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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.

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

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Abbreviations: AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care; ANOVA, analysis of variance; IACUC, Institutional Animal Care and Use Committee; ISI, inter-stimulus interval; ITI, inter-trial interval; LSD, least squared difference; PTSD, post-traumatic stress disorder; SD, Sprague Dawley; S–R, stimulus–response; US, unconditional stimulus; WKY, Wistar–Kyoto.

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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.