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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 s), each co-terminating with a foot shock (1.0 mA, 0.5 s), at 30-s intervals. In the shock stress procedure, animals received 10 foot shocks at 30-s intervals, without tones. All subjects underwent a tone-only test both 24 h (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.

1. Introduction

Posttraumatic stress disorder (PTSD) is an anxiety disorder that can develop after a terrifying experience (American Psychiatric Association, 2000, 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, 2000, 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,b).

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, 2008; Sanford et al., 2003). However, findings from studies in

Abbreviations: 5-HT, serotonin; ACTH, adrenocorticotropic 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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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, 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,b; 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 ± 2 °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-h light/dark cycle, with lights on at 0700 h. Rats within each strain were assigned to either the CFC group ($N = 6$–7/strain) or the SS group ($N = 4$/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 isofluorane 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-h 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 to 3 PM. In addition, Dugovic et al. (2000) reported 24-h sleep recording in WKY and WIS rats, confirming that 11 AM to 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 (1 m^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-h light/dark cycle, with lights on at 0700 h, and temperature was controlled at $22° \pm 2$ °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 h (11 AM–3 PM) each day over 3 days. The next day (Baseline), animals had a baseline 4-h 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 s) co-terminating with a mild electric foot shock (US: 1.0 mA, 0.5 s) at 30-s intervals. In the SS procedure, rats received 10 mild electric foot shocks (1.0 mA, 0.5 s) at 30-s 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 h 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-s 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-h sleep recording (11 AM–3 PM) was begun 5 min 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