covers of the same size in the window without a strobe light behind them in order to account for differences in ambient light due to the positioning of the lights in the experimental condition.

2.3. Procedure

Escape–avoidance sessions occurred 3 days per week (with a minimum 1 day in-between each session). Each session involved 20 trials. A trial consisted of a 60 s warning signal tone that was followed by 0.5 s intermittent footshocks every 3 s. This occurred over 5 min if a lever-press did not occur. A lever-press during the 60 s prior to shock presentation was labeled an "avoidance" response. Any lever-press during the subsequent 5 min was labeled an "escape" response. Following any of these responses (or the culmination of 5 min), a 3 min ITI occurred. For those subjects that were provided the flashing light during the ITI, the light flashed at a rate of 5 Hz. A rat that failed to emit a lever press response by the end of the fourth training session was removed from the study. After 10 acquisition sessions, the shock was removed from the testing situation in order to assess the extinction of the acquired lever-press response. For those subjects that were exposed to the flashing light during the ITI, the light no longer flashed during the ITI period in the extinction sessions. Hence, all groups were equivalent in the stimulus presentations during the extinction sessions.

Eyeblink conditioning occurred over 1 week. Following an initial 30 min acclimation session to the chamber and EMG signal-check (Day 1), three consecutive days of 100 CS–US pairings occurred. For this experiment, a delay eyeblink conditioning protocol was used where the 500 ms CS coterminated with the 10 ms US (eliciting an unconditional blink). Each of the paired trials was separated by a pseudorandom ISI, ranging in duration from 20 to 40 s. On the last day, 40 CS–US paired trials were followed by 60 CS-alone trials (extinction). Thus, all subjects experienced 340 CS–US paired trials prior to 60 extinction trials.

2.4. Data analysis

For escape/avoidance training, several measures were obtained using Graphic State computer interface with the behavior testing chambers (Coulbourn Instruments, Langhorn, PA). Using S-plus (TIBCO Software Inc, Palo Alto, CA), the session data was tabulated on a trial basis for whether an escape response was emitted, latency of an escape response, whether an avoidance response was emitted, latency of an avoidance response, and number of lever presses for each of the 3 min of the ITI periods (crossed indexed with whether they followed an escape or avoidance response during the acquisition phase).

The eyeblink conditioning EMG signals were filtered and analyzed for emissions of CRs and URs. Activity during the CS that exceeded 6 times the standard deviation of the rectified baseline period (the absolute value of the signal) and prior to the US onset of each trial was recorded as a CR. The baseline period was the 200 ms immediately prior to CS onset. URs were assessed on the first trial for each of the sessions, and the method for assessing and quantifying the UR has been described elsewhere (Servatius, 2000). Briefly, the UR was quantified by measuring the maximum amplitude of the rectified EMG signal starting 20 ms after US cessation following filtering of the raw EMG signal using the loess function in S-plus statistical software (TIBCO, Somerville, MA). Four rats were excluded due to poor EMG signals.

Statistical analyses of the compiled data were conducted using NCSS 2000 (Number Cruncher Statistical Systems, Kaysville, Utah) for each of the 3 experiments. For avoidance learning, each experiment included a single sex; therefore, Sex was not in the statistical models per se. Latency measures and the percentage of trials an avoidance response was emitted during the acquisition phase were analyzed via a 2 (Strain) × 2 (ITI-signal) × 10 (Sessions) repeated measures ANOVA. For trials which ended without a lever press, the maximum value of 396 s was substituted for the response latency. During the acquisition phase, ITI responses were analyzed via a 2 (Strain) × 2 (ITI-signal) × 2 (Reinforced Response) × 3 (ITI-Minute) repeated measures ANOVA. During the extinction phase, response latency was analyzed via a 2 (Strain) × 2 (ITI-signal) × 6 (Sessions) repeated measures ANOVA, and the number of ITI-responses were analyzed via a 2 (Strain) × 2 (ITI-signal) × 6 (Sessions) × (ITI-Minute) repeated measures ANOVA.

For Experiment 3 (classical conditioning), there were 2 primary measures of analysis: percentage of CRs emitted and UR amplitude. For the CR measure, daily trials were segregated into blocks of 20 trials. For the initial 3 days of conditioning, a 2 (Strain) × 2 (Light) × 3 (Day) × 5 (Block) repeated measures ANOVA was utilized to detect differences in acquisition. A separate 2 (Strain) × 2 (Light) × 5 (Block) repeated measures ANOVA was utilized to assess differences on Day 4, given the mixture of CS–US paired and CS-alone trials. UR amplitude was assessed using trial 1 on each of the 4 training days. This was accomplished using a 2 (Strain) × 2 (Light) × 4 (Day) repeated measures ANOVA. Differences across Day would be suggestive that the UR changed as a function of associative or non-associative learning (e.g. long-term habituation). Three SD rats (1 in the flashing-light condition) and 1 WKY rat (also in the flashing light condition) were removed from the study because of poor signal or insufficient muscle stimulation to elicit reliable blink responses.

A criterion for significant differences in the ANOVA main effects and interactions was set at $p < 0.05$. The same level was used for the post-hoc tests (Fishers Least-Squared Difference). All data are expressed as means ± the standard error of the mean.

3. Results

3.1. Experiment 1 — strain differences in ITI-signal influence on male rats

3.1.1. Acquisition

All groups showed a reduction in response latency as they progressed through the 10 sessions of escape–avoidance training

(see Fig. 1, top). This impression was confirmed by a significant main effect of Session, $F(9, 350) = 12.4$, $p < 0.001$. In addition, main effects of Strain, $F(1, 43) = 4.3$, $p < 0.05$ and ITI-signal, $F(1, 43) = 5.1$, $p < 0.05$, suggest the presence/absence of the ITI-signal effected the 2 strains similarly, which themselves exhibit a difference in overall response latency. In general, the presence of the ITI-signal facilitated acquisition, as indexed by response latency, but, the response latencies were clearly much more reduced in the WKY strain when the ITI-signal was present during training.

With regards to the specific acquisition of the avoidant behavior, all groups increased the percentage of avoidance responses emitted over the 10 acquisition sessions, as evidenced by a main effect of Session, $F(9, 387) = 47.7$, $p < 0.001$. In addition, the presence of an ITI-signal had a significant effect on the acquisition of the avoidance response. Both a main effect of ITI-signal, $F(1, 43) = 9.7$, $p < 0.005$, and a Strain × ITI-signal interaction, $F(1, 43) = 5.0$, $p < 0.05$, were significant.
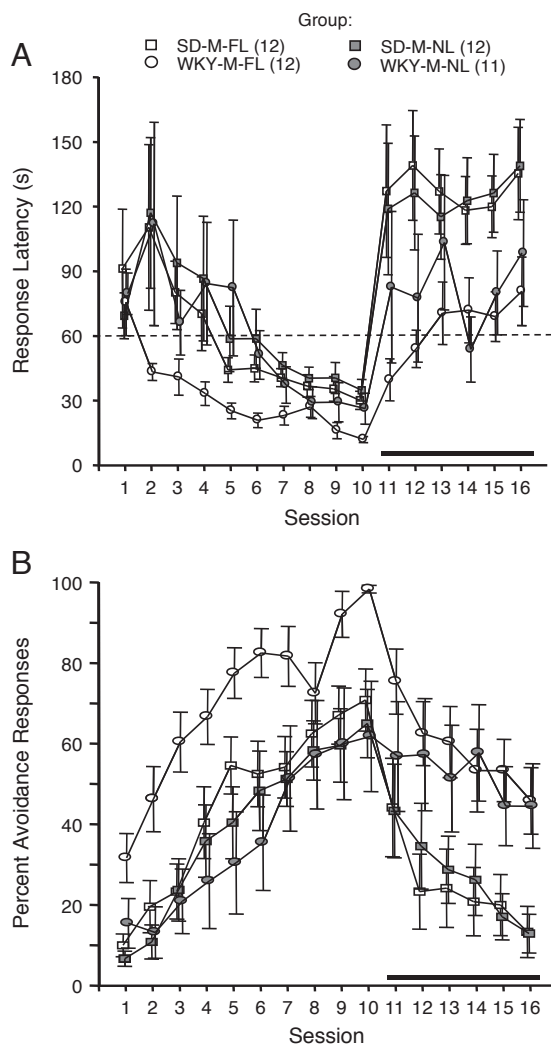

Fig. 1. Panel A shows a reduction in lever-press response latency across 10 sessions of acquisition. Male WKY rats that were exposed to an explicit ITI-signal exhibited the quickest reduction in response latency. Six subsequent extinction sessions (with removal of shock and ITI-signal) are denoted by the black line above the x-axis. Increases in response latency occurred more rapidly in male SD rats over these trials. Panel B illustrates the specific proportion of avoidance responses emitted as a function of acquisition sessions and extinction sessions (same designations as Panel A). Male WKY rats exhibited quicker acquisition of lever-press avoidance when trained with an ITI-signal. However, during extinction, WKY rats showed more avoidance responses than SD rats, regardless of whether they had been trained with or without and ITI-signal. See text for statistical details.

As shown in Fig. 1 (bottom), WKY rats trained in the presence of the ITI-signal exhibited significantly more avoidance responses over the 10 sessions of acquisition.

Within session analyses were separately conducted on the first 5 sessions of acquisition and the last 5 sessions of acquisition. For the first half of acquisition sessions, all rats exhibited a general increase in avoidance responding, which is confirmed by a main effect of Trial, $F(19, 817) = 6.4$, $p < 0.001$. As shown in Fig. 2A, within session acquisition was more rapid in WKY rats trained with the ITI-signal, with a main effect of Strain $F(1, 43) = 4.4$, $p < 0.05$ and a Strain × Trial interaction, $F(19, 817) = 2.0$, $p < 0.005$. However, it also appears that SD rats trained with the flashing light also acquired the avoidance behavior somewhat quicker. These impressions were confirmed by a significant main effect of ITI-signal, $F(1, 43) = 12.4$, $p < 0.001$, and a significant ITI-signal × Trial interaction, $F(19, 817) = 1.7$, $p < 0.05$, but the influence of the ITI-signal was substantially more upon the WKY strain, which was confirmed by a significant Strain × ITI-signal interaction, $F(1, 43) = 6.2$, $p < 0.01$. In the second half of acquisition (Fig. 2B), there continued to be differences between the WKY-ITI-signal trained group and the others. This difference was reflected in a significant main effect of ITI-signal, $F(1, 43) = 5.3$, $p < 0.05$, and a significant Strain × Trial interaction, $F(19, 817) = 4.1$, $p < 0.001$.

As shown in Fig. 3, non-reinforced responses were analyzed based on whether they followed an escape or avoidance response during acquisition. ITI responses showed a time-dependent reduction over the 3 min of the ITI that began with an initial difference in responding during the first minute due to the form of the prior reinforced response. These impressions were confirmed by a significant main effect of ITI-minute, $F(2, 86) = 597.9$, $p < 0.001$ and a significant ITI-minute × Reinforced Response interaction, $F(2, 86) = 12.1$, $p < 0.001$. Further, those rats that were exposed to a flashing light during the ITI exhibited more non-reinforced lever-press responding during the first minute of the ITI than those that were not exposed to a flashing light during the ITI, as evidenced by a significant ITI-signal × Reinforced Response interaction, $F(1, 43) = 6.1$, $p < 0.05$. This pattern occurred as WKY rats generally emitted more responses during the ITI than SD rats. This impression was confirmed by a main effect of Strain, $F(1, 43) = 9.4$, $p < 0.005$ and a Strain × ITI-signal × Reinforce Response × ITI-minute interaction, $F(2, 86) = 3.1$, $p < 0.05$. In short, under conditions where a flashing light is presented during the ITIs, WKY rats exhibit more non-reinforced responses than SD rats, most notably during the first 2 min. The absence of a flashing light during the ITI substantially reduces this difference.

3.1.2. Extinction

The majority of males of both strains showed a very rapid increase in response latency during the first extinction session; afterwards very little change was observed across the remaining 5 extinction sessions (see Fig. 1, top). As a result, there was not a significant main effect of Session or any significant interactions with Session. Yet, there was a main effect of Strain, $F(1, 43) = 8.9$, $p < 0.005$, with SD rats exhibiting much longer response latencies over the 6 extinction sessions.

When we examine responses occurring within the first 60 s of the now non-predictive warning signal, there is a overall decrease in responding during that period of time, as evidenced by a main effect of Session, $F(5, 215) = 19.5$, $p < 0.001$. However, the response curves of the two strains quickly separate after the second extinction session. This difference was confirmed by a main effect of Strain, $F(1, 43) = 10.9$, $p < 0.005$. In fact, in the last extinction session, the WKY rats are responding during the first 60 s of warning 40% more often than the SD rats.

The within-session examination of the extinction of lever-press responses within the first 60 s of warning illustrated the major difference between the male SD and WKY rats. As shown in Fig. 2C, SD rats exhibited an increase in responding through the first 3 trials
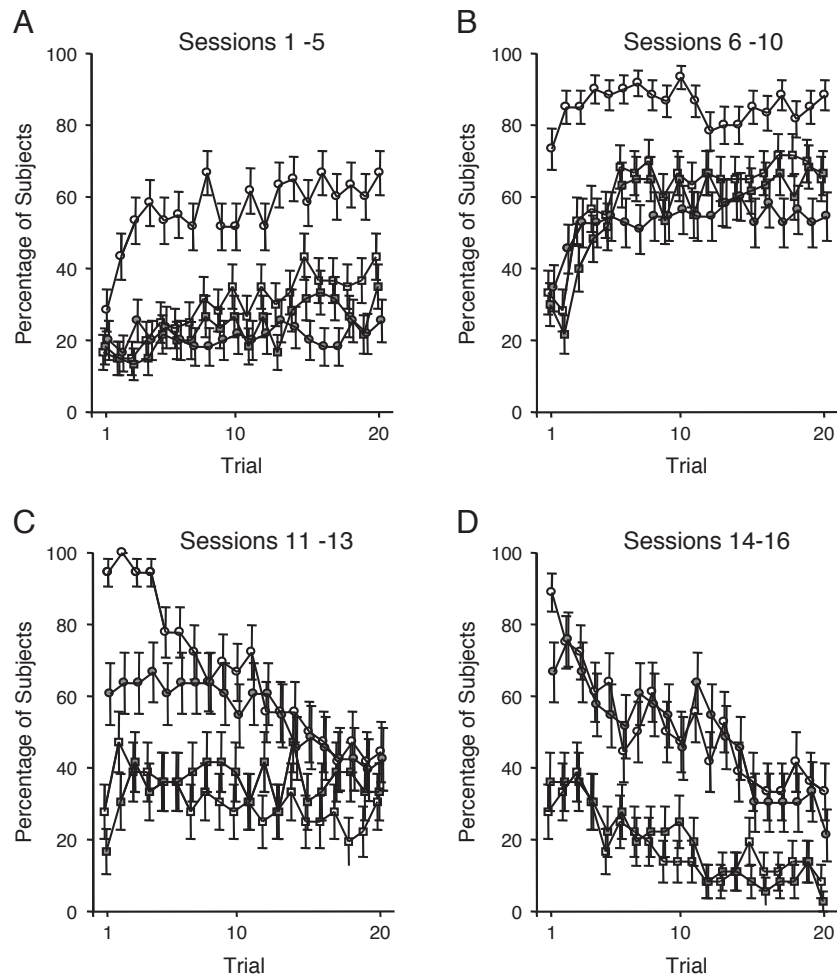
Fig. 2. Panels A and B show the average percentage of subjects emitting a lever-press avoidance response across the 20 trials within each acquisition session. Group designations are the same as Fig. 1. Significantly more male WKY rats (trained with an ITI-signal) show avoidance responses over trials compared to all other groups. Panels C and D show the percentages of subjects emitting responses during the first 60 s of warning, in the absence of the shock, across the 20 trials within each extinction session. Although significant differences between WKY groups are evident early in extinction, they quickly dissipate, leading to a general strain difference in the number of subjects emitting responses during the "avoidance" window of time.

before a gradual reduction in those responses occurred, while the WKY rats began from the highest level of responding they would show across trials. This initial difference in responding shows why the 2 SD groups begin to differ from the WKY group that was trained without the ITI-signal (despite exhibiting very similar acquisition rates). These impressions were confirmed by main effects of Strain, $F_{(1, 43)} = 7.3$, $p < 0.01$, and Trial, $F_{(19, 817)} = 7.5$, $p < 0.001$, that were additionally coupled with Strain×Trial, $F_{(19, 817)} = 4.7$, $p < 0.001$, and ITI-signal×Trial, $F_{(19, 817)} = 3.0$, $p < 0.001$ interactions. As extinction sessions continued, the influence of having been trained with the ITI-signal diminished, while the influence of Strain clearly remained, as evidenced by significant main effects of Strain, $F_{(1, 43)} = 14.9$, $p < 0.001$, and Trial, $F_{(19, 817)} = 12.7$, $p < 0.001$, and a significant Strain×Trial interaction, $F_{(19, 817)} = 1.7$, $p < 0.05$.

In the absence of shock, there is no difference in the outcome of responding to the warning signal during extinction sessions; therefore, ITI responses during these sessions were only analyzed with respect to minute and group designation and not on the timing of the response (i.e. avoid versus escape). These analyses yielded simple strain differences in responding as presses decreased over the 3 minute intervals. Thus, a main effect of Strain, $F_{(1, 43)} = 18.0$, $p < 0.001$ showed that WKY rats responded significantly more during the flashing light-absent ITIs (1.0 versus 0.7 responses per min). This difference was superimposed on a main effect of ITI-minute, $F_{(2, 85)} = 511.9$, $p < 0.001$, which saw

responses dropping significantly from minute 1 (1.8 responses per min) to minutes 2 and 3 (0.4 responses per min each). No other differences due to prior ITI-signal exposure were found.

3.2. Experiment 2 — strain differences in ITI-signal influence on female rats

3.2.1. Acquisition
All groups showed a reduction in response latency as they progressed through the 10 sessions of escape–avoidance training (see Fig. 4, top). This impression was confirmed by a significant main effect of Session, $F_{(9, 248)} = 38.9$, $p < 0.001$. However, as evidenced by the reduction in the between-group differences in mean response latencies from the early blocks (1–3) to the later blocks (4–10), there was a significant Strain×ITI-signal×Session interaction, $F_{(9, 248)} = 3.8$, $p < 0.001$. Thus, there were differences in the initial acquisition of the lever-press response that were attributable to differences in the strain as well as the presence/absence of the ITI-signal. The ITI-signal was associated with quicker initial-block response times in female WKY rats, whereas the ITI-signal was associated with slower initial-block response times in female SD rats. Still, these differences were no longer apparent by session 4, and differences remained absent throughout the remaining acquisition sessions.
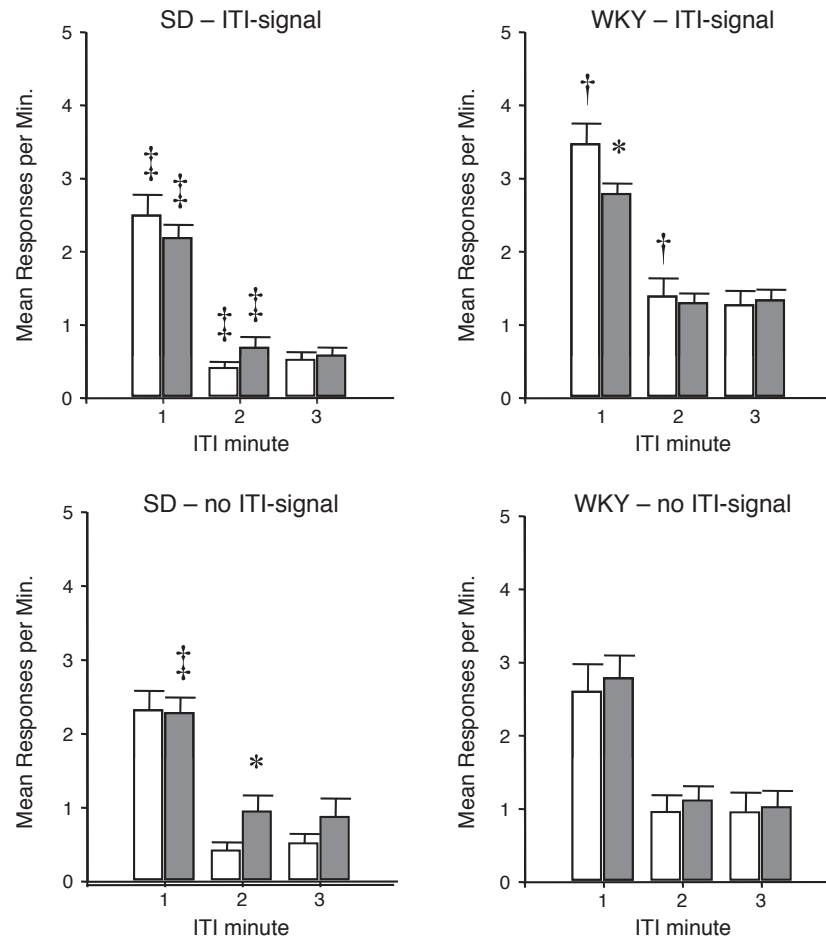
Fig. 3. Depicted is the mean number of lever-press responses emitted per subject during each one of the 3 min of the ITI during the 10 sessions of acquisition in male SD and WKY rats trained with or without an ITI-signal. Responses are segregated further by whether they occurred following either an escape (open bar) or avoidance (filled bar) response. An asterisk (*) represents a significant difference between responses following either an escape and avoidance response during the same minute. A cross (†) represents a significant difference within the same ITI-signal condition (across strain). A double-cross (‡) represents a significant difference within the same strain (across ITI-signal condition).

With respect to the specific acquisition of avoidant behavior (see Fig. 4, bottom), only a main effect of Session reached significance, $F_{(9, 252)} = 52.8$, $p < 0.001$. All groups showed a similar increase in the percentage of trials where an avoidance response was emitted.

Similar to the between-session analyses, the within-session analyses did not detect any significant differences between conditions. As shown in Fig. 5A and B, all groups showed similar within session acquisition rates. This was confirmed by only detecting significant main effects of Trial during early, $F_{(19, 532)} = 7.7$, $p < 0.001$, and late acquisition, $F_{(19, 532)} = 2.9$, $p < 0.001$.

Non-reinforced responses, emitted during the ITI, generally decreased over the 3 min of the ITI. This was confirmed by a significant main effect of ITI Minute, $F_{(2, 56)} = 417.0$, $p < 0.001$. However, there were differences in the level of responding, dependent upon the prior reinforced response, most notably in the first minute of the ITI (see Fig. 6). This was confirmed by a significant main effect of Reinforced Response, $F_{(1, 28)} = 8.0$, $p < 0.01$, and a significant Reinforced Response × ITI Minute interaction, $F_{(2, 56)} = 30.8$, $p < 0.001$. No differences were apparent between the 2 strains.

3.2.2. Extinction

In the absence of shocks and ITI-signal, response latencies increased over the last 6 sessions of the experiment (see Fig. 4, top). This was confirmed by a main effect of Session, $F_{(5, 139)} = 23.2$, $p < 0.001$. However, influences of Strain and prior training with the ITI-signal were evident as well in the form of significant ITI-signal × Session,

$F_{(5, 139)} = 2.9$, $p < 0.05$, and Strain × ITI-signal × Session, $F_{(5, 139)} = 2.4$, $p < 0.05$, interactions. In short, during the early extinction sessions, there were sessions where either the SD or WKY rats exhibited longer response latencies if they had been trained with the ITI-signal (which was now absent). These differences switched in the SD rats by the last extinction session, with SD rats trained in the absence of the ITI-signal showed the longest response latencies.

An examination of the responses occurring during the first 60 s of the now non-predictive warning signal yielded similar results as the response latency analysis. A main effect of Session, $F_{(5, 139)} = 66.7$, $p < 0.001$ was coupled with significant ITI-signal × Session, $F_{(5, 139)} = 3.0$, $p < 0.01$, and Strain × ITI-signal × Session, $F_{(5, 139)} = 2.3$, $p < 0.05$, interactions. As with response latency, those groups that had the ITI-signal removed with the beginning of extinction sessions reduced their responding during the first 60 s more rapidly than those groups that did not have any change in visual stimuli.

Within-session analyses of responding during the first 60 s of the warning signal found slower extinction in female WKY rats that were not trained with the ITI-signal (see Fig. 5C and D). In fact, during the initial 3 sessions of extinction, both the WKY and SD rats were slower in reducing their responding if they had been trained without an ITI-signal. Further, the WKY rats trained with the ITI-signal reduced their responding more than all other groups in the later trials of those sessions. These impressions were confirmed by significant main effects of ITI-signal, $F_{(1, 28)} = 5.3$, $p < 0.05$, and Trial, $F_{(19, 532)} = 23.2$, $p < 0.001$, and a significant Strain × Trial interaction, $F_{(19, 532)} = 4.2$,
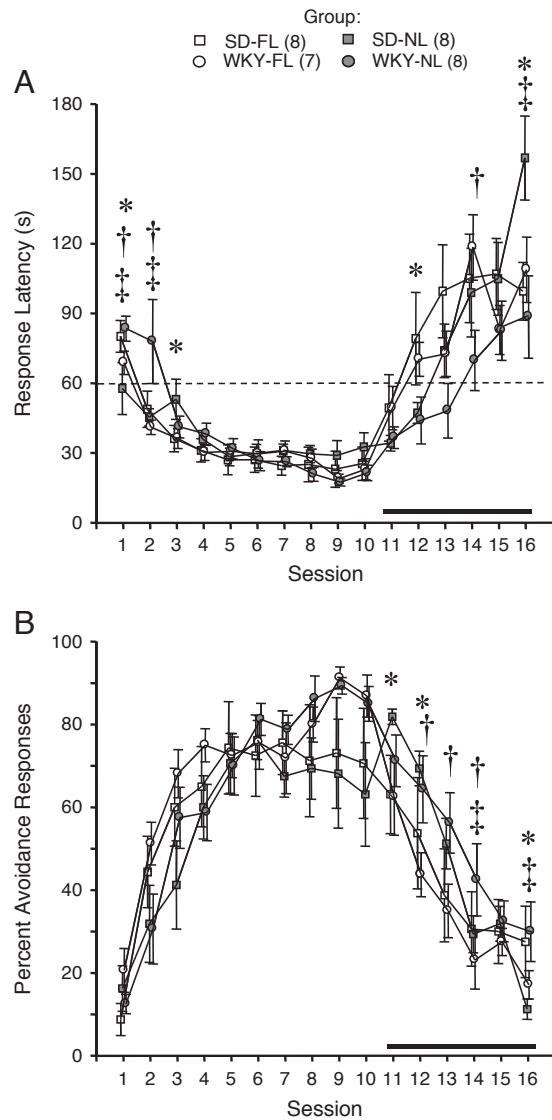
Fig. 4. Panel A shows a reduction in lever-press response latency across 10 sessions of acquisition. Differences in acquisition were evident early in acquisition, with female WKY rats being slower to reduce response latency. The six subsequent extinction sessions are denoted by the black line above the x-axis and reflect a general trend for female WKY rats that acquired the behavior in the absence of the ITI-signal to show a slower lengthening of response latency. Panel B illustrates the specific proportion of avoidance responses emitted as a function of acquisition sessions and extinction sessions (same designations as Panel A). Female SD and WKY rats exhibited more avoidance responses during extinction if they had acquired the avoidance response in the absence of an ITI-signal. An asterisk (*) represents a significant difference within the SD strain. A cross (†) represents a significant difference within the WKY strain. A double-cross (‡) represents a significant difference between the no-light (NL) conditions.

p<0.001. By the second half of extinction sessions, the influence of ITI-signal was reduced. Basically, the initial responses in each strain differed, although the effect was clearly more apparent in the WKY rats not trained with an ITI-signal. However, by the last 10 trials, no differences existed between groups. These impressions were confirmed by a significant main effect of Trial, F (19, 532) = 32.5, p<0.001 and a significant Strain × Trial interaction, F (19, 532) = 1.7, p<0.05.

As with the males, in the absence of shock, ITI responses during these sessions were only analyzed with respect to minute and group designation. As observed during acquisition, there was a main effect of ITI-minute, F (2, 56) = 810.9, p<0.001, with the first minute responses (1.9 responses per min) being significantly greater than those of minute 2 (0.5 responses per min) and minute 3 (0.5 responses

per min). A main effect of having been trained with or without an ITI-signal was also significant, F (1, 28) = 5.0, p<0.05, in that, those rats that had been trained with a flashing light during the ITIs generally responded more during the ITIs in extinction when the light was now absent for them as well (1.0 responses per min versus 0.9 responses per min). However, a closer inspection of the data finds that this interpretation is only applicable to WKY rats (1.1 responses per min versus 0.8 responses per min) and not SD rats (1.0 responses per min for both groups), which was supported by a significant Strain × ITI-signal interaction, F (1, 28) = 6.7, p<0.01. Thus, prior training with an ITI-signal led to greater responding selectively in WKY rats during extinction, when both the shock and the ITI-signal were absent.

3.3. Experiment 3 — strain differences in classical conditioning

3.3.1. Acquisition

In order to determine whether the faster acquisition observed in male WKY rats was due to paradigm-independent enhancement of associative learning because of the visual stimulus used as the ITI-signal, we provided a 5 Hz visual stimulus throughout 3 consecutive eyeblink conditioning training sessions. Male SD and WKY rats showed differential acquisition of a conditioned eyeblink response over 3 days of 300 CS–US paired trials (see Fig. 7). As evidenced by significant main effects of Day, F (3, 90) = 53.0, p<0.001 and Block, F (4, 120) = 14.4, p<0.001, all groups exhibited an increase in CR emission across and within training sessions. Further, as training progressed, differences between groups decreased, as evidenced by significant interactions of Strain × Day × Block, F (12, 258) = 4.2, p<0.001 and Light × Day × Block, F (12, 258) = 2.2, p<0.01. Post-hoc analyses found the presence of the flashing light facilitated performance selectively in SD rats, such that those SD rats trained in the presence of the flashing light did not differ from either WKY group. The flashing light did not appreciably influence acquisition in WKY rats, both WKY groups exhibited faster acquisition compared to SD rats trained without the flashing light. Thus, SD rats were more affected by the presence/absence of a flashing light present during eyeblink conditioning.

3.3.2. Extinction

We removed the US and the visual stimulus after the first 2 blocks (40 trials) of eyeblink conditioning on the 4th day of training. Initial paired trials were included in an attempt to equate all groups in performance prior to the removal of the US and visual stimulus. As evidenced in Fig. 7, both groups of SD rats began Day 4 emitting significantly less CRs than either comparison WKY group, and, although the percentage of CRs increased in both SD groups, only the no-light SD group attained the same level of performance as the WKY groups. Subsequently, following US removal, both WKY groups extinguished slower than both SD groups. These impressions were confirmed by significant main effects of Strain, F (1, 30) = 12.3, p<0.001 and Block, F (4, 120) = 27.6, p<0.001, as well as significant Strain × Block, F (4, 120) = 3.0, p<0.05, and Strain × Light × Block, F (4, 120) = 3.0, p<0.05, interactions. The flashing light only affected SD rats in the first 2 blocks of paired trials. Once removed, with the US, extinction was similar within each strain. Thus, extinction of a classically conditioned eyeblink response exhibited a similar strain difference as previously observed in the extinction of lever-press escape–avoidance behavior in male rats, and, as also observed in avoidance learning, removal of the light did not differentially affect the rate of extinction of the behavior in either strain of male rats.

4. Discussion

4.1. Facilitation of avoidance learning in vulnerable male rats

Understanding the proposed functionality of avoidance learning "safety signals" is important for any learning-based approach to the
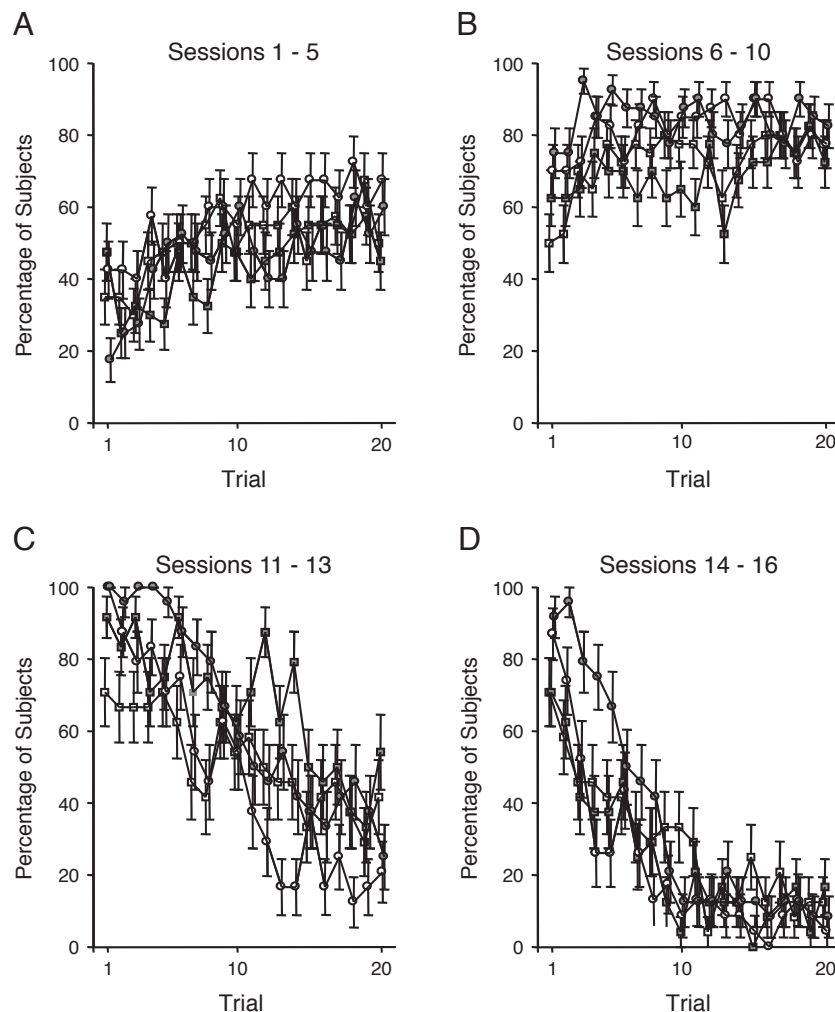
Fig. 5. Panels A and B show the average percentage of subjects emitting a lever-press avoidance response across the 20 trials within each acquisition session. Group designations are the same as Fig. 4. No differences across groups were evident in the acquisition of the avoidance behavior. Panels C and D show the percentages of subjects emitting responses during the first 60 s of warning, in the absence of the shock, across the 20 trials within each extinction session. For the females trained without an ITI-signal, a greater percentage of subjects emitted responses in the "avoidance" window of time, compared to those that were trained with the ITI-signal (now absent), during the first half of extinction sessions.

study of anxiety vulnerability, for perpetuation of anxiety disorder symptoms may be highly influenced by the perceived safety signals in the patient's environment (Grillon et al., 1994; Rachman, 1984; Schmidt and Telch, 1994; Schmidt et al., 2006). Therefore, the principal goal of this study was to determine if susceptibility to acquire active avoidance behaviors in vulnerable populations is influenced by the presence of a discrete visual stimulus presented during the non-threat ITIs. This was clearly the case, as only male WKY rats exhibited a difference in acquisition of lever-press avoidance based on the presence/absence of the ITI-signal. The absence of the flashing light was associated with slower acquisition in male WKY rats, and, in fact, led to a similar acquisition curve as male SD rats. Thus, the expressed vulnerability of male WKY rats to acquire conditioned avoidant behaviors quicker than male SD rats appears to be linked to the presence of a discrete ITI-signal. No other group (male or female) showed any differential acquisition of lever-press avoidance due to the presence or absence of an ITI-signal.

In past experiments, male WKY rats exhibited more non-reinforced responses during the ITIs, compared to male SD rats, but this was always in the presence of the ITI-signal (Beck et al., 2010; Servatius et al., 2008). Here, in addition to slowing acquisition of avoidance responding, we found the absence of the ITI-signal for male WKY rats also was associated with less lever-pressing in the first minute of the ITIs, specifically following the emitting of escape

responses. In fact, much like avoidance acquisition, male WKY rats trained without the ITI-signal were no different than male SD rats in first-minute ITI responding following shock exposure. These results would seem to suggest that the ITI-signal facilitates bar-pressing at an early stage of acquisition, when escape responding is more predominant. Why this would be the case is still not entirely clear, but one hypothesis is that the ITI-signal increases arousal during the non-threat periods, thereby enhancing the associative learning processes. Yet, when tested in a separate learning paradigm (Experiment 3), the flashing light did not selectively facilitate the acquisition of a conditional response in male WKY rats. This finding does not support a general arousal hypothesis for the role of the flashing light ITI-signal in male WKY rats. Still, we cannot rule out that there is an interaction between having been just shocked, subsequently seeing the ITI-signal, and overall arousal, or arousal that becomes associated with the ITI-signal. In order to test that hypothesis, we would have to provide shocks and limited ITI-signal presentation selectively during the ISIs during eyeblink conditioning. The problem with that approach is that mere exposure to shocks, or presumably any stimuli that increase extra-hypothalamic corticotrophin releasing hormone levels in the brain or peripheral pro-inflammatory cytokine activity, facilitates the acquisition of conditioned eyeblink responses (Servatius and Beck, 2003; Servatius et al., 2005; Shors et al., 1992). Nonetheless, based on the current data we can still rule-out an enhanced avoidance learning
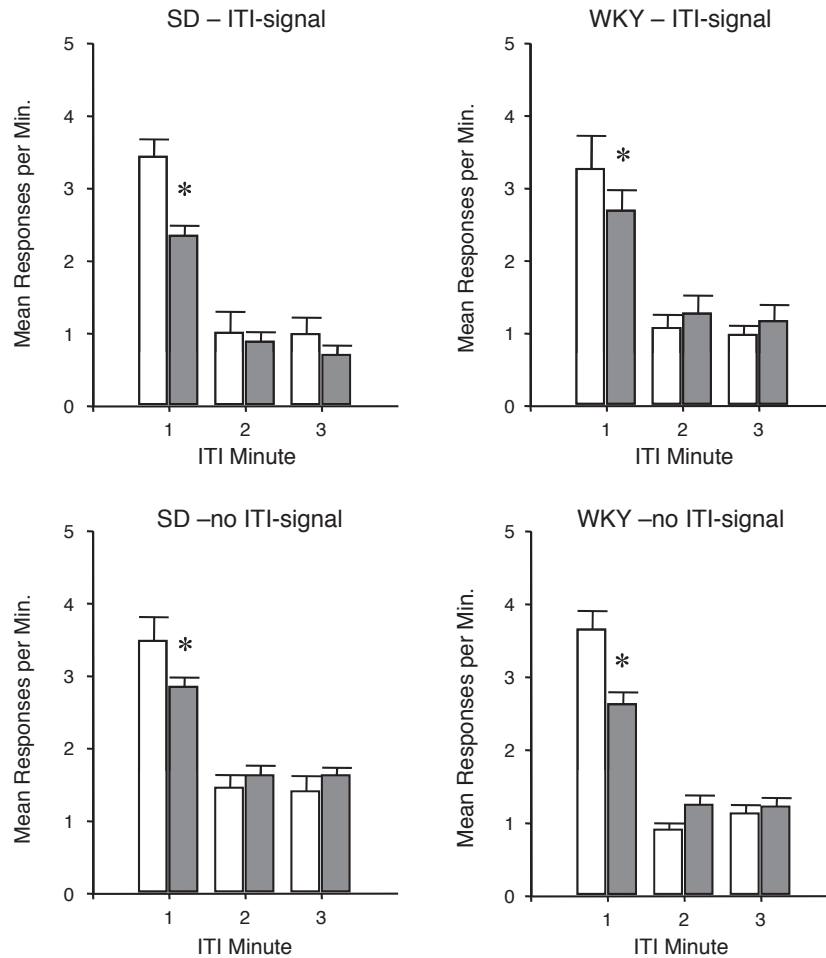
Fig. 6. Depicted is the mean number of lever-press responses emitted per subject during each one of the 3 min of the ITI during the 10 sessions of acquisition in female SD and WKY rats trained with or without an ITI-signal. Responses are segregated further by whether they occurred following either an escape (open bar) or avoidance (filled bar) response. An asterisk (*) represents a significant difference between responses following either an escape and avoidance response during the same minute. No other differences across strain or ITI-signal condition were detected.

hypothesis that is solely based on male WKY rats having a greater sensitivity to the flashing light that then leads to faster associative learning.

The hypothesis that the ITI-signal is perceived as a safety signal during acquisition does not garner much support from the current data. One feature that should have differed, if it was perceived as a safety signal, is the distribution of responses across the 3 min of the ITI. Assuming that non-reinforced lever-presses is a sign of continued fear or anxiety in the rats (Brennan et al., 2003), then a reduction in non-reinforced responding should have occurred in the presence of the ITI-signal, because it would be serving as a conditioned inhibitor of the fear elicited by the threat. This pattern was observed, but, it was observed in all conditions — regardless of explicit ITI-signal. Further experimentation could test whether prior acquisition of avoidance behavior in the presence of the flashing light ITI-signal causes retardation or summation effects in eyeblink conditioning, 2 accepted criteria for identifying conditioned inhibitors (Rescorla, 1969), but the current data are not suggestive of the ITI-signal exhibiting properties of a conditioned inhibitor of fear. In addition, an assessment of arousal during the avoidance procedure would provide evidence as to whether the ITI-signal is perceived as a safety signal, as safety signals reduce arousal under conditions of threat in humans (Carter et al., 1995; Grillon et al., 1994). At this juncture, it appears that the mere absence of the warning signal decreases responding over the 3 min of the ITI. Exposure to an additional stimulus may increase the overall amount of responses emitted, but a similar reduction in responding over time still occurs.

These do not appear to be the properties of a stimulus-specific conditioned inhibitor.

4.2. Extinction of active avoidance behavior

Although consistency was not observed across groups in acquisition of avoidance behavior, within-sex consistencies were observed in the extinction sessions. For the males, the latency to lever-press during the warning signal lengthened and the amount of elicited lever-presses to the warning signal decreased more rapidly in SD rats. These expressions of the extinction process were less evident in male WKY rats. Further, despite the differences observed during acquisition due to the presence/absence of an ITI-signal, there were no differences in the lever-press responses during the extinction phase that were attributable to the prior exposure to the ITI-signal during acquisition. Hence, we can conclude that the predominant influence of the ITI-signal upon avoidance behavior in male WKY rats is to facilitate the acquisition of the behavior, not the maintenance of the instrumental response in the absence of shock.

The resistance for male WKY rats to extinguish the avoidance instrumental response may be caused by a strain difference in the strength of Pavlovian conditioning to the warning signal. At asymptotic performance, well-trained rats emit the requisite instrumental response during the initial minute of warning signal exposure (prior to the onset of the first shock). This stimulus–response (S–R) relationship could reflect an aversion to the warning signal, an interpretation that
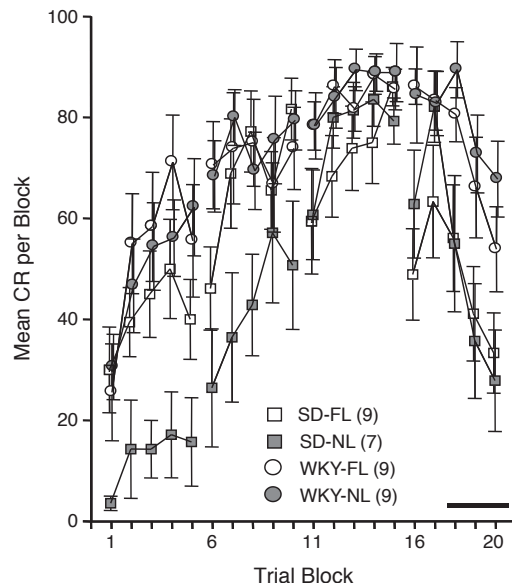
Fig. 7. Depicted is the mean number of conditioned eyeblink responses detected per each of 4 daily 20-trial blocks in male SD and WKY rats. On the last 3 blocks on the 4th day, the unconditioned stimulus was removed (denoted by the black line above the x-axis). Strain differences in the acquisition of the conditioned reflex responses are influenced by the presence/absence of a constant 5 Hz flashing light. During acquisition the light generally facilitates acquisition, albeit to a much greater degree in SD rats. However, during extinction, only a strain difference is evident, with SD rats extinguishing more rapidly than WKY rats.

would mean the aversive properties of the shock have transferred to the warning signal, and the rats are exhibiting instrumental control over the duration of this aversive stimulus. In other words, they are escaping an aversive signal. On the other hand, the animals could be emitting the response because they learned the warning signal is a *predictor* of future shock. This interpretation is founded in a Pavlovian/ classical conditioning framework, and the eyeblink conditioning experiment provides support for this hypothesis. As in the avoidance paradigm, male WKY rats were slower than male SD rats to reduce their blink responses to the CS (in the absence of the US) during the last day of eyeblink conditioning. This demonstration is quite important because it shows that WKY rats are more apt to continue to emit conditioned responses even in a learning paradigm where the continued presence or absence of the unconditioned stimulus is evident on all trials, regardless of any behavioral response. Male WKY rats are more sensitive to contingency differences during the acquisition of a classically conditioned eyeblink response than are male SD rats (Ricart et al., 2011b), but, we do not know if those differences influence the *extinction* of the behavior or if the male WKY rats are slow to extinguish any classically conditioned responses once asymptotic performance is attained.

Trait anxiety in humans is associated with slower extinction of Pavlovian fear conditioning and differential activation of amygdala and prefrontal cortex (Sehlmeyer et al., 2011), areas known to be required for the extinction of conditioned fear in rats (Milad et al., 2004; Quirk et al., 2003; Santini et al., 2004; Sierra-Mercado, Jr. et al., 2006) and recently discovered to show differential c-fos activation during extinction when male WKY rats are compared to male SD rats (Jiao et al., 2011). Compared to SD rats, WKY rats exhibit less c-fos activation in the anterior cingulate, prelimbic, and infralimbic cortices, as well as, less c-fos activation of GABA-labeled cells in the basolateral amygdala, following several extinction sessions of lever-press avoidance behavior (Jiao et al., 2011). Further work will need to determine whether these differences in neuronal activation in prefrontal-amygdala areas in male WKY rats are the cause of slower extinction of lever-press avoidance, and a determination, as such, would allow for future testing of site-

specific pharmacological interventions that could help extinguish avoidance behavior in avoidance-susceptible, anxiety vulnerable, populations.

In contrast to the males, females in both strains exhibited differences in behavior during the extinction phase that could be attributable to prior exposure to the ITI-signal during acquisition. Those females that had acquired the avoidance behavior in the presence of an explicit ITI-signal exhibited longer response latencies and a quicker reduction of avoidance-timed responses during the extinction phase. This suggests the ITI-signal acquired some association to the lever-press behavior in the female rats, despite not influencing acquisition rates or the amount of non-reinforced responding during acquisition. One explanation for this effect, the ITI-signal becomes associated with a "threat context" (i.e. if the ITI-signal is there, then there is a high probability that the shock is there as well). Contextual stimuli that cue an animal that a specific CS– US or S–R contingency is present are termed *occasion setters* (Holland, 1989; Schmajuk et al., 1998). Because of the manner in which we have the flashing light signal only presented during the ITI, there is no flashing light that serves as an occasion setter prior to the first trial. So, it may be that the first trial is ambiguous to the rats but subsequent trials are "cued" by the preceding ITI-signal following the previous trial. If this were occurring, the absence of the ITI-signal following trials 2–20 should cause rapid within-session extinction curves. In fact, the within-session analyses show rapid extinction in those previously trained with the ITI-signal present; it is as early as trials 4–5 in female WKY rats and trials 6–8 in female SD rats. Moreover, these data also suggest that the ITI-signal could be serving as a *sex-specific* occasion setter. To our knowledge, there has not been any systematic study that has identified sex differences in the acquisition or utilization of occasion setters in classical or instrumental conditioning. This may be a fertile area of investigation with regards to understanding how males and females use different stimuli in the environment when encoding associations.

In a sample of predominantly female undergraduates, Lovibond found creating compound conditional stimuli during extinction, using either previously excitatory or inhibitory stimuli, increased the rate of extinction of a fear response (Lovibond et al., 2000). The implication is that any change in stimulus properties of a fear-inducing situation may cause the female brain to re-evaluate the contingencies previously associated with threat. Additional experimentation will have to determine if female rats, trained with the flashing light present prior to the first trial of each acquisition session, exhibit quicker extinction of initial-trial responses following the removal of the light during extinction. Moreover, the Lovibond study would suggest *introducing* an ITI-signal during extinction should cause the female rats trained in the absence of an ITI-signal to re-re-evaluate the contingencies and extinguish quicker as well. The introduction of such an occasion setter prior to the first trial could be particularly advantageous to female WKY rats, which exhibit warm-up decrements. If female WKY rats are more attuned to contextual stimuli serving as occasion setters for future harm, they may be also more sensitive to occasion setters that are specifically associated with the absence of harm. Thus, it may be worthwhile to test the effectiveness of a "safety" occasion setter, present at the beginning of extinction sessions, as a tool to work against the WKY rats' warm-up decrements, which make them relatively immune to contingency changes that are present on the first trial of each session.

4.3. Signals and implications for anxiety vulnerability

A significant problem for individuals with anxiety disorders is the occurrence of avoidant behaviors. The adoption of avoidant strategies and resistance to utilize non-avoidant strategies encompasses the 2-step process we are trying to model in our current active avoidance protocol. The current study illustrates the importance of signals to this 2-step process. Importantly, though, the influence of these signals (be it threat-predictive, safety-predictive, arousing, inhibiting, etc.) is not

the same across the vulnerability factors under examination here: behavioral inhibition and female sex.

After manipulating the predictive value of a warning signal, Sidman and Borman concluded that the avoidance behavior displayed by their male albino rats was more likely due to the instrumental control of the situation, rather than a Pavlovian predictive relationship, because of how the responding waxed and waned as the shocks were avoided then subsequently experienced again, once the assumed reinforcement of the behavior decreased in the absence of shock (Sidman and Boren, 1957). When one considers the warm-up effect observed in male SD rats in the current experiment, a similar conclusion could be drawn; there appears to be an ongoing balance between experience with the shock and emission of the lever-press behavior. This constant balancing process makes them more attuned to changes in the CS–US contingency (i.e. shock removal). Importantly, this balance may not be as evident in the vulnerable populations. Clearly, male WKY rats are sensitive to the presence of the ITI-signal. It does not appear to be arousing in nature, but a possible role as a safety signal is not obvious from these data either. Somehow the signal is promoting rapid within session acquisition of the avoidance behavior. Acquisition rate is not usually tracked in fear conditioning because it is so rapid, but the acquisition of avoidance behavior suggests individuals with behavioral inhibition may acquire perceived threat associations quicker. Our lab has recently discovered that people with high behavioral inhibition scores acquire conditioned eyeblink responses quicker than those with low to normal behavioral inhibition (Myers et al., accepted for publication). This further supports our theory that there is an intrinsic factor associated with behavioral inhibition that influences the rate at which conditioned behaviors are acquired. Conversely, for females, the recognition and encoding of perceived occasion setters, during conditioning of threat-based associations, could be the key vulnerability factor for rapidly adopting avoidant behaviors. As with behavioral inhibition, the role for occasion setters would be in addition to known differences in the acquisition of conditioned behaviors; both women and female rats are reported to have faster acquisition of conditioned eyeblink responses, although the sex differences in learning are more state dependent due to fluctuations in ovarian hormones (Beck et al., 2008; Holloway et al., 2011; Shors et al., 1998; Wood and Shors, 1998). Gaining an understanding of how each of these vulnerability factors differentially modulate the acquisition and retention of threat-based associations is important in order for clinicians to be able to impart the most efficient and effective cognitive-behavioral therapeutic approaches to extinguish overly avoidant coping responses.

Still, the understanding of how the behavior is adopted (i.e. acquired) is only the first part of the problem; for it is the extinction of the behavior (or thoughts) that is of immediate importance to the therapist. The current study suggests the contextual cues may play a significant role in maintaining avoidant behaviors in females and may be a worthwhile target for exposure therapy, whereas, individuals with a behaviorally inhibited temperament may be a more difficult problem. The high level of responding on the first few extinction trials, throughout extinction sessions, in male and female WKY rats suggests that within session extinction trials are not sufficient to reduce the initial emitting of the avoidant behavior when subsequently faced with the warning signal on a different day. It appears the behavioral response is spontaneously recovered each new session, and there may be a neurobiological explanation for this finding.

WKY rats are more prone to encode associations as S–R relationships, versus contextual or spatial representations (Clements et al., 2007). The implication is that the S–R association may not be well-linked to a specific time or place. Thus, each time a WKY rat is placed in a box with the sound and the lever, the immediate reaction is to emit the avoidance behavior, regardless of the fact that it may have shown significant within session extinction of behavior just 2 days earlier. If this is due to the creation of a very strong S–R association between the warning signal and behavior, then a more dominant caudate-dependent memory system (over hippocampal-dependent memory system) may be the

root of this vulnerability (Gabriele and Packard, 2006; Packard, 2009; Packard and Gabriele, 2009; Poldrack and Packard, 2003). WKY rats have significantly less dopamine D1 and D2 receptor binding in the caudate-putamen (Novick et al., 2008; Yaroslavsky et al., 2006). Moreover, D1 and D2 receptors are involved in plasticity processes in the dorsal striatum (namely long-term potentiation and long-term depression, respectively) (Lovinger, 2010), and reduced D2 receptor availability has been linked to compulsive behavior (Volkow et al., 2001). Therefore, in the case of the behaviorally inhibited phenotype (male or female), an additional pharmacotherapy may be required in order to break the cycle of responding to the previously acquired warning signal, whereas, exposure or other forms of cognitive-behavioral therapy may be sufficient for others.

4.4. Conclusions

In the current study, we showed that animals used to model different anxiety vulnerabilities utilize signals differently when acquiring active avoidance behaviors. Specifically, behaviorally inhibited, male WKY rats are more susceptible to acquire active avoidance behaviors when an additional signal is presented during the ITIs, possibly by facilitating the predictive association between the perceived warning signal and the subsequent shock. Still, regardless of the increased susceptibility caused by the additional signal, male WKY rats were slower to extinguish the acquired active-avoidance response, suggesting that once the behavior is acquired it is difficult to cease its occurrence. On the other hand, the female vulnerability to acquire avoidance responses quicker was not influenced by the presence of the ITI-signal, but, in its absence during extinction, response rates reduced more rapidly, suggesting the ITI-signal is encoded as an occasion setter for the presence of the shock. In all, these data illustrate that susceptibility to adopt avoidant behaviors may not be simply founded in the strength of the warning-response associations created but in qualitative differences in the encoding of other stimuli present during the acquisition process. In some cases, differences in neurobiology may necessitate pharmacotherapy to weaken these associations. In general, our data suggest that a thorough understanding of individual differences in how threat-response coping responses are adopted, as well as the context under which they are adopted, may be crucial when determining the most appropriate therapeutic approaches to extinguishing unwanted avoidance strategies in those individuals inflicted with anxiety disorders.

Acknowledgments

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