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Review

# Anxiety vulnerability in women: A two-hit hypothesis

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ABSTRACT

Females are twice as likely to develop an anxiety disorder compared to males, and thus, are believed to possess an innate vulnerability that increases their susceptibility to develop an anxiety disorder. However, studies using aversive learning paradigms to model anxiety disorders in humans and animals have revealed contradictory results. While females exhibit the ability to rapidly acquire stimulus–response associations, which may result from a greater attentional bias towards threat, females are also capable to readily extinguish these associations. Thus, there is little evidence to suggest that the female sex represents a vulnerability factor of anxiety, per se. However, if females are to possess a second vulnerability factor that increases the inflexibility of stimulus–response associations, then an anxiety disorder may be more likely to develop. Behavioral inhibition (BI) is a vulnerability factor associated with the formation of inflexible stimulus–response associations. In this "two hit" model of anxiety vulnerability, females possessing a BI temperament will rapidly acquire stimulus–response associations that are resistant to extinction, resulting in the development of an anxiety disorder. In this review we explore evidence for a "two-hit" hypothesis underlying anxiety vulnerability in females. We explore the literature for evidence of a sex difference in attentional bias towards threat that may lead to the facilitated acquisition of stimulus–response associations in females. We also provide evidence that BI is associated with inflexible stimulus–response association formation. We conclude with data generated from our laboratory that highlights the additive effect of the female sex and behavioral inhibition vulnerabilities using a model behavior for anxiety disorder-susceptibility, active avoidance.

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**Contents**



## Introduction

Stress and anxiety are often assumed to coincide, and while stress is a major factor in the development of anxiety, thankfully, not every individual exposed to stress will develop a disorder (Mineka and Zinbarg, 2006). So what makes individuals that develop an anxiety disorder unique? The answer likely lies in the concept of vulnerability. While several vulnerability factors of anxiety disorders have been identified, one of the most predominant vulnerability factors is simply being female (Alonso et al., 2004; Kessler et al., 1995, 2012; Stinson et al., 2007). Compared to men, women are two times more likely to be diagnosed with an anxiety disorder (Alonso et al., 2004; Bekker and van Mens-Verhulst, 2007; Kessler et al., 2012). In generalized anxiety disorder (GAD), women make up 55–60% of the clinical patient population (American Psychiatric Association, 2013) and in post traumatic stress disorder (PTSD), a disease with stress exposure as diagnostic criteria (American Psychiatric Association, 2013), females express greater lifetime prevalence (Kessler et al., 1995) and are symptomatic for longer periods of time compared to males (American Psychiatric Association, 2013). While the overwhelming

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sex bias in anxiety disorders is recognized in the field of anxiety research, the mechanism underlying anxiety vulnerability in women is currently unknown.

Animal models of anxiety incorporate various learning paradigms. These paradigms include non-associative learning (e.g. fear potentiated startle) (Brown et al., 1951; Davis, 1986), classical conditioning (e.g. Pavlovian fear conditioning) (Pavlov, 1927; Watson and Rayner, 2000), and instrumental learning tasks (e.g. active lever-press avoidance) (Miller, 1948; Mowrer, 1951). Despite the diversity in paradigms used to model anxiety, few have extensively been studied in females (Lebron-Milad and Milad, 2012), at least to the extent sex-specific neurological causes are identified. Furthermore, studies that incorporate females have revealed conflicting results that call into question the idea of the female sex as a vulnerability factor in and of itself.

Significant sex differences exist in the acquisition of several classical and instrumental learning paradigms used in anxiety research in both animals (Beck et al., 2011; Dalla and Shors, 2009; Dalla et al., 2009; Van Oyen et al., 1981) and humans (Spence and Spence, 1966). In rodents and humans, females acquire eyeblink conditioning quicker than males (Bangasser and Shors, 2007; Dalla et al., 2009; Spence and Spence, 1966; Wood and Shors, 1998). In active lever press avoidance females acquire avoidant behavior faster and to a greater asymptotic level compared to males (Beck et al., 2010, 2011; Van Oyen et al., 1981). This is interesting given the fact that individuals possessing identified anxiety vulnerability factors also rapidly acquire classically conditioned behaviors (Holloway et al., 2012; Myers et al., 2012). The ability of females and individuals with anxiety to rapidly acquire stimulus–response associations may be due to their increased attentional bias towards threat. Females have been shown to be more vigilant than males (Beck et al., 2002), and there is some evidence that suggests that females may in fact have a greater difficulty disengaging attention from threatening stimuli (Goos and Silverman, 2002; McClure, 2000; Tan et al., 2011).

However, rapid acquisition of stimulus–response associations is not necessarily pathological, per se, unless these associations are inflexible. Traditionally the inability to successfully extinguish a learned fear response is interpreted as anxiety-like behavior (Milad and Quirk, 2012; Milad et al., 2006; Sotres-Bayon et al., 2004). Given that females possess an innate vulnerability to develop anxiety, one might expect that they naturally exhibit perseveratory behavior, but this is not the case. Extinction of certain behaviors, such as conditioned fear or avoidance, do not consistently exhibit a sex difference (Baran et al., 2009, 2010; Beck et al., 2011), unless animals are previously exposed to stress (Baran et al., 2009). As shown in Fig. 1, female Sprague Dawley rats extinguish an acquired lever-press avoidance response quicker if they had previously experienced uncontrollable shock prior to any avoidance training. Thus, contrary to the female vulnerability hypothesis, the proactive effects of stress facilitate extinction of both conditioned fear and avoidance in females but not males (Baran et al., 2009). Furthermore, estrogen has been shown to facilitate extinction training (Chang et al., 2009; Gupta et al., 2001; Milad et al., 2009) and has been proposed as a potential treatment for PTSD in women (Glover et al., 2012). These examples from the literature suggest that neither the extinction of a classically fear conditioned reflexive response nor an instrumentally conditioned avoidance response appears to be a primary source of anxiety disorder vulnerability, which is dependent upon the acquisition of associations.

The results from the animal literature suggest that being female is not pathological. However, if women express a second vulnerability factor that increases the inflexibility of stimulus–response associative learning, an anxiety disorder may be more likely to develop. One such vulnerability factor, which is associated with inflexible stimulus–response associations is behavioral inhibition (BI), which is characterized as an extreme behavioral withdrawal in the presence of novel social and nonsocial stimuli (Kagan et al., 1987; Kagan et al., 1989). Children expressing a BI temperament are more likely to develop anxiety disorders as adults (Fox et al., 2005; Kagan et al., 1987, 1989a; Rosenbaum et al., 1993; Schwartz et al., 2003) and more likely to exhibit an enhanced reactivity to stressors (Smoller et al., 2005). Animal models of BI show not only a rapid acquisition of stimulus–response associations (similar to the female phenotype) (Beck et al., 2010; Servatius et al., 2008), but also an inability to modify or adapt these associations (Jiao et al., 2011; Servatius et al., 2008). Thus, we are proposing that females with a second vulnerability factor (such as BI temperament) represent the actual "female vulnerability" supported by the epidemiological statistics. In this "two hit hypothesis", being female contributes an attentional bias towards threat that allows for the rapid formation of stimulus–response associations, and, when combined with an inflexible BI temperament, bestows a higher susceptibility to develop an anxiety disorder. As shown in Fig. 2, female sex is associated with rapid acquisition of conditional responses, but, without the added temperament of BI, the response readily extinguishes. Thus,

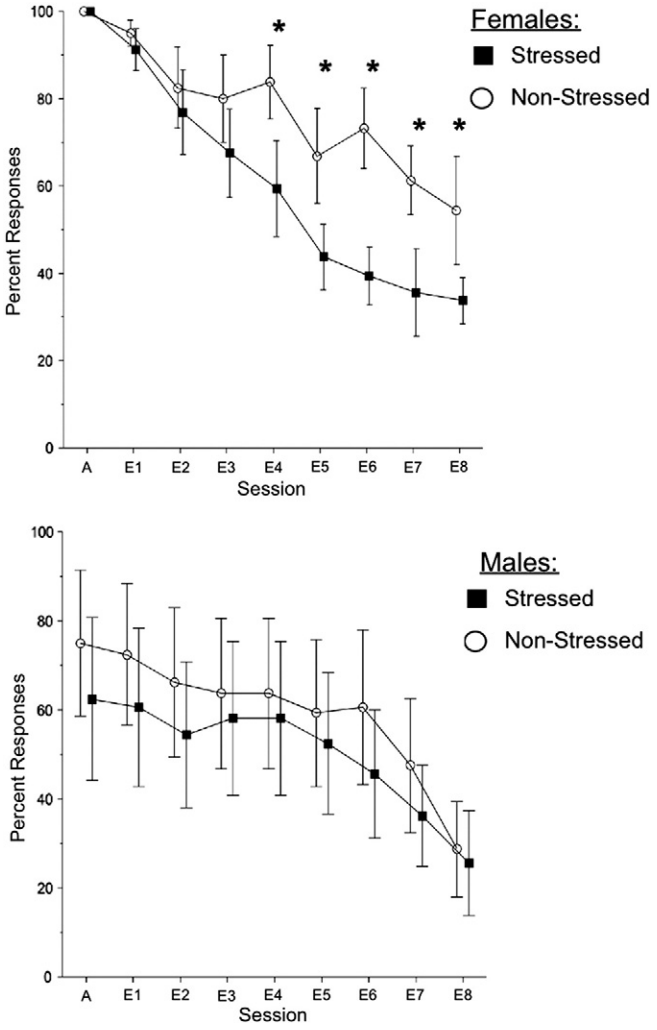


**Fig. 1.** Proactive effects of stress on extinction of active lever-press avoidance. These graphs represent the mean percentage of extinction session lever-presses emitted during the trial periods that previously coincided with reinforcement (i.e. warning signal and/or shock periods). Prior to any avoidance learning, "stress" rats were exposed to 2 h of intermittent tailshock (40 shocks, 3 s duration). Acquisition of lever-press avoidance followed over several weeks, in order to obtain asymptotic performance levels. The upper graph shows that "stress" female Sprague Dawley rats exhibited facilitated extinction rates. This was confirmed by a significant Stress × Session interaction, $F(8, 112) = 2.1$, $p < .05$. However, the "stress" male rats were not different than their counterparts that were similarly trained in lever-press avoidance in the absence of a prior stressor exposure. An asterisk (*) represents a significant difference between stress and control conditions, as determined by Fisher's Least Significant Difference ($p < .05$).