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Animal Models of Anxiety Vulnerability - The Wistar Kyoto Rat

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1. Introduction

1.1 Anxiety

Anxiety disorders are the most common psychiatric disorders with a worldwide lifetime prevalence of 16-29% (Kessler et al., 2005; Somers et al., 2006). People with anxiety disorders are likely to suffer from depression and drug (or alcohol) abuse in an effort to gain relief from their symptoms, therefore, eliciting secondary disorders (Kessler et al., 2005). Although each subtype (i.e. generalized anxiety disorder, obsessive-compulsive disorder (OCD), panic disorder, post traumatic stress disorder (PTSD) and social phobia) has unique features, the core symptom of all anxiety disorders is excessive avoidance. The etiology of anxiety disorders remains elusive (the presumed role of trauma in PTSD notwithstanding). What is abundantly clear is anxiety disorders arise as a complex interaction of genetic, epigenetic, sociocultural factors with life experiences; that is, anxiety disorders are best explained with diathesis models (Kendler et al., 2002; Mineka and Zinbarg, 2006; Zinbarg and Barlow, 1996).

Among a variety of neurobiological and neurobehavioral factors representing a source of risk for anxiety disorders, inhibited temperament is consistently linked to anxiety disorders (Biederman et al., 1993; Fox et al., 2005a; Hirshfeld et al., 1992; Hirshfeld-Becker et al., 2007; Kagan et al., 1987; Rosenbaum et al., 1993; Smoller et al., 2003). Behavioral inhibition is characterized as extreme withdrawal in the face of social and nonsocial challenges (Fox et al., 2005b; Kagan et al., 1989; Rosenbaum et al., 1991). Those with inhibited temperament exhibit excessive physiological reactivity to environmental challenges (Kalin et al., 2000; Kalin and Shelton, 2003; Keltikangas-Jarvinen et al., 1999; Perez-Edgar et al., 2007; Schwartz et al., 2003; Smoller et al., 2005; Tyrka et al., 2006; Tyrka et al., 2008).

Although there is support for temperament as a risk factor, the translation of risk to actualized disorder is unclear. Acquisition, expression and retention of avoidance may be the final common pathway to anxiety disorders. The particularly debilitating feature of avoidance is that, left untreated, avoidance increases over time and leads to a worsening of symptoms. Avoidance acquisition is more apparent in PTSD; the growth of avoidance traces the full expression of PTSD (Karamustafaloglu et al., 2006; Kashdan et al., 2006; North et al., 2004; O'donnell et al., 2006a). Given this prominent position, avoidance learning may represent an endophenotype for anxiety disorders (Gould and Gottesman, 2006).
Here, we present the case for inbred WKY rats to serve as a model for risk of anxiety disorders. Evidence is presented for the concordance of neurobehavioral, neuroendocrine, and neurochemical features to that observed in humans at risk for expressing an anxiety disorder. Particular emphasis is placed on enhanced avoidance acquisition and resistance to extinction as an endophenotype for vulnerability to anxiety disorders. Implications for treatment and efficacy are discussed.

2. Putative animal model for vulnerability to anxiety disorders - The WKY rat

The Wistar Kyoto (WKY) rat strain was first developed as a normotensive control strain for the spontaneously hypertensive rat (SHR) strain derived from the Wistar (WIS) rat (Okamoto and Aoki, 1963). Unlike its parent strain WIS rat, the WKY rat exhibits many unique behavioral characteristics differing from an out-bred rat strain. The most significant features are behavioral withdrawal, propensity to avoid, hyper-responsiveness to stress and hypervigilance (Drolet et al., 2002; Lemos et al., 2011; McAuley et al., 2009; Pare, 1992a; Pare, 1989b; Pare, 1992b; Pare, 1993; Solberg et al., 2001).

2.1 Temperament: Behavioral inhibition

The WKY rat displays features of inhibited temperament in a variety of situations (Braw et al., 2008; Ferguson and Cada, 2004; Malkesman et al., 2005; Pare, 1992b; Pare, 1994; Pare, 1996; Pare et al., 2001; Servatius et al., 1998; Tejani-Butt et al., 2003). For example, Figure 1 depicts activity in the open field test comparing WKY rats to outbred SD rats. Upon placement into the center of the open field (which is brightly lit), the WKY rat remains immobile for a period of time; the latency to leave the center segment is often 2-3 times as long as exhibited by outbred strains (Drolet et al., 2002; Ferguson and Cada, 2003; Nosek et al., 2008). This reluctance to leave the center segment is followed by slow deliberate exploration. However, activity will generally increase over several minutes. Hypolocomotion is not

![Open field activity in male and female WKY and SD rats](open_field_activity.png)

Fig. 1. Both female and male WKY rats display longer latencies to leave the center segment and overall lower numbers of segments crossed in a 2-min open field test. These data represent several studies (male: N = 60/strain; female: N=25/strain) and are obtained as the initial assessment of phenotype two weeks after delivery from breeders.
secondary to a motoric disturbance, in that WKY rats exhibit normal motor activity in a running wheel (Ferguson and Cada, 2003), a rotarod (Ferguson et al., 2003b) and a turning wheel avoidance task (Pare, 1992a). Together, these data suggest that the lack of movement is not physical, but psychological. Moreover, inhibited temperament is displayed by both female and male WKY rats compared to their outbred counterparts.

Inhibited temperament extends to more explicit nonsocial and social threats. In terms of social interactions, WKY rats generally exhibit normal play behaviors with conspecifics (Braw et al., 2006; Malkesman et al., 2006b), but reductions in play and more subordinate-type behaviors when faced with outbred rats (Ferguson and Cada, 2004). In response to an electrified probe, normal rats bury the probe; WKY rats simply freeze (Ahmadiyeh et al., 2005; Carr and Lucki, 2010; Gutiérrez-Mariscal et al., 2008; Pare, 1994). Thus, WKY rats represent an animal model of behavioral withdrawal in the face of social and non-social challenges.

2.2 Anxiety signs and symptoms
As stated earlier, the core feature of anxiety disorders is avoidance. There are a variety of expressions of avoidance; however, all will have a common process of acquisition and resistance to extinction. In addition to avoidance as a learned response, common features of anxiety disorders are altered arousal, social interaction, communication, attention, learning and memory. Still, each anxiety disorder has distinct features. Thus, endophenotypes may relate to the core features of avoidance learning, common characteristics (e.g., heightened arousal), or disorder-specific features (e.g., compulsions). Accordingly, behavioral assessments may be sensitive to a particular aspect or a general process concordant with anxiety.

2.2.1 Arousal
Arousal has two general aspects, the basal or undisturbed state and the relative magnitude of response to challenges. Moreover, arousal may be indexed through neuroendocrine or neurobehavioral measures. For each, the WKY has documented abnormalities.

Neuroendocrine and neurochemical. Within the hypothalamic-pituitary-adrenal axis (HPAA), levels of corticosterone (CORT) and adrenocorticotropic hormone (ACTH) are measured to evaluate arousal levels as affected by circadian rhythms and stress (Ottenweller et al., 1994). WKY rats has been proposed as a model of stress vulnerability, exhibiting exaggerated HPAA responses to stress regimens compared to common rat strains (Pare et al., 1999b; Pare and Kluczynski, 1997; Pare and Redei, 1993b; Redei et al., 1994). Basal peripheral ACTH and CORT levels are generally higher in WKY rats and remained significantly higher after the diurnal peak as compared to WIS rats (Solberg et al., 2001). Moreover, WKY rats exhibit a sustained CORT response to acute stress and an enhanced plasma ACTH response to various stressors (De La Garza II and Mahoney III, 2004; Malkesman et al., 2006a; Pare and Redei, 1993a; Rittenhouse et al., 2002). Others reported that CRH content and mRNA binding are not different in WKY rats relative to other strains suggesting that a defective negative feedback system may contribute to hyperresponsive HPAA in WKY rats (Gomez et al., 1996; Redei et al., 1994). Together, neuroendocrine evidence suggests that WKY rats are inherently hyperresponsive to stress. Exaggerated HPAA activity is reminiscent of inhibited temperament (Smoller et al., 2003; Smoller et al., 2005).
In general the neurochemical profile of the WKY rat is aberrant compared to outbred rats. WKY rats have inherently low butyrylcholinesterase level (Figure 2) and activity (Servatius et al., 1998), leading to greater sensitivity to cholinomimetics (Beck et al., 2001). Among the neurotransmitters, WKY rats have altered levels of monoamines, namely norepinephrine (NE), dopamine (DA) and serotonin (5-HT), and their metabolites as compared to out-bred strains with a high degree of specificity in various regions (De La Garza II and Mahoney III, 2004; Ferguson et al., 2003a; Pardon et al., 2003; Scholl et al., 2010). Moreover, these monoamine systems show greater responsiveness in the face of acute stress (Pardon et al., 2002; Pardon et al., 2000) and chronic stress (Pardon et al., 2003). We will discuss this point in a later section (section 5).

![Plasma butyrylcholinesterase in male SD and WKY rats](image)

Fig. 2. WKY rats exhibit significant lower level of butyrylcholinesterase compared to SD rats. (n=19-20/strain, p<.05)

Neurobehavioral. The acoustic startle response (ASR) is a simple reflex used to index arousal and vigilance in mammals (Ardekani et al., 1989). The ASR can be used to reveal differences in sensitivity (threshold to elicit a reflex response), responsivity (the magnitude of response), latency, as well as nonassociative processes of habituation, dishabituation and sensitization.

We and others demonstrated that WKY rats made larger startle responses to a white noise within a wide range (92dB to 120dB) as compared to other inbred and outbred rat strains (Glowa and Hansen, 1994; McAuley et al., 2009; Servatius et al., 1998). Of 45 inbred and outbred rat strains including SD rats, male WKY rats exhibited the highest ASR magnitude when exposed to 8 trials of 110dB white noise (Glowa and Hansen, 1994). We found that WKY rats exhibited significantly higher startle responsivity at 92 and 102dB white noise after correction for each subject’s body weight (Figure 3a). In addition to greater startle responsivity, male WKY showed higher sensitivity compared to male SD rats, measured by a multi-intensity startle test (3-ASR) (Figure 3b). Although both male and female WKY exhibit substantially higher ASRs compared to SD rats, only male rats demonstrate habituation when single intensity startle test (1-ASR) was used (Figure 4). Yet others reported that WKY show similar or lower ASR magnitude compared to SD rats (Buuse,
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We reasoned the inconsistency may be due to variant procedures used and whether subjects’ body weight was factored into the startle response.

![Graph](image)

**Fig. 3.** WKY rats display higher startle magnitude compared to the same sex outbred SD rats regardless of sex (n=12-17/strain/sex) (a). WKY rats of both sexes also exhibited greater sensitivity to respond, responding more to acoustic stimuli of moderate intensity (b).

WKY rats exhibited exaggerated stress response and elevated arousal following stress stimulation. Stress has been described as one of the key risk factors of anxiety disorders (Chantarujikapong et al., 2001; Grillon et al., 2007b; Mineka and Zinbarg, 1996). As described in previous literatures, WKY rats are behaviorally hyperresponsive to stress with the stress-induced exaggerated HPAA response (Redei et al., 1994; Solberg et al., 2001). Inasmuch as the basal behavioral state of WKY is abnormal, assessing the impact of stress on behavioral reactivity has been problematic. For example, assessing freezing to context or to cues is difficult given the propensity to freeze in novel situations. However, prior acute stress have been noted to increase freezing behavior and reduce activity in the OFT and elevated plus maze in WKYs (Nosek et al., 2008). When challenged in the forced swim test (FST), the WKY rat predominantly exhibits floating behavior and fewer struggling responses compared to other strains. The lack of struggling has been interpreted as ‘behavioral despair’, a sign of depression-like behavior in rodents (Malkesman and Weller, 2009; Pare, 1992a; Pare and...
Startle habituation in male and female SD and WKY rats

![Graph showing startle habituation in SD and WKY rats](image)

Fig. 4. Habituation of acoustic startle responses in WKY and SD rats: nonassociative processes. Although WKY rats generally display larger ASRs than SD rats, habituation appears normal (n=8/strain/sex).

Redei, 1993a). The heightened stress reactivity is most clearly evident in the enhanced susceptibility to develop stress-induced ulcers in WKY rats compared to outbred strains (Pare, 1989a; Pare, 1989c; Pare and Schimmel, 1986). Pretreatment with drugs that elevate central monoamines reduce the severity of ulceration (Pare et al., 1999a; Tejani-Butt et al., 2003). Evidence from elevated arousal or exaggerated stress response in WKY rats may provide insight to the alterations in the CNS neurochemistry that may be responsible for anxiety. The effects of stress are discussed in the following sections in more detail.

2.2.2 Sleep disturbances

Sleeping disruption is one of the major symptoms of anxiety disorder and a hallmark of PTSD (Ross et al., 1989). WKY rats exhibit altered sleep-wake cycle and longer rapid eye movement sleep (REMS) episodes compared to other strains (Dugovic et al., 2000). REMS fragmentation was significantly altered following stress in WKY rats compared to control strain (DaSilva et al., 2011; Dugovic et al., 2000; Laitman et al.). Altered sleep patterns may also preexist as a vulnerability to anxiety, that are further disturbed after exposure to psychological distress.

2.2.3 Avoidance

As the core symptom of all anxiety disorders, avoidance behavior differs between patients with anxiety disorder and normal population (Foa et al., 2006; O’Donnell et al., 2006b). In
humans avoidance is characterized in the form of emotions, ideations, and behaviors. In animal models, avoidance is characterized as passive (withholding a likely response to avoid aversive stimulation) and active (performing a target response to prevent aversive stimulation). Early work showed WKY rats exhibit superior acquisition in passive avoidance tasks compared to SD or WIS rats (Pare 1993; Pare 1996). Given that being immobile/freeze is the dominant coping strategy for WKY rats, superior acquisition of passive avoidance is not a surprise. However, when tested with a wheel-turning avoidance task, WKY performed equally well compared to WIS rat (Pare, 1992a). In contrast, Berger and Starzec found that WKY rats performed poorly in lever-press avoidance task compared to SHR rats (Berger and Starzec, 1988). As an arbitrary target response, a leverpress is not among the species specific defense reactions (Bolles, 1970). We reasoned that the inconsistency between studies and laboratories may due to the procedure applied, the nature of the test and the reference strain to which WKYs were compared.

In our institute, we utilized a signalled lever-press avoidance paradigm to study anxiety and its vulnerability in rats. It is known that anxiety is a disorder that develops over a period of time, so is the avoidance. Thus, a lever-press avoidance learning model allows the acquisition of avoidance to develop over an extended period of time, mimicking the developmental process of anxiety in humans. Our data indicated that the learning of WKY rats is superior in the lever-press avoidance task compared to a noninhibited reference strain, the SD rat (Beck et al., 2010; Jiao et al., 2011; Servatius et al., 2008). The superior active avoidance performance of WKY rats is in stark contrast to other rat strains with features of trait behavioral inhibition such as the Maudsley High Reactive (Blizard and Adams, 2002), which are generally poor in active avoidance. Moreover, rats bred for superior active avoidance are typically the least behaviorally inhibited (Syracuse high avoiders, Roman high avoiders and Australian high avoiders) (Aguilar et al., 2004; Brush, 2003; Driscoll, 1986; Overstreet et al., 1990; Overstreet et al., 1992).

Over the past several years we have amassed a substantial database concerning the avoidance performance of WKY rats. For one, the avoidance performance of WKY rats reaches asymptotic levels that approach unity; that is, once acquired WKY rats typically exhibit near perfect avoidance (Figure 5.). That perfect avoidance begins with the first trial of a session. Outbred rats display a typical pattern of avoidance performance in which each session begins with escape responses, although avoidance was expressed at the end of the previous session (i.e., warm up) (Hineline, 1978a; Hineline, 1978b). WKY rats generally do not exhibit warm up as acquisition progresses. This near perfect expression of avoidance resembles human avoidance. Facilitated avoidance acquisition is apparent in both female and male rats compared to their respective outbred counterparts. The near perfect avoidance behavior also insulates the rat from experiencing changes in shock contingencies. Accordingly, WKYs display perseveration of avoidance responding in the absence of shock, but continued presence of the explicit safety signal (Servatius et al., 2008). Perseveration/resistance to extinction has been implicated in neuropathology of anxiety (Barad, 2005; Myers and Davis, 2002).

WKY rats also display another interesting and potentially clinically-relevant feature. Each training session begins with a 60-s stimulus free period prior to the first warning signal. As WKY rats acquire avoidance they emit bar presses, which are not reinforced, during this period (Figure 6.). This pattern of response is only exhibited prior to the first trial; nonreinforced responses are rarely displayed on subsequent trials. These nonreinforced
responses may be akin to worry (Mineka, 2004), accompanying avoidance acquisition only in those at risk.

Acquisition and extinction in a lever-press avoidance task

Fig. 5. Avoidance responses made during acquisition and extinction in WKY and SD rats. WKY rats acquired lever-press avoidance faster and to a higher degree (sessions 1-10). However, WKY rats extinguished slower during early extinction phase while the transition between acquisition and extinction was more significant in SD rats (sessions 11-23, shock-off, safety signal on; sessions 24-28, shock-off, safety signal off). (N=8/strain)

Lever-press during the 1st min of each session in male and female SD and WKY rats

Fig. 6. WKY rats emitted more anticipatory lever-presses during the initial minute of each session during acquisition. (N=8-10/strain/sex)

On the other hand, stress intensity is often cited as a contributing factor in the development of anxiety disorders (Braunstein-Bercovitz et al., 2001; Foa et al., 2006; Grillon et al., 2007b; Grillon et al., 2007a; Mineka and Zinbarg, 1996; Silver et al., 2002). Given the relationship between anxiety disorders and avoidance, one expects stress to accelerate avoidance acquisition, raise the asymptotic levels, or affect extinction. The results from a recent study
suggest that stressor intensity only affects extinction. Training with a greater shock intensity than our standard, did not affect the acquisition curves of either SD or WKY rats (Figure 7.) (Jiao et al., 2011). Of course, there is little room to enhance asymptotic performance of WKY rats, but the rate to reach this level could differ. However, WKY rats trained with higher intensity shock exhibited perseveration of avoidance response during extinction compared to WKY rats trained with the lower intensity shock; extinction curves of SD rats resembled WKY rats trained with a lower intensity.

A long standing discrepancy between the basic science literature and clinical descriptions concerns the relationship between avoidance acquisition and arousal. In rats, arousal decreases as avoidance is acquired (Coover et al., 1973). However, arousal is sustained in humans with anxiety disorders and who are employing avoidance. Therefore, we assessed ASRs prior to avoidance acquisition and toward the end of acquisition training. Whereas the ASRs of SD rats are virtually unchanged between the measures, the ASRs of WKY rats increase (Figure 8.). The increase is evident at a period of training in which WKY rarely, if at all, experience shock. This increase is beyond the basal exaggerations normally noted between strains. There is emerging data that exaggerated ASRs in PTSD may be increases beyond preexisting ASR differences; that is, exaggerated ASRs are an amplification of a preexisting condition (Guthrie and Bryant, 2005). These data suggest that exaggerated ASRs are an interaction of subject vulnerabilities and avoidance acquisition.

**LEVER-PRESS RESPONSE AS A FUNCTION OF SHOCK INTENSITY**

![Graph showing lever-press response as a function of shock intensity](image)

Fig. 7. WKY rats acquired lever-press avoidance faster and to a higher degree regardless of shock intensity. However, WKY rats trained with 2.0-mA foot-shock resisted extinction while the transition between acquisition and extinction was more significant in all SD rats and WKY rats trained with 1.0-mA foot-shock (Jiao et al., 2011).
Fig. 8. WKY and SD rats were tested for 1-ASR before and after 10 sessions of avoidance acquisition. In both tests, WKY startled with a higher magnitude than SD rats. WKY rats exhibited elevated startle response following acquisition training while SD showed similar startle magnitude in both tests. (N=24/strain; strain difference, p<.05; test difference, p<.05)

3. Genetic components

Genetic components (trait vulnerability) play an important role in various psychiatric disorders, including anxiety disorders. Quantitative trait loci (QTL) analysis indicated that common loci, which influence certain behavioral characteristics tested by OFT and defensive bury test in rats, may represent genetic factors contributing to anxiety and depression (Ahmadiyeh et al., 2005; Boyle and Gill, 2001; Cloninger et al., 1998; Henderson et al., 2000; Solberg et al., 2004). Several QTL (Imm 1 D2Rat188, Imm3 D5Rat40, Imm6 D16Arb5, Climb2 D1Rat147, FST1 D16Rat75) were identified for climbing, immobility and swimming in WKY rats, sharing common target regions with susceptibility loci mapped by genome scan analyses for emotionality QTL in rodents and human genetic linkage to emotional disorder (Solberg et al., 2004). Significance of microarray analysis (SAM) revealed that expression of 66 genes was increased in the locus coeruleus (LC) of WKY compared to SD rats (Pearson et al., 2006), including genes that encoded for enzymes involved in NE turnover. Moreover, the mRNA of catechol-O-methyltransferase (COMT), a key enzyme in the catabolism of catecholamines, was found at levels four- to sevenfold higher in the cerebral cortex in WKYS than SDs (Walker et al., 2004). Thus, this rat strain may be genetically predisposed to psychiatric disorders that are linked to altered monoaminergic system.

4. Brain anatomy and neuronal activity

Converging data from structural and functional magnetic resonance imaging (MRI) studies suggest that differential patterns of anatomical brain abnormalities appear to be involved in mood and anxiety disorders (Brambilla et al., 2002; Lind et al., 2006; Milad et al.; Ohara et al., 2004). Abnormalities were found in orbital frontal lobe, basal ganglia, temporal lobe and hippocampus in patients with various subtypes of anxiety (Brambilla et al., 2002; Bystritsky et al., 2001; McEwen, 2005). Moreover, imaging data from humans suggest that medial prefrontal cortex, amygdala, thalamus and periaqueductal gray are involved in avoidance
acquisition (Mobbs et al., 2007; Samanez-Larkin et al., 2008; Simmons et al., 2006; Straube et al., 2009; Suslow et al., 2009). However, the neurocircuitry underlying avoidance extinction and its perseveration in anxiety states is largely a matter of speculation. Mainly supported by fear extinction studies, mPFC plays a key role in both humans and experimental animals (rodents). Anxiety patients exhibit reduced activity of the anterior cingulate cortex and thalamus during episodes of re-experiencing (Hopper et al., 2007; Lanius et al., 2003), leading to a suggestion that reduced cortical influence on structures such as the amygdala may explain the resistance to extinction. Reduced cortical influence on the amygdala is also presumed to underlie the persistent expression of heightened arousal (e.g., increased acoustic startle) observed in PTSD patients (Nutt and Malizia, 2004).

The knowledge of anatomical difference between WKY and other rats is quite limited. A recent volumetric study evaluated hippocampus in female rats in which the hippocampal volume of WKY rats is 20% less than that of WIS rats (Lemos et al., 2011). On the other hand, alterations in neuronal activation are demonstrated in the expression of c-Fos or brain derived neurotrophic factor (BDNF) in various brain regions in WKY rats compared to out-bred rat strains (Ma and Morilak, 2004; O’Mahony et al., 2011). We recently reported that the c-Fos immunoreactivity is lower in the medial prefrontal cortex in the WKY rat than the SD rat at the end of extinction of a lever-press avoidance task, whereas a reduced GABAergic activation was found in basal amygdala in the WKY rat trained with higher shock intensity (Jiao et al., 2011).

However, the structures that are critical for acquisition and extinction of lever press avoidance are relatively unknown. In our initial work, we assessed c-Fos immunoreactivity in various regions in the SD rat at multiple time points within the phases of acquisition and extinction. Our results indicated that there is a trend of increased c-Fos expression in the prefrontal regions as extinction starts and proceeds. Interestingly, a similar pattern was observed in the lateral and basolateral amygdala where a reduced activity was expected during extinction. A further analysis targeting GABAergic neuron revealed that GABAergic activation arises during the extinction phase. An elevated GABAergic activation (detected by the double staining for c-Fos and parvalbumin (PV, a calcium binding protein that is expressed in 50-60% amygdalar GABAergic neurons (Kemppainen and Pitkanen, 2000)) in the basolateral and lateral amygdala would reduce the excitatory output from the amygdala as GABA neurons in the basolateral amygdala are mainly interneurons that make synaptic contact on projection neurons (Rosenkranz and Grace, 2001). Thus an elevated GABAergic activity may be responsible for the increased neuronal activation during extinction phase in the basolateral amygdala. However, this assumption needs further investigation since parvalbumin positive neurons represent 50-60% of GABAergic neurons in the basolateral amygdala (Kemppainen and Pitkanen, 2000). We do not as yet know whether these neuronal alterations are present or different in WKY rats.

From a different perspective, enhanced avoidance learning may be secondary to deficits in neurotrophins. Converging data supports the role of neurotrophins in mood disorders; one of the latest theories of the neuropathology of anxiety and depression disorders (Chen et al., 2006; Martinowich et al., 2007). For example, low levels of the brain derived neurotrophic factor (BDNF) are found in stress-related disorders in humans (Duman and Monteggia, 2006). In general, lower BDNF is related to anxiety and depression disorders that are not responsive to serotonergic antidepressant treatment (Duman, 2004; Kaluelf et al., 2006). Consistent with this perspective, a recent study illustrated that BDNF levels are significantly lower in the CA3 of hippocampus in WKY rats compared to SD rats (Malkesman et al., 2009).
5. Potential mechanisms in psychopharmacology

Alterations in neurochemistry in the CNS are implicated in various psychiatric disorders (i.e. depression, addiction and anxiety). Recent investigations targeting central neurochemical pathways have enhanced our understanding of anxiety disorders.

5.1 Serotonin (5-HT)

Evidence for the association of altered serotonergic activity in anxiety, that decreasing serotonergic function is anxiogenic and increasing it anxiolytic, is mostly supported by the use of selective serotonin reuptake inhibitors (SSRIs) in various sub-types of anxiety disorders (Bremner, 2006; Vaswani et al., 2003). WKY rats showed significant lower basal 5-HT tissue level in limbic regions and cell body area compared to WIS or SD rats (De La Garza II and Mahoney III, 2004; Scholl et al., 2010). Acute stress elicits an increased tissue level of 5-HT in the amygdala in WIS but not in WKY rats, it increased 5-HT turnover rate in the mPFC only in WKY rats (De La Garza II and Mahoney III, 2004). When exposed to chronic stress, WKY rats failed to show stress-induced reduction of 5-HT tissue level as SD rats did, whereas the turnover rate was increased in both WKY and SD rats (O’Mahony et al., 2011). Previous studies showed increased binding of 5-HT₁₄ receptors in hippocampus and hypothalamus, but decreased binding of 5-HT transporters in the cell body area in WKY rats following chronic stress, compared to SD rats (Pare and Tejani-Butt, 1996). However WKY rats are insensitive to serotonergic drugs (e.g. SSRIs and receptor agonists) in terms of activity in the EPM and OFT, immobility in the FST, or severity of gastric ulceration after stress (Chaouloff et al., 1998; Griebel et al., 1999; Lahmame and Armario, 1996; Lopez-Rubalcava and Lucki, 2000; Pare et al., 2001; Tejani-Butt et al., 2003), suggesting serotonergic manipulation may not affect the temperamental behaviors of these rats.

5.2 Dopamine (DA)

Converging literatures demonstrate that an aberrant DA circuitry is associated with anxiety disorders (Gendreau et al., 1998; Hamner and Diamond, 1996; Mathew et al., 1981; Taylor et al., 1982). WKY rats exhibit altered dopaminergic function in various brain regions. DA levels in WKY rats do not differ between WIS or SD rats in most brain areas; DA turnover is higher in the nucleus accumbens shell in the WKY rat compared to the WIS rat (De La Garza II and Mahoney III, 2004; Ferguson et al., 2003a; Scholl et al., 2010). Receptor and transporter binding studies show that WKY rats have altered dopaminergic pathways compared to control strains. The results from those studies reveal a significant strain difference, with WKY rats exhibiting lower levels of D₁ receptor binding in the caudate putamen and nucleus accumbens core, but higher binding levels in the substantia nigra pars reticulata compared to WIS rats (Novick et al., 2008). D₁ receptors in the substantia nigra are involved in mediating the startle response (Meloni and Davis, 1999), thus a higher D₁ receptor level in this region may lead to heightened ASR magnitude in WKY rats. Results from a recent study demonstrated that WKY rats exhibited higher D₂ receptor binding levels in the nucleus accumbens shell and ventral tegmental area, but lower D₂ receptor binding in the caudate putamen, nucleus accumbens core and hypothalamus compared to WIS rats (Yaroslavsky et al., 2006). It is known that the D₁ and D₂ receptors represent critical sites where DA acts to modify behavior related to anxiety and reward; the altered expression of this receptor in the WKY rat may be reflective of the anxiety susceptibility noted in this rat strain. Moreover, DA transporter binding levels were lower in the nucleus accumbens core,
amygdala and cell body regions (ventral tegmental area and substantia nigra), but higher in the hippocampus and hypothalamus compared to SD and WIS rats (Jiao et al., 2003). The observed differences in the density and distribution of DAT sites in WKY rats may lead to altered modulation of synaptic DA levels in the cell body and mesolimbic regions, thereby contributing to the noted anxiety- and depression-like behaviors reported in this rat strain. This speculation was supported by a further study in which some of the alterations in DAT binding was reversed by chronic nomifensine (i.e. a dopamine transporter blocker) treatment (Jiao et al., 2006). Moreover, after 8-12 days of nomifensine administration, WKY rats showed significantly increased head poke responses in the emergence test, reduced latency to leave the center in an open field and increased activity in the FST (Tejani-Butt et al., 2003). Therefore, the WKY rat may represent a good model for a sub-type of anxiety disorder exhibiting imbalanced DA distribution in the CNS.

5.3 Norepinephrine (NE)
Defective noradrenergic function is one of the major mechanisms in the neuropathology of anxiety in human (Bremner et al., 1996; Charney and Redmond, Jr., 1983; Hamner and Diamond, 1996; Neumeister et al., 2005; Sullivan et al., 1999). The WKY appears to have normal tissue levels of NE (De La Garza II and Mahoney III, 2004; O'Mahony et al., 2011; Scholl et al., 2010) and tyrosine hydroxylase (TH, the rate-limiting enzyme in NE synthesis) (Mann and Bell, 1991; Vachette et al., 1993). In response to acute stress, noradrenergic reactivity appears to be blunted. For example, acute stress-induced increases of NE tissue levels in the lateral bed nucleus of the stria terminalis (BSTL) were significantly lower in WKY compared to SD rats (Pardon et al., 2002; Pardon et al., 2003). Moreover, the acute stress-induced increases in neuronal activity (cFos expression) in medial amygdala and locus ceruleus (LC) was lower in WKY rats compared to SD rats (Ma and Morilak, 2004). Alternatively, NE reactivity may be delayed. Acute stress-induced increases of TH mRNA level in WKY rats were apparent, but delayed by 2-hr in WKY rats compared to control strains (Pardon et al., 2002; Sands et al., 2000). Although reactivity to acute stress is blunted, chronic stress appears to sensitize NE in WKY rats. After chronic exposure to cold stress, increased BSTL NE release was induced by acute stress challenge only in WKY rats, but not in SD rats (Pardon et al., 2003). Chronic stress decreases NE transporter binding in the cell body area and decreases α2 and β receptor binding in terminal regions, suggesting aberrant NE modulatory responses towards stress in WKY rats (Tejani-Butt et al., 1994; Zafar et al., 1997). Although the NE response to acute stress is blunted, WKY rats may exhibit less habituation to stress appearing as larger levels of reactivity as the reactivity of SD rats declines. A sustained NE response may underlie hypervigilance and elevated arousal in response to specific or generalized stress (Cameron et al., 2004; Lahdesmaki et al., 2002; Maes et al., 2002; Schramm et al., 2001). The efficacy of NE-targeted drugs in altering behaviors relevant to anxiety is limited. Much of the past research has focused on altering behaviors in the FST. Drugs which act by blocking NE transporters reduced immobility in FST and increase activity in the OFT in WKY rats (Lahmame and Armario, 1996; Lucki and Nobler, 1985; Pare, 1992b; Pare et al., 2001; Will et al., 2003). Thus, increasing NE availability affects inhibited temperament of WKY rats.

5.4 Others
There is a dearth of knowledge about strain difference between the WKY rat and comparison strains in the glutamatergic and the GABAergic pathways. A recent
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An autoradiographic study demonstrated that WKY rats exhibited lower N-methyl-d-aspartate (NMDA) receptor binding in anterior cingulate cortex, caudate putamen, nucleus accumbens, CA1 of hippocampus and substantia nigra compared to WIS rats (Lei et al., 2009). Interestingly, chronic stress increased NMDA receptor binding in the prefrontal cortex, caudate putamen and nucleus accumbens only in the WKY rat (Lei and Tejani-Butt, 2010). Thus the authors speculated that NMDA receptors in these regions may be more sensitive to stress in WKY rats. Consistent with the previous report (Jiao et al., 2011), our recent preliminary data suggest that naïve WKY rats exhibited altered density of PV immunoreactive cells in the amygdala and prefrontal cortex compared to SD rats (Table 1.). Although basal tissue level of GABA does not differ between WKY and WIS rats, stress increased it only in WKY rats (O’Mahony et al., 2011). Moreover, the levels of GABA-A receptor binding are higher in amygdala, caudate putamen, CA2 and CA3 of hippocampus, periaqueductal gray and substantia nigra in WKY compared to WIS rats (Lei et al., 2009). Given the role of GABA and GABA-A receptor in the pathophysiology of stress and anxiety, future investigation should emphasize GABAergic system in order to understand the unique behavioral aspects in WKY rats, a field that has not been studied sufficiently in this strain. So far only one report demonstrated the effect of diazepam on WKY rats measured by emergence test (Pare et al., 2001). Single dose of diazepam reduced WKY rats’ activity in an OFT. Thus the effects of pharmacological manipulation on GABAergic system need further investigation in WKY rats.

<table>
<thead>
<tr>
<th>REGIONS</th>
<th>STRAIN</th>
<th>MEAN PV (ir) CELL DENSITY ± S.E.M. (CELL COUNTS/mm3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior Cingulate</td>
<td>SD</td>
<td>2208.08 ± 716.27</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>3503.53 ± 111.97</td>
</tr>
<tr>
<td>Prelimbic Cortex</td>
<td>SD</td>
<td>2364.65 ± 281.96</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>3181.51 ± 241.74</td>
</tr>
<tr>
<td>Infalimbic Cortex</td>
<td>SD</td>
<td>2891.72 ± 408.16</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>2989.72 ± 442.44</td>
</tr>
<tr>
<td>Basal Amygdala</td>
<td>SD</td>
<td>2939.07 ± 211.16</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>1786.03 ± 445.31</td>
</tr>
<tr>
<td>Lateral Amygdala</td>
<td>SD</td>
<td>2294.93 ± 251.59</td>
</tr>
<tr>
<td></td>
<td>WKY</td>
<td>1427.58 ± 265.70</td>
</tr>
</tbody>
</table>

Table 1. PV (ir) positive cell densities in the prefrontal cortex and amygdala in WKY and SD rats. (N=4/strain)

5.5 Summary

The WKY rat exhibits a neurochemical profile reminiscent of anxiety disorders. The profile is also reminiscent of behavioral inhibition in nonhuman primates (Kalin, 2004; Kalin and Shelton, 2003; Kalin et al., 2007). Past research has focused on the WKY as animal model of depression owing to the stress reactivity, susceptibility to ulcers, and reduced FST. Faster avoidance acquisition, avoidance perseveration and sustained arousal induced by avoidance learning are strong signs of anxiety vulnerability. Thus, investigation of psychotropic drugs...
in their ability to affect avoidance may have relevance for treatment of human anxiety. Recent findings of GABAergic activity selective to avoidance acquisition and extinction in WKY rats suggest a novel target for this class of anxiolytics.

6. Overall summary
Anxiety disorders develop as an interaction of trait vulnerabilities (e.g., behavioral inhibition), early life experiences and environmental exposures. The interplay of these influences determines success or failures to effectively cope, especially under stress. WKY rats model trait inhibited temperament, a risk factor for anxiety disorders. Accordingly, WKY rats have neurobehavioral, neuroanatomical, neurochemical and neuropharmacological features consistent with inhibited temperament. In particular, WKY rats acquire active avoidance faster; avoidance that is resistant to extinction. Moreover, WKY rats display two behavioral features which are hallmarks of anxiety as avoidance develops: worry and increased arousal. In the respect of neurochemistry and neuroendocrine, WKY rats demonstrated over stimulated NE circuitry and exaggerated HPAA activity in response to stress stimulation, and innate 5-HT deficit together with altered receptors function in monoamine systems and GABAergic system, which all contribute to hyper-reactivity towards stress and the phenotypes that related to anxiety behavior in this rat strain. Together, these significant findings suggest that the WKY rats should be studied as an animal model of vulnerability to develop anxiety disorders.

7. Acknowledgement
This research was supported by NIH grants and a Department of Veterans Affairs Merit Review research program and the Stress & Motivated Behavior Institute of the New Jersey Medical School. The described experimentations were conducted with approval by the VANJHCS Institutional Animal Care and Use and Research and Development Committees. The authors would like to thank the technical expertise of Toni Marie Dispenziere, Tracey Longo and Amanda Agley in the behavioral testing of the animals in these experiments.

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Anxiety, whether an illness or emotion, is a term with historical roots even in the Bible, but it was not popular until the modern age. Today, we can group, diagnose and treat several anxiety disorders to an extent, but the assessment of symptoms and severity, dealing with resistant conditions, new treatment modalities and specific patient population, such as children, are still the challenging aspects of anxiety disorders. This book intends to present anxiety disorders from a different view and discuss a wide variety of topics in anxiety from a multidimensional approach. This Open Access book addresses not only psychiatrists but also a broad range of specialists, including psychologists, neuroscientists and other mental health professionals.

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