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Fear conditioning fragments REM sleep in stress-sensitive Wistar-Kyoto, but not Wistar, rats

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Abstract

Pavlovian conditioning is commonly used to investigate the mechanisms of fear learning. Because the Wistar-Kyoto (WKY) rat strain is particularly stress-sensitive, we investigated the effects of a psychological stressor on sleep in WKY compared to Wistar (WIS) rats. Male WKY and WIS rats were either fear-conditioned to tone cues or received electric foot shocks alone. In the fear-conditioning procedure, animals were exposed to 10 tones (800 Hz, 90 dB, 5 sec), each co-terminating with a foot shock (1.0 mA, 0.5 sec), at 30-sec intervals. In the shock stress procedure, animals received 10 foot shocks at 30-sec intervals, without tones. All subjects underwent a tone-only test both 24 hrs (Day 1) and again two weeks (Day 14) later. Rapid eye movement sleep (REMS) continuity was investigated by partitioning REMS episodes into single (inter-REMS episode interval > 3 min) and sequential (interval ≤ 3 min) episodes. In the fear-conditioned group, freezing increased from baseline in both strains, but the increase was maintained on Day 14 in WKY rats only. In fear-conditioned WKY rats, total REMS amount increased on Day 1, sequential REMS amount increased on Day 1 and Day 14, and single REMS amount decreased on Day 14. Alterations were due to changes in the number of sequential and single REMS episodes. Shock stress had no significant effect on REMS microarchitecture in either strain. The shift toward sequential REMS in fear-conditioned WKY rats may represent REMS fragmentation, and may provide a model for investigating the neurobiological mechanisms of sleep disturbances reported in posttraumatic stress disorder.

Keywords

Fear conditioning; Posttraumatic Stress Disorder; REMS microarchitecture; Sleep; Wistar-Kyoto rat

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1. Introduction

Posttraumatic Stress Disorder (PTSD) is an anxiety disorder that can develop after a terrifying experience (American Psychiatric Association, DSM-IV-TR). The diagnosis of PTSD requires that the individual has been exposed to a traumatic event and has experienced symptoms, for at least one month, within each of three symptom clusters, re-experiencing, avoidance, and hyperarousal (American Psychiatric Association, DSM-IV-TR). It has been argued previously that the sleep disturbance in PTSD is the hallmark of the disorder (Ross et al., 1989), entering into the diagnostic criteria twice: 1) as hyperarousal in the form of insomnia, and 2) as re-experiencing the traumatic event in the form of repetitive nightmares. A greater number of REMS interruptions has been observed in PTSD patients (Breslau et al., 2004; Habukawa et al., 2007; Mellman et al., 2002), and increased REMS phasic muscle activity has been reported in combat veterans with PTSD (Mellman et al., 1997; Ross et al., 1994a, 1994b).

Animal models have been widely used in behavioral research to exploit genetic differences in key components of the stress response, such as anxiety-like behavior and hypothalamo-pituitary-adrenal axis (HPA) function (Shepard and Myers, 2008). Pavlovian conditioning is commonly used in rodent studies to investigate mechanisms involved in associative learning. For example, cued fear conditioning (CFC) utilizes the pairing of a neutral conditioned stimulus (CS), a tone for example, with an aversive unconditioned stimulus (US), such as an electric foot shock, so that the CS acquires fear-inducing properties similar to those produced by the aversive stimulus. In rodents, alterations in rapid eye movement sleep (REMS) have been proposed as a sensitive index of fear conditioning (Jha et al., 2005; Pawlyk et al., 2005; Pawlyk et al., 2008; Sanford et al., 2003). However, findings from studies in rodents of the effects of stress on sleep-wake behavior have been shown to vary depending on the stress paradigm, species, strain, and gender (Andersen et al., 2009; Gómez et al., 1998; Jha et al., 2005; Papale et al., 2005; Pawlyk et al., 2005; Pawlyk et al., 2008).

The Wistar-Kyoto (WKY) rat strain is known to be particularly sensitive to stress. For example, WKY rats compared to other strains have a greater susceptibility to stress-induced gastric ulcers (Paré, 1990, 1992, 1994a, 1994b; Paré and Redei, 1993) and demonstrate greater immobility in the Forced Swim Test (FST) (Armario et al., 1995; Paré, 1992, 1994a; Tejani-Butt et al., 1994). They also exhibit higher levels of emotionality and freezing behavior in stressful conditions and lower exploratory behavior in the Open Field Test (OFT) (Paré, 1992, 1994a; Tejani-Butt et al., 1994). WKY rats easily develop signs consistent with anhedonia in a novel environment (Paré, 1993), and they spend longer time in the closed arm in the Elevated-Plus Maze Test (Paré, 1992; Paré et al., 1999). Although WKY rats readily acquire avoidance behavior, compared to control strains they are more resistant to behavioral extinction (Berger and Starzec, 1988; Paré, 1993, 1996; Servatius et al., 2008).

The present study investigated the effects of CFC on sleep-wake behavior in WKY rats compared to a control, Wistar (WIS), rat strain. To confirm that the alterations observed were due to fear conditioning and not due to a residual effect of shock stress (SS), we studied the effects of shock alone in an additional group of animals. We hypothesized that CFC would produce greater long-term alterations in anxiety-related freezing behavior and REMS microarchitecture in WKY rats, as compared to WIS rats. In addition, we hypothesized that alterations in sleep-wake behavior from SS alone would be less pronounced than those alterations produced by CFC in WKY and WIS rats.

2. Methods

2.1. Subjects

Male WKY and WIS rats, 8 weeks of age, were purchased from Charles River Laboratories. Upon arrival, animals were individually housed for a 1-week acclimation period in a temperature ($22 \pm 2^\circ \text{C}$) and humidity ($45 \pm 15\%$) controlled animal colony located in the University of Pennsylvania School of Veterinary Medicine. Subjects were given ad lib access to food and water, except during the 10-min training period, and they were maintained on a 12-hr light/dark cycle, with lights on at 0700 hrs. Rats within each strain were assigned to either the CFC group ($N = 6\text{--}7/\text{strain}$) or the SS group ($N = 4/\text{strain}$). All experimental procedures were approved by, and conducted in accordance with, the Institutional Animal Care and Use Committee of the University of Pennsylvania.

2.2 Surgical Procedure

All surgical procedures were performed stereotaxically under aseptic conditions. A mixture of ketamine (85 mg/kg, i.m.) and xylazine (15 mg/kg, i.m.) was injected to induce anesthesia, which was then maintained with isoflurane gas (0.25%). Following the induction of anesthesia, the surgical field was clipped and thoroughly cleaned with betadine and alcohol and then draped. The animal's head was placed in the stereotaxic apparatus and secured using blunt ear bars. A midline incision exposed the skull and dorsal cervical musculature to implant electrodes for chronic electroencephalogram (EEG) and electromyogram (EMG) recording. Two pairs of stainless steel screw electrodes were affixed to the skull above the frontal and sensorimotor cortices for recording the EEG, and one single screw electrode was implanted as a reference. Two insulated stainless steel wire electrodes were attached bilaterally to the dorsal neck muscles for recording the EMG. Leads from the electrodes were routed to a 9-pin miniature connector and cemented onto the skull with dental acrylic. Animals were given meloxicam (0.2 mg/kg, i.m.) as an analgesic, both prior to surgery and again 24-hrs post-surgery. Gentamicin (5 mg/kg, s.c.) was diluted in lactated Ringer's solution and given post-surgery as an antibiotic. Animals had a 1-week post-surgery recovery period.

2.3. Sleep Recording and Signal Processing

Based on previous observations from this laboratory (Jha et al., 2005; Madan et al., 2008; Pawlyk et al., 2005), we chose to record sleep from 11 AM – 3 PM. In addition, Dugovic et al. (2000) reported 24-hr sleep recording in WKY and WIS rats, confirming that 11 AM – 3 PM is the time in which both strains exhibit the greatest amount of sleep and the least amount of wakefulness. During sleep recording, rats remained individually in their home cage, which was placed in a sound-dampened chamber (1m^3). They were attached to a cable counter-weighted and connected to a 12-channel, freely rotating swivel (SL6C, Plastics One). The light in the recording chamber was maintained on a 12-hr light/dark cycle, with lights on at 0700 hrs, and temperature was controlled at $22^\circ \pm 2^\circ \text{C}$. EEG and EMG data were collected using a Grass Model 7 polygraph (Grass-Telefactor, USA) amplifier system, and recorded on a PC using Spike 2 software (Cambridge Electronics, UK). EEG and EMG signals were amplified using high-pass (EEG: 0.3 Hz; EMG: 10 Hz) and low-pass (EEG: 100 Hz; EMG: 100 Hz) filters and digitized by CED Power-1401 (Cambridge Electronics, UK) as used by Madan et al. (2008). Behavior was recorded via mini-video cameras mounted inside the recording chamber.

2.4. Cued Fear Conditioning (CFC) and Shock Stress (SS) Procedures

Following surgery and a 1-week recovery period, rats were habituated to handling and to the sleep recording procedure in a recording room for 4 hrs (11 AM – 3 PM) each day over 3

days. The next day (Baseline), animals had a baseline 4-hr sleep recording (11 AM – 3 PM) in this room. One day later (Training Day), animals were entered into either the CFC or the SS protocol. In the CFC procedure, rats received 10 presentations of a tone (CS: 800 Hz, 90 dB, 5 sec) co-terminating with a mild electric foot shock (US: 1.0 mA, 0.5 sec) at 30-sec intervals. In the SS procedure, rats received 10 mild electric foot shocks (1.0 mA, 0.5 sec) at 30-sec intervals, without tones. Foot shocks were transmitted through the grid floor of a Coulbourn Instruments Habitest operant cage placed in a training chamber in a training room different from the recording room. Tones were produced by a tone generator (Coulbourn Instruments Precision Shock Generator). Then, 24 hrs after training (Day 1), and again 2 weeks later (Day 14), animals in both the CFC and SS groups were returned to the recording chamber in the recording room for a test recording, which followed exposure to 3 tone presentations, without foot shock, at 30-sec intervals. To minimize contextual effects, the CFC and SS procedures (Training Day) were conducted in an environment different from the sleep recording and fear testing (Baseline, Day 1, and Day 14) environment. Freezing behavior was measured over a 5-min observation period on the Baseline day and again immediately after the tone presentations on Day 1 and Day 14. Then, rats were connected to the recording cable and the 4-hr sleep recording (11 AM – 3 PM) was begun 5 mins after the tone presentations; (this delay permitted the measurement of freezing behavior). Circadian factors were minimized by carrying out each study at the same time each day.

Sleep was not recorded on the Training Day in either group because this would assess the effects of stress imposed by shock, rather than the effects of fear conditioning or stress sensitization. In the CFC study, the aim was to determine if the psychological stress of being reminded of a fearful experience would affect sleep-wake behavior, even in the absence of the physical stressor. In the SS study, the aim was to determine if previous exposure to a stressor would affect sleep-wake behavior when animals were exposed to stimuli unrelated to the initial stressor (Stam, 2007). Because the diagnosis of PTSD requires that the individual experience symptoms for at least 1 month, both short- and long-term sleep-wake responses to CFC and SS were investigated. Thus, in the present study, Day 1 data indicate the short-term effect of CFC and SS on sleep-wake behavior, while Day 14 data indicate the long-term effect of CFC and SS on sleep-wake behavior.

2.5. Data Analysis

Freezing behavior was calculated as total time (sec) spent freezing as a percent of the 5-min observation period. Using Spike 2 software, myoclonic twitches (MT) during REMS (as a measure of REMS phasic activity) were visually analyzed from the EMG channel via conversion into an event channel. A window was created to capture only MT by setting a lower horizontal line above the electrocardiogram and an upper horizontal line to exclude artifacts. An event raster was then generated to indicate each twitch (Madan et al., 2008), and the frequency of MT occurrence was calculated (total number of MT/total REMS time). Somnologica software (Flaga hf. Medical Devices, Reykjavik, Iceland) was used for sleep scoring, and EEG traces were scored manually in 10-sec epochs. Sleep latency (time to sleep onset), sleep efficiency (total sleep time/total recording time), and total time (mins) spent in REMS and non-REMS (NREMS) were evaluated. REMS was further assessed by partitioning episodes into one of two groups, those with a long inter-REMS episode interval (> 3 min) and those with a short inter-REMS episode interval (≤ 3 min), the former being defined as single (siREMS) and the latter as sequential (seqREMS) REMS (Amici et al., 1994). Using these parameters, total time (mins) spent in siREMS and seqREMS, as well as the number and mean duration of siREMS and seqREMS episodes, were calculated using a program created by Dr. Aaron Pawlyk (Pawlyk et al., 2005). All scoring was blinded; this

was achieved by having a non-scoring individual randomly assign code names to all data and video files.

2.6. Statistical Analyses

Statistical analysis was performed using SAS 9.1. Sleep-wake measures were compared using a repeated measures with between subjects factor analysis of variance model (between subjects factor: Strain [WIS and WKY]; within subjects factor: Condition [Baseline, Day 1, and Day 14]). Baseline strain differences were determined using one-way ANOVA. Differences were considered significant at $p < .05$.

3. Results

Behavior Following Cued Fear Conditioning

Freezing behavior—Both strains froze more on Day 1 than at Baseline (WKY: $F_{(2,22)} = 74.62$; $p < .001$; WIS: $F_{(2,22)} = 53.49$; $p < .001$); this difference from Baseline was maintained on Day 14 in WKY rats only, $F_{(2,22)} = 32.67$; $p < .001$. A significant Strain x Condition interaction was found for percent of time spent freezing, $F_{(2,22)} = 10.40$; $p < .001$. As compared to WIS rats, WKY rats froze more at Baseline, $F_{(1,11)} = 11.66$; $p < .05$, and on Day 14, $F_{(1,11)} = 5.63$; $p < .05$ (see Figure 1A).

MT during REMS—As compared to WIS rats, WKY rats exhibited significantly less frequent MT during REMS at Baseline, $F_{(1,11)} = 22.03$; $p < .001$, and on Day 14, $F_{(1,11)} = 14.03$; $p < .01$ (see Figure 1B).

Sleep Architecture Following Cued Fear Conditioning

Sleep macroarchitecture—As compared to WIS rats, WKY rats had a longer sleep latency at Baseline, $F_{(1,11)} = 6.20$; $p < .05$. WKY rats in the CFC group spent more time in REMS on Day 1 than at Baseline, $F_{(2,22)} = 7.61$, $p < .05$ (see Table 1).

REMS microarchitecture—A representative hypnogram of a WIS rat 14 days following CFC depicts a relatively unfragmented REMS pattern (see Figure 2A). A representative hypnogram of a WKY rat 14 days following CFC depicts a relatively fragmented REMS pattern (see Figure 2B).

seqREMS amount—CFC WKY rats spent more time in seqREMS on Day 1, $F_{(2,22)} = 74.62$; $p < .001$, and on Day 14, $F_{(2,22)} = 8.50$; $p < .05$, than at Baseline. A significant Strain x Condition interaction was found for seqREMS amount, $F_{(2,22)} = 12.28$; $p < .001$. As compared to WIS rats, WKY rats spent a greater amount of time in seqREMS on Day 1, $F_{(1,11)} = 26.34$; $p < .001$, and on Day 14, $F_{(1,11)} = 12.87$; $p < .01$ (see Figure 3A).

seqREMS episode number—CFC WKY rats had a greater number of seqREMS episodes on Day 1, $F_{(2,22)} = 12.01$; $p < .01$, and on Day 14, $F_{(2,22)} = 12.00$; $p < .01$, than at Baseline. A significant Strain x Condition interaction was found for the number of seqREMS episodes, $F_{(2,22)} = 15.99$; $p < .001$. As compared to WIS rats, WKY rats had a greater number of seqREMS episodes on Day 1, $F_{(1,11)} = 23.66$; $p < .001$, and on Day 14, $F_{(1,11)} = 22.88$; $p < .001$ (see Figure 3B).

seqREMS episode duration—In both strains, CFC had no significant effect on the average duration of a seqREMS episode (see Figure 3C).

siREMS amount—CFC WKY rats spent less time in siREMS on Day 14 than at Baseline, $F_{(2,22)} = 6.89$; $p < .05$. A significant Strain x Condition interaction was found for siREMS

amount, $F_{(2,22)} = 6.59$; $p < .01$. As compared to WIS rats, WKY rats spent less time in siREMS on Day 14, $F_{(1,11)} = 18.88$; $p < .01$ (see Figure 3D).

siREMS episode number—CFC WKY rats had a smaller number of siREMS episodes on Day 14 than at Baseline, $F_{(2,22)} = 5.43$; $p < .05$ (see Figure 3E).

siREMS episode duration—In both strains, CFC had no significant effect on the average duration of a siREMS episode (see Figure 3F).

Behavior Following Shock Stress

Freezing behavior—As compared to WIS rats, WKY rats froze more at Baseline, $F_{(1,6)} = 19.45$; $p < .01$ (see Figure 4A).

MT during REMS—As compared to WIS rats, WKY rats had less frequent MT during REMS at Baseline; however, variability together with a small sample size precluded significance (see Figure 4B).

Sleep Architecture Following Shock Stress

In both strains, SS had no significant effect on sleep macroarchitecture and REMS microarchitecture in either strain (see Table 2).

4. Discussion

The present study characterized the alterations in sleep architecture and freezing behavior on Day 1 and Day 14 post-CFC and post-SS, in WKY and WIS rats. Our results indicate that while fear conditioning increased freezing behavior from baseline in both strains, the increase was maintained on Day 14 in WKY rats only. Fear conditioning had no effect on the frequency of MT during REMS in either strain; however, WKY rats exhibited significantly less frequent MT under all experimental conditions. In cued fear-conditioned WKY rats, total REMS amount increased on Day 1, seqREMS amount increased on Day 1 and Day 14, and siREMS amount decreased on Day 14. Shock stress was found to have no significant effect on freezing behavior, MT, or REMS microarchitecture in either strain.

The strain difference in freezing behavior indicated that WKY rats froze more than WIS rats under baseline conditions, which confirmed the anxious phenotype of this rat strain. In the CFC group, the percent of time spent freezing increased from Baseline in both strains but was maintained on Day 14 only in the WKY rats, suggesting that WKY rats had a more sustained response to the fearful tone reminder than did WIS rats. Furthermore, in agreement with previous reports (Beck et al., 2010; Servatius et al., 2008), CFC WKY rats did not extinguish the freezing response when they were presented with the CS on Day 14, whereas fear-conditioned WIS rats showed a clear extinction curve for freezing behavior.

A recent study in fear-conditioned Sprague-Dawley (S-D) rats reported an increase in REMS phasic activity (measured as total number of nuchal MT during REMS) two weeks post-CFC, with a positive correlation between the number of MT during REMS and the percent of time spent freezing (Madan et al., 2008). Therefore, we investigated the occurrence of nuchal MT during REMS in our CFC and SS animals, postulating a positive correlation between MT during REMS and freezing behavior. Contrary to our hypothesis, CFC WKY rats, compared to CFC WIS rats, had a lower total number of MT during REMS. Cued fear-conditioned WKY rats exhibited a small increase in the total number of MT on Day 1; this was due to an increase in total REMS amount, and the number did not exceed the number of MT in CFC WIS rats (data not shown). When the frequency of MT occurrence

during REMS was calculated, CFC WKY rats, compared to CFC WIS rats, had significantly less frequent MT in all conditions. It is possible that evaluating the frequency distribution of MT within each REMS episode, just prior to REMS termination, for example, may provide additional insight into the relationship between REMS phasic activity and REMS microarchitecture.

Re-exposure to the CS altered total REMS amount and REMS microarchitecture in fear-conditioned WKY rats, but not WIS rats, confirming the stress-resilient phenotype of the latter strain (Rosen et al., 2006). The increase in total REMS amount on Day 1 was found to be due to a significant increase in seqREMS amount, while the decrease in siREMS amount on Day 14 accounted for the absence of an increase in total REMS amount in WKY rats. The mean durations of seqREMS and siREMS episodes were not changed; the changes in seqREMS and siREMS amounts were due to changes in the number of episodes. Several studies in rats have reported that fear conditioning produced long-term alterations in both the amount and microarchitecture of REMS, with little effect on sleep efficiency and NREMS amount (Jha et al., 2005; Pawlyk et al., 2005; Sanford et al., 2003; Tang et al., 2005). In agreement, we observed that sleep efficiency and total NREMS amount were not altered in the CFC groups.

While the significance of REMS fragmentation following the experience of a stressor is not fully understood, it has been hypothesized that, because there is an absence of homeostatic control during REMS, brief interruptions within a cluster of seqREMS episodes may provide homeostatic regulation while recovery REMS is obtained (Amici et al., 1994, 1998; Zamboni et al., 2001). Amici et al. (1994) have reported that environmental stressors differentially alter seqREMS and siREMS distribution. For example, exposure to cold stress resulted in REMS suppression; during recovery sleep at the normal ambient temperature, an increase in REMS was due to an increase in the number of seqREMS episodes. Investigations of seqREMS and siREMS under control conditions have found that the average seqREMS episode duration is approximately 80% of that of a siREMS episode; however, seqREMS episodes usually occur in clusters, such that a cluster of seqREMS episodes has nearly double the duration of a siREMS episode (Amici et al., 1994, 1998; Zamboni et al., 2001). Thus based on our results, it is possible that physiologic challenges to homeostasis during REMS in cued fear-conditioned WKY rats could be managed via a mechanism of REMS fragmentation.

We recognize, from the work of Amici et al. (1994), that the increase in seqREMS on Day 1 in cued fear-conditioned WKY rats may be attributed to REMS deprivation during the 24 hrs after training. However, this would not explain the similar increase in seqREMS on Day 14. Also, because SS had no significant effect on REMS microarchitecture in either strain, the shift toward seqREMS in cued fear-conditioned WKY rats cannot be explained as a residual effect of shock stress. Alterations in REMS microarchitecture in the WKY rat strain appear to be specific to the anticipatory stress produced by the presentation of fearful CSs. REMS continuity may be important in the processing of fearful stimuli and in associative learning generally (Datta, 2000; Mavanji and Datta, 2003; Walker and van der Helm, 2009), and REMS fragmentation has been reported in humans with PTSD (Breslau et al., 2004; Habukawa et al., 2007; Mellman et al., 2002). It has been hypothesized that REMS discontinuity in the early aftermath of trauma may predict the later development of PTSD (Mellman et al., 2002). In the current study, the increased number of seqREMS episodes suggests that the WKY rats developed more fragmented REMS after CFC. Sustained sleep-wake alterations in CFC WKY rats may represent fear incubation, in which mild pathological symptoms become progressively more severe over time (Pickens et al., 2009).

The WKY rat strain, compared to WIS and S-D rats, demonstrates prolonged stress-induced elevations in adrenocorticotrophic hormone (ACTH) and corticosterone (CORT) levels, suggesting a decreased sensitivity of the HPA axis to glucocorticoid feedback regulation (De La Garza and Mahoney 2004; Malkesman et al., 2006; Pardon et al., 2003; Redei et al., 1994; Solberg et al., 2001). Furthermore, while WKY rats exhibited low baseline noradrenergic activity, repeated stress exposure resulted in heightened and maladaptive noradrenergic activation (Morilak et al., 2005; Pardon et al., 2002; Pardon et al., 2003). Prolonged increases in ACTH and CORT, together with heightened noradrenergic activity, support the hypothesis that the exaggerated HPA axis response to stress in the WKY rat strain may be due to a dissociation of the HPA axis and central noradrenergic feedback mechanisms (Pardon et al., 2002; Pardon et al., 2003; Redei et al., 1994). Norepinephrine (NE) neurons in the locus coeruleus (LC) (Aston-Jones and Bloom, 1981; Gervasoni et al., 1998; Reiner, 1986) and serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN) (McGinty and Harper, 1976; Ursin, 2002) decreased their firing rates from wake to NREMS, and became virtually inactive during REMS. Thus, it is possible that heightened noradrenergic activity in the fear-conditioned WKY rat disrupts the maintenance of REMS episodes, leading to an overall fragmented REMS pattern.

Dugovic and coworkers (2000) hypothesized that the higher amount of REMS in WKY rats may be reflective of its unique NE and 5-HT profile. In agreement, we have recently reported that WKY rats had lower baseline levels of NE in the LC when compared to WIS rats, and exhibited lower baseline levels of 5-HT in the DRN when compared to S-D rats (Scholl et al., 2009). In contrast to our observation that CFC increased seqREMS in WKY rats, we reported in a previous study that CFC decreased seqREMS in S-D rats (Madan et al., 2008). While Madan et al. (2008) suggested that the decrease in seqREMS in S-D rats involved changes in 5-HT levels, we have previously reported that antidepressant drugs targeting the NE reuptake system but not the 5-HT reuptake system, attenuated anxiety-like behavior (as measured by the OFT and the FST) in WKY rats (Tejani-Butt et al., 2003). Thus heightened noradrenergic reactivity in WKY rats could be one mechanism to explain the REMS fragmentation in the aftermath of fear conditioning. This interpretation would be consistent with recent reports that the alpha-1 adrenergic receptor antagonist prazosin shows efficacy in ameliorating the sleep and nightmare disturbance in individuals with PTSD (Raskind et al., 2003, 2007; Taylor et al., 2006, 2008; Taylor and Raskind, 2002).

5. Conclusions

Genetic factors play a role in the pathophysiology of anxiety disorders, including PTSD (Davidson et al., 1989; Lyons et al., 1993; True et al., 1993; Skre et al., 1993; Yehuda, 1999). This supports the validity of investigating sleep changes in fear-conditioned WKY rats as a possible animal model of the sleep disturbances observed in PTSD. Although both WKY and WIS rats showed evidence of an anxiety-like response to fearful tones on Day 1 post-conditioning, sustained long-term alterations in freezing behavior and REMS microarchitecture indicate that a tone reminder is significantly more stressful to WKY rats on Day 14. While WIS rats show a clear extinction curve, WKY rats failed to extinguish the sleep-wake response to CFC at Day 14. The REMS fragmentation observed following CFC in this stress-sensitive rat strain resembles the REMS discontinuity that has been described in PTSD (Breslau et al., 2004; Habukawa et al., 2007; Mellman et al., 2002). Additional experiments are necessary to determine how the failure of WKY rats to mount a strong phasic REMS response in the early aftermath of a stressful experience might relate to the increase in REMS phasic activity that has been observed in humans with chronic PTSD (Mellman et al., 1997; Ross et al., 1994a, 1994b).

Abbreviations

5-HT	serotonin
ACTH	adrenocorticotrophic hormone
CFC	cued fear conditioning
CORT	corticosterone
CS	conditioned stimulus
EEG	electroencephalogram
EMG	electromyogram
FST	forced swim test
HPA	hypothalamo-pituitary-adrenal axis
LC	locus coeruleus
MT	myoclonic twitches
NE	norepinephrine
NREMS	non-rapid eye movement sleep
OFT	open field test
PTSD	posttraumatic stress disorder
REMS	rapid eye movement sleep
S-D	Sprague Dawley
seqREMS	sequential rapid eye movement sleep
siREMS	single rapid eye movement sleep
SS	shock stress
US	unconditioned stimulus
WIS	Wistar
WKY	Wistar-Kyoto

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Figure 1. Behavioral measurements recorded from cued fear-conditioned WIS and WKY rats. (A) Percent time spent freezing [N= 6–7/strain]. (B) Frequency of MT occurrence (number of twitches/min) during REMS [N= 6/strain]. Data expressed as mean $\pm$ S.E.M. Significant differences within subjects: * $p < .05$, ** $p < .01$, *** $p < .001$. Significant differences between subjects: # $p < .05$, ## $p < .01$, ### $p < .001$.

A) Representative hypnogram of a WIS rat 14 days following CFC.

B) Representative hypnogram of a WKY rat 14 days following CFC.

Figure 2.

A representative hypnogram of a cued fear-conditioned WIS rat and of a cued fear-conditioned WKY rat. The ordinate depicts sleep architecture as REMS (PS), NREMS (DS), and wake (AW). (A) Hypnogram of a WIS rat on Day 14: the red bars depict relatively unfragmented REMS. (B) Hypnogram of a WKY rat on Day 14: the red bars depict relatively fragmented REMS.

Figure 3. REMS microarchitecture recorded from cued fear-conditioned WIS and WKY rats. (A) Total amount of time (mins) spent in seqREMS. (B) Number of seqREMS episodes. (C) Average seqREMS episode duration (mins). (D) Total amount of time (mins) spent in siREMS. (E) Number of siREMS episodes. (F) Average siREMS episode duration (mins). Data expressed as mean $\pm$ S.E.M, [N= 6–7/strain]. Significant differences within subjects: * $p < .05$, ** $p < .01$, *** $p < .001$. Significant differences between subjects: # $p < .05$, ## $p < .01$, ### $p < .001$.

Figure 4.

Behavioral measurements recorded from shock-stressed WIS and WKY rats. (A) Percent time spent freezing [N= 4/strain]. (B) Frequency of MT occurrence (number of twitches/min) during REMS [N= 4/strain]. Data expressed as mean $\pm$ S.E.M. Significant differences within subjects: * $p < .05$, ** $p < .01$, *** $p < .001$. Significant differences between subjects: # $p < .05$, ## $p < .01$, ### $p < .001$.

Table 1

Differences in sleep architecture at Baseline, Day 1, and Day 14 post-conditioning

Sleep architecture in cued fear-conditioned WIS and WKY rats. Data expressed as mean ± S.E.M., [N= 6–7/strain].

	Baseline		Day 1		Day 14	
	WIS	WKY	WIS	WKY	WIS	WKY
Sleep Efficiency (%)	70.6 ± 2.2	65.9 ± 3.2	68.0 ± 1.7	72.3 ± 2.4	64.2 ± 1.9	63.3 ± 4.9
Sleep Latency (min)	15.3 ± 3.2	24.1 ± 3.9 #	23.9 ± 6.1	20.1 ± 3.5	22.8 ± 6.6	24.6 ± 8.1
NREMS Amount (min)	132.8 ± 6.2	122.5 ± 5.2	126.3 ± 5.0	126.8 ± 3.6	118.9 ± 3.7	115.6 ± 6.9
REMS Amount (min)	36.7 ± 4.5	35.5 ± 2.6	36.9 ± 2.6	46.8 ± 2.9 *	35.1 ± 2.5	36.3 ± 5.0

Significant differences within subjects:

- *
 $p < .05$,
- **
 $p < .01$,
- ***
 $p < .001$.

Significant differences between subjects:

- #
 $p < .05$,
- ##
 $p < .01$,
- ###
 $p < .001$.

Table 2
Differences in sleep architecture at Baseline, Day 1 and Day 14 post-shock stress
Sleep architecture in shock-stressed WIS and WKY rats. Data expressed as mean $\pm$ S.E.M., [N= 4/strain].

	Baseline		Day 1		Day 14	
	WIS	WKY	WIS	WKY	WIS	WKY
Sleep Architecture:						
Sleep Efficiency (%)	69.3 $\pm$ 2.5%	63.0 $\pm$ 4.1%	64.4 $\pm$ 8.1%	60.6 $\pm$ 1.7%	55.0 $\pm$ 8.3%	67.9 $\pm$ 4.3%
Sleep Latency (min)	12.8 $\pm$ 3.6	16.3 $\pm$ 8.7	35.0 $\pm$ 10.5	22.4 $\pm$ 4.8	34.3 $\pm$ 11.9	13.1 $\pm$ 6.7
NREMS Amount (min)	124.3 $\pm$ 5.8	120.5 $\pm$ 7.1	122.2 $\pm$ 14.5	111.5 $\pm$ 1.8	103.5 $\pm$ 14.4	132.0 $\pm$ 6.5
REMS Amount (min)	42.0 $\pm$ 2.8	30.6 $\pm$ 4.5	32.3 $\pm$ 5.9	34.0 $\pm$ 3.7	28.5 $\pm$ 6.4	31.0 $\pm$ 5.7
REMS Microarchitecture:						
<u>seqREMS</u>						
Amount (min)	18.0 $\pm$ 5.1	13.7 $\pm$ 3.0	13.0 $\pm$ 5.6	9.3 $\pm$ 5.4	15.5 $\pm$ 5.4	10.9 $\pm$ 3.0
Number of episodes	10.0 $\pm$ 2.1	7.0 $\pm$ 1.3	6.8 $\pm$ 2.6	5.5 $\pm$ 3.1	10.3 $\pm$ 3.3	8.3 $\pm$ 2.3
Episode duration (min)	1.8 $\pm$ 0.3	2.2 $\pm$ 0.8	1.4 $\pm$ 0.5	1.3 $\pm$ 0.5	1.4 $\pm$ 0.1	1.3 $\pm$ 0.3
<u>siREMS</u>						
Amount (min)	24.0 $\pm$ 6.0	16.9 $\pm$ 3.0	19.3 $\pm$ 3.9	24.7 $\pm$ 4.5	13.0 $\pm$ 3.6	20.1 $\pm$ 5.7
Number of episodes	9.3 $\pm$ 2.0	7.3 $\pm$ 1.4	7.3 $\pm$ 1.1	10.8 $\pm$ 1.5	7.8 $\pm$ 2.0	11.3 $\pm$ 1.5
Episode duration (min)	2.5 $\pm$ 0.1	2.4 $\pm$ 0.2	2.6 $\pm$ 0.3	2.3 $\pm$ 0.2	1.7 $\pm$ 0.2	1.6 $\pm$ 0.4

Significant differences within subjects:

* $p < .05$,
 ** $p < .01$,
 *** $p < .001$.

Significant differences between subjects:

$p < .05$,
 ## $p < .01$,
 ### $p < .001$.