A Rodent Model of Sleep Disturbances in Posttraumatic Stress Disorder: The Role of Context After Fear Conditioning

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Background: *A prominent sleep disturbance, likely including a disruption of rapid eye movement sleep (REMS) continuity, characterizes posttraumatic stress disorder (PTSD). We set out to develop a fear conditioning paradigm in rats that displays alterations in sleep architecture analogous to those in PTSD.*

Methods: *Baseline polysomnographic recordings of rats were performed in a neutral context to which the rats had been habituated for several days. Rats were then shock- or mock-trained in a distinctly different context, and their sleep was studied the following day in that context. A separate group of rats was shock-trained and studied in the neutral context on the following 2 days.*

Results: *Rats that slept in the neutral context exhibited a REMS-selective increase in sleep 24 hours after training and increases in REMS and non-REMS 48 hours after training. In contrast, rats that slept in the presence of situational reminders of the training context exhibited a REMS-selective decrease in sleep 24 hours later. Animals that were mock-trained showed no changes in sleep.*

Conclusions: *Shock training induced days-long changes in sleep architecture that were disrupted when the animal was exposed to situational reminders of the training context.*

Key Words: Fear, fear conditioning, PTSD, anxiety, sleep, REMS

The anxiety disorders are the most prevalent of the psychiatric disorders (Lepine 2002). Difficulty sleeping is a diagnostic criterion for three of them: posttraumatic stress disorder (PTSD), acute stress disorder, and generalized anxiety disorder (American Psychiatric Association 1994). We and others have suggested that sleep disturbances, especially recurrent anxiety dreams, are a hallmark of PTSD (Harvey et al 2003; Ross et al 1989). The exact relationship between sleep and the anxiety disorders is poorly understood (Kryger et al 2000), and most animal models of anxiety fail to specifically address sleep disturbances (Shekhar et al 2001; Uys et al 2003).

Pavlovian fear conditioning, by which emotionally neutral stimuli that become associated with unconditionally aversive events come to elicit fearful or anxious responses, has provided important experimental paradigms for exploring learned fear and anxiety in animals (Grillon 2002). Fear conditioning might be particularly pertinent to the study of PTSD, which by definition is a disorder occurring in the aftermath of a psychologically traumatic event and must therefore involve learned neurobehavioral responses. Fear conditioning paradigms in which situational reminders are used, without complete replication of the aversive event, have been viewed as an ideal means of modeling PTSD in humans, for whom situational reminders, and not complete reexperiencing, are more clinically relevant (Pynoos et al 1996).

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Various stress-inducing paradigms have been used to investigate the mechanisms through which fear and anxiety influence behavior during wakefulness (WAKE) (Uys et al 2003). We and others have reported that a single shock-training session results in a reduction of rapid eye movement sleep (REMS) in the sleep period immediately after shock training in both the rat (Adrien et al 1991; Datta 2000; Mavanji et al 2003; Sanford et al 2001; Vazquez-Palacios and Velazquez-Moctezuma 2000) and mouse (Sanford et al 2003a, 2003b, 2003c). Interestingly, paradigms involving stress due to either immobilization or brief presentation of ether resulted in an increase in REMS (Bodosi et al 2000; Dewasmes et al 2004; Vazquez-Palacios and Velazquez-Moctezuma 2000). These disparate effects on REMS, and in some cases non-REMS (NREMS) (Bonnet et al 1997; Sanford et al 2003a; Vazquez-Palacios and Velazquez-Moctezuma 2000), indicate that there is not a simple relationship between stress and sleep. Effects on REMS microarchitecture of stress in rats and reports of REMS microarchitecture changes in patients with PTSD, in the form of increased rapid eye movements (Mellman et al 1997; Ross et al 1994), highlight the necessity for analyses of sleep microarchitecture in animal models of anxiety.

To provide insights into the neural substrates of disturbed sleep in PTSD, we have extended our previous studies using shock training in rats (Sanford et al 2001). We sought a paradigm in which the recall of fearful memories would influence sleep only when situational reminders are present and in which animals that receive no shock but are otherwise treated identically would not show any changes in sleep architecture.

Methods and Materials

Subjects and Surgical Procedures

Subjects were 20 male Sprague-Dawley rats weighing 300–450 g at the time of the study. They were maintained on ad libitum food and water on a 12:12 light/dark cycle, with lights on at 7:00 AM. All procedures were approved by the Institutional Animal Care and Use Committees of the Philadelphia VA Medical Center and of the University of Pennsylvania.

Anesthesia was initiated with ketamine (85 mg/kg, IM) and xylazine (15 mg/kg, IM), and maintained by administration of isoflurane through a face mask. Stainless steel screw electrodes

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Table 1. Sleep Parameters for Shock-Trained Rats Studied in the Training Context

	Baseline	First Posttraining Day	Effect of Day (*p*)	Effect of Day × Hour (*p*)
Sleep Efficiency (%)	62.8 ± 4.6	51.0 ± 5.3	.003[a]	.260
WAKE-T (min)	22.3 ± 2.8	29.4 ± 3.2	.003[a]	.287
WAKE-N	16.1 ± 1.1	14.5 ± 1.4	.267	.016[a]
WAKE-EL (min/episode)	2.3 ± .7	8.8 ± 3.5	.042[a]	.005[a]
NREMS Latency (min)	23.4 ± 8.0	46.4 ± 9.5	.036[a]	N/A
NREMS-T (min)	30.4 ± 2.1	27.2 ± 2.8	.093	.059
NREMS-N	16.1 ± 1.2	14.4 ± 1.5	.261	.015[a]
NREMS-EL (min/episode)	2.1 ± .2	1.8 ± .2	.316	.110
REMS Percentage	17.1 ± 1.9	8.2 ± 1.6	.0003[a]	.156
REMS Latency (min)	57.2 ± 6.5	120.6 ± 21.4	.011[a]	N/A
REMS-T (min)	7.4 ± 1.0	3.4 ± .7	.0007[a]	.210
REMS-N	3.6 ± .4	2.6 ± .5	.051	.493
REMS-EL (min/episode)	1.9 ± .2	.9 ± .1	.002[a]	.542
sinREMS-T (min)	4.7 ± .7	2.2 ± .4	.003[a]	.016[a]
sinREMS-N	1.9 ± .3	1.6 ± .3	.364	.081
sinREMS-EL (min/episode)	2.1 ± .3	1.0 ± .2	.017[a]	.378
seqREMS-T (min)	2.7 ± .7	1.2 ± .4	.085	.519
seqREMS-N	1.8 ± .4	1.0 ± .3	.221	.786
seqREMS-EL (min/episode)	.7 ± .2	.4 ± .1	.049[a]	.942
clsREMS-T (min)	3.8 ± .9	1.9 ± .6	.141	.838
clsREMS-N	.9 ± .2	.4 ± .1	.142	.606
clsREMS-EL (min/cluster)	2.3 ± .5	1.5 ± .4	.164	.578

REMS and NREMS latencies are presented as minutes ± SEM after the start of the recording period. All other values are presented as an hourly mean ± SEM for the entire 4-h recording period. WAKE, wakefulness; REMS, rapid eye movement sleep; NREMS, non-REMS; Sleep Efficiency, percentage of total recording time spent in REMS and NREMS; REMS Percentage, percentage of total sleep time spent in REMS; sinREMS, REMS occurring in single episodes; seqREMS, REMS occurring in sequential episodes; clsREMS, clusters of seqREMS and intervening WAKE/NREMS; -T, average hourly time in state; -N, average hourly number of episodes of state; -EL, average episode length of state.

[a]Significant main or interaction effect.

were implanted in the skull (mediolateral [ML] and anteroposterior [AP] coordinates: ML 2.0, AP 2.0, and ML −2.0, AP −2.0) for recording the frontoparietal electroencephalogram, and a pair of stainless steel wire electrodes was implanted in the neck muscles for recording the nuchal electromyogram. A grounding screw was placed in the skull just rostral to the braincase. Postoperative pain and potential infection were controlled with butorphanol (.35 mg/kg, IM) and gentamicin (4.5 mg/kg, IM), respectively. Animals were allowed to recover from surgery for a minimum of 1 week.

Shock Training and Fear Conditioning Protocols

Animals were habituated to tethering in a recording cage placed in a recording chamber (neutral context) for 6 hours on each of 4–5 consecutive weekdays. After the weekend, which rats spent in their home cage in the animal colony, they were again habituated to the neutral context for 1 day. On the following day, a baseline sleep recording was performed in the neutral context from 11 AM to 3 PM. The next day, rats were either shock-trained or mock-trained (identical to shock training except that no shock was delivered) in a distinctly different context (training context). To differentiate the two contexts, several situational reminders were incorporated: visibly distinct recording chambers were used for the different contexts for each rat; illumination was kept at 20–25 Lux and 60–75 Lux for the neutral context and training context, respectively; different transport routes and animal handlers were used for each context; for the neutral context, the recording chamber and recording cage were cleaned with Original Windex (SC Johnson and Son, Racine, Wisconsin), and fresh bedding was used every day, whereas for the training context the bedding from the previous day's shock or mock training was used.

During training, animals were placed in the recording chamber in a Coulbourn Habitest cage (E10-18RF) (Coulbourn Instruments, Allentown, Pennsylvania) equipped with a grid floor used to deliver footshock. A Coulbourn Precision Regulated Animal Shocker (E13-14) was used to manually administer five scrambled footshocks (.5 mA; .5 sec) randomly at 3–6-min intervals over a 30-min period to the shock-trained group. No shocks were administered during the first 3 min of the shock training, to allow for contextual recognition (Lattal and Abel 2001). The mock-trained group was treated the same as the shock-trained group, except that no shocks were administered. The following day, sleep studies were performed from 11 AM to 3 PM. Shock-trained animals were studied in either the neutral context (ST/NC-1, n = 8) or the training context (ST/TC, n = 7). Mock-trained animals were only studied in the training context (MT/TC, n = 5). The ST/NC animals were studied on an additional posttraining day (ST/NC-2, n = 8) to evaluate further the long-term effects of shock on sleep architecture.

We chose the .5-mA shock intensity, with a .5-sec duration, as within the range that has been shown to elicit immediate and posttraining changes in freezing—a common indicator of fear conditioning in rodents—in a similar, purely contextual, paradigm (Cordero et al 1998). Pilot studies in a series of nonimplanted rats demonstrated that rats exhibited freezing and ultrasonic vocalizations in both the identical training context and in the presence of situational reminders of the training context (data not shown). Unfortunately, the tethering of rodents during polysomnographic studies results in a several-minutes-long pos-