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Severe and protracted sleep disruptions in mouse model of post-traumatic stress disorder FREE

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Abstract

Increasing evidences suggest that the predator threat model is a valid animal model of post-traumatic stress disorder (PTSD). However, sleep has never been examined in this model. Since sleep disturbances, including insomnia and excessive daytime sleepiness, are severe and protracted symptoms of PTSD, we hypothesized that mice exposed to predator odor trauma (POT) will display contextual fear conditioning along with severe and protracted sleep disruptions. Adult male C57BL/6J mice, instrumented with wire electrodes (to record hippocampal local field potentials [LFP] and nuchal muscle [electromyogram, EMG] activity), were exposed to contextual conditioning using soiled cat litter as unconditional stimulus (US). On day 1, fear memory acquisition (FMA) training was performed by exposing mice to contextual cage (conditional stimulus; CS) for 30 min followed by exposure to CS + US for 90 min. On day 5, fear memory recall (FMR) testing was performed by exposing mice to CS (without US) for 120 min. LFP and EMG were recorded continuously for 5 days. Mice exposed to POT displayed as follows: (1) hyperarousal coupled with electrophysiological indicators of memory acquisition and retrieval (increased hippocampal θ and γ power) during FMA and FMR; (2) increased nonrapid eye movement (NREM) δ and rapid eye movement θ power during sleep post FMA, indicating memory consolidation; (3) protracted sleep disturbances as evident by increased wakefulness, reduced NREM sleep and NREM δ power, increased NREM β power during light (sleep) period, and increased sleep during dark (active) period. Based on these results, we suggest that mice exposed to POT display severe and protracted sleep disturbances mimicking sleep disturbance observed in human PTSD.

predator odor trauma, contextual fear conditioning, sleep disturbances, insomnia, memory, hippocampal, θ, gamma activity

Statement of Significance

Post-traumatic stress disorder (PTSD) is a psychiatric condition that is experienced after exposure to a life-threatening event. PTSD is characterized by several interrelated symptom clusters; however, hyperarousal, insomnia, nightmares, and excessive daytime sleepiness are amongst the most distressing and persistent symptoms. Several animal models have been developed and used to understand the pathophysiology of PTSD. The predator threat model is one such reliable model of human PTSD that satisfies several criteria of PTSD as described in Diagnostic and Statistical Manual of Mental Disorders 5. However, sleep has never been examined in this model. Since sleep disturbances are severe and protracted symptoms of PTSD, we hypothesized that mice exposed to predator odor will display contextual fear conditioning along with severe and protracted sleep. The results of our study suggest that mice exposed to predator odor trauma display severe and persistent sleep disturbances, mimicking sleep disturbance observed in human PTSD, along with electrophysiological indicators of memory acquisition, consolidation, and recall. Further ongoing work will examine cellular and molecular substrates underlying sleep disturbances

observed in the predator threat model of PTSD.

Introduction

Post-traumatic stress disorder (PTSD) is a complex psychiatric condition that is experienced by a subset of individuals after exposure to a life-threating event that elicits fear, helplessness, and/or horror. PTSD is characterized by several inter-related symptom clusters such as hyperarousal symptoms (e.g. sleep difficulties, insomnia, hypervigilance, and exaggerated startle), re-experiencing symptoms (e.g. recurrent nightmares, flashbacks, distress, and physiological reactivity upon exposure to trauma cues), and avoidance and emotional numbing symptoms such as avoidance of traumatic reminders, anhedonia, detachment from others, restricted emotional experiences, and sense of foreshortened future [1].

Sleep disruptions including frequent awakenings, nightmares, and reduced slow-wave sleep are amongst the most prominent, distressing, and persistent symptoms experienced by patients with PTSD [2–4]. Sleep disruptions in patients with PTSD negatively affect their ability to recover from PTSD, as sleep serves as a restorative function and sleeplessness represents a physiological stressor that can lead to impaired function and health. Additionally, although sleep facilitates emotional processing of traumatic experiences, sleep disruptions contribute to impaired fear extinction and fear extinction consolidation, similar to what is exhibited by PTSD patients [5–8]. Thus, sleep disturbances are not just secondary symptoms rather, a core feature of PTSD [9–11].

Several animal models have been developed and used to understand the pathophysiology of PTSD. The predator threat model is one such model that meets several diagnostic criteria as described in Diagnostic and Statistical Manual of Mental Disorders 5 [12–15]. In this model, rodents (rats or mice) are exposed to predator stimuli (cat, cat odor, coyote odor, or fox odor; obtained either from natural or synthetic sources) in an inescapable environment [16–18]. Exposure to predator odor provokes fear and anxiety, induces stress, and produces long-lasting endocrine changes [15]. However, the effects of predator odor trauma (POT) on sleep–wakefulness have never been examined. Thus, the overall focus of this study was to examine the effect of POT on sleep–wakefulness. We hypothesized that mice exposed to POT will display contextual conditioning along with severe and protracted sleep disruptions.

Materials and Methods

Animals

Adult male C57BL/6J mice (7 to 8 weeks old; 22 to 26 g; Jackson Laboratories, Bar Harbor, ME) were used to test our hypothesis. Mice were housed, four per cage, in a sleep recording room, maintained at ambient temperature (25 ± 2°C) with 12:12 hr light-dark cycle (light onset at 06:00 am). All animals had ad libitum access to food and water. Ambient room temperature, with ad libitum access to standard laboratory chow and water, was maintained during the entire experiment. All experimental procedures met NIH guidelines for appropriate care and use of animals in research. All protocols were approved by local committees at Harry S. Truman Memorial Veterans' Hospital.

Experimental design and statistical analysis

A priori power analysis (based on our preliminary data; $\alpha = 0.05$; power ≥ 0.9 [G*Power; Ref. 19]) was performed to calculate the sample size. All subsequent statistical analyses were performed by Prism software (Graphpad Software, Inc., La Jolla, CA).

We used contextual fear-conditioning paradigm with contextual cage as the conditional stimulus (CS) and soiled cat litter (two scoops, used by cat for 2 days and sifted for stools; obtained from School of Veterinary Medicine on the day of the experiment) as unconditional stimulus (US). The CS was very similar to mouse's recording cage (contained one scoop of mouse's own bedding) except aluminum foil was wrapped from outside on all four sides and the bottom, covering approximately half cage. Fear memory retrieval or recall (FMR) was tested on day 5 by exposing mouse to CS (contextual cage) without US (no cat litter). Three groups were used as follows: no odor control (NOC) = mice exposed to two scoops of unused fresh cat litter. Nonpredator odor control (NPOC) = mice exposed to two scoops of cat litter used as a bedding by a different mouse for 2 days. POT = mice were exposed to two scoops of US. All control and experimental protocols were performed in parallel to maximize comparability and repeated at least three times.

Surgery

All stereotaxic surgeries were performed under sterile conditions and inhalation (isoflurane) anesthesia. Mice were stereotaxically implanted with stainless steel tube (27 gauge; length = 13.5 mm) containing three formvar-insulated stainless wire electrodes (100 μm diameter) in the pyramidal layer of the hippocampal CA1 region (coordinates AP −1.9; ML ±1.0; DV −1.3; from bregma [20]) to record hippocampal local field potentials (LFP). Three flexible stainless steel wire electrodes were secured to the neck (nuchal) muscle to record muscle activity [electromyogram (EMG)]. Two anchors were also fixed onto the skull. All LFP and EMG electrodes were connected to a multichannel electrode pedestal (MS363, Plastics One, Inc., Roanoke, VA), and the entire assembly was secured to the skull with dental cement. The wound was sutured. Animals were continuously monitored until ambulatory. Flunixin (2.5 mg/kg/12 hr for 1 day), administered subcutaneously, was used as a postsurgical analgesic.

Postoperative recovery and habituation

Following surgery, mice were housed individually and allowed to undergo postoperative recovery for 48 hr in sleep recording cages (similar to normal shoebox home cages except taller [height = 10″] with open top and a grommeted hole on one [shorter] side of the cage for dispensing water [15 mL bottles fitted with metal sipper tubes]). Next, mice were tethered to lightweight sleep recording cables (Plastics One, Inc., Roanoke, VA). Mice were unrestrained and were able to move freely. They were allowed to habituate with the sleep recording set up until a stable sleep–wakefulness cycle was established. Once a stable sleep–wakefulness cycle was established, the experiment was begun by recording baseline sleep–wakefulness for 24 hr.

Fear memory acquisition

On the following day, 1 hr after light onset, mice were divided into three groups as described above. Next, each mouse was untethered from the recording cable and its recording cage was replaced with a CS. Mice were retethered and allowed to explore the CS for 30 min. Subsequently, soiled (POT) or control litter (NOC or NPOC) was gently introduced and spread to cover the entire cage. Mice were allowed to remain in this environment for 90 min (CS + US). On completion, the CS (+US) was replaced with animal's own sleep recording cage. Thus, the total time for fear memory acquisition (FMA) training was 120 min (CS = 30 min; CS + US = 90 min). On completion, animals were left undisturbed (except checking for food and water) until tested on day 5. Sleep–wakefulness was continuously recorded for 5 days.

Fear memory recall

On test day (day 5), 1 hr after light onset, recording cage of each mouse was replaced with CS; no US was introduced. Mice were housed in this environment for 2 hr. Sleep–wakefulness was recorded continuously.

Data acquisition and analysis

Behavioral state–related rhythms, such as δ, θ, and γ activities, recorded by surface EEG, are also present in subcortical areas such as hippocampus and behave in similar fashion to cortical rhythms [21–26]. Therefore, we used LFP, along with EMG, to identify sleep–wake states. LFP (1–100 Hz) and nuchal EMG (30–300 Hz) were filtered at 60 Hz (notch filter) and acquired with a 16 channel, bipolar Physiodata Amplifier System (Model 15LT) with 4 Quad Neuroamplifiers (Model 15A54; Grass Technologies, West Warwick, RI). The acquired data were visually scored in 10 s epochs as (1) wakefulness (active and quiet), (2) nonrapid eye movement (NREM) sleep, or (3) rapid eye movement (REM) sleep. Wakefulness was identified by the presence of low voltage fast activity (desynchronization) in LFP coupled with high (active W) or reduced (quiet W) EMG activity. NREM sleep was identified by the predominance of slow wave, synchronized activity in the LFP with reduced EMG. REM sleep was identified by the concomitant presence of θ activity in the LFP with no muscle tone [27]. NREM and REM sleep latency (defined as amount of time between light onset or FMA training and first noninterrupted 60 s NREM or 30 s REM sleep bout), bout frequency, and average duration of each bout (for all three states) were also determined [28]. Spectral analysis of LFP was also performed to examine δ (1–4 Hz), θ (5–9 Hz), β (12–20 Hz), and γ (35–50 Hz) activities.

Statistical analysis

One-way ANOVA followed by Dunnett's post hoc test was used to examine the effects of POT on sleep–wakefulness and electrophysiological parameters during (1) FMA, (2) 9 hr of light period post-FMA, and (3) FMR. Two-way repeated measure ANOVA with time (two levels: days 2 and 4) as within-subject repeated measure and treatment (three levels: NOC, NPOC, and POT) as between-subject measure followed by Bonferroni's

post hoc test was used to examine the effects of POT on sleep–wakefulness and electrophysiological parameters on days 2 and 4 light period. Similarly, two-way repeated measure ANOVA with time (three levels: days 1, 2, and 4) as within-subject repeated measure and treatment as between-subject measure (three levels: NOC; NPOC; POT) followed by Bonferroni's post hoc test was used to examine the effects of POT on sleep–wakefulness and electrophysiological parameters on days 1, 2, and 4 dark period.

Results

Baseline day

Baseline sleep–wakefulness was comparable between all three groups (Table 1; $N = 5$ per group)

Table 1.

Sleep–wakefulness during baseline

		Wakefulness	NREM	REM
Light period	NOC	38.9 ± 1.3	53.7 ± 1.3	7.2 ± 0.6
	NPOC	39.6 ± 1.4	53.3 ± 1.1	6.8 ± 0.7
	POT	38.9 ± 0.7	52.6 ± 1.3	7.6 ± 0.7
Dark period	NOC	75.9 ± 1.5	21.4 ± 1.5	2.7 ± 0.3
	NPOC	70.0 ± 1.3	26.5 ± 0.8	3.3 ± 0.5
	POT	70.6 ± 4.1	26.1 ± 3.6	3.3 ± 0.6

NOC = no odor control; NPOC = nonpredator odor control; POT = predator odor trauma.

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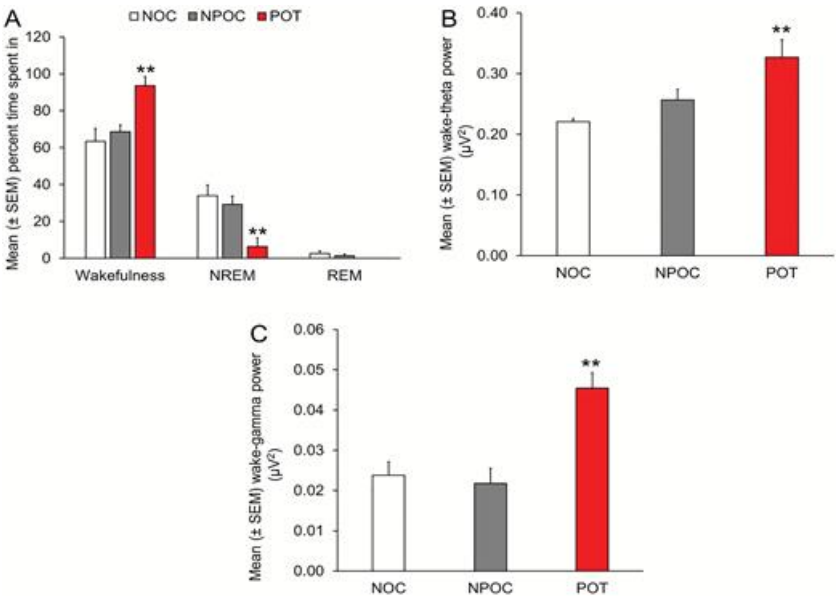
Electrophysiological indicators of memory encoding observed during FMA training

Sleep–wakefulness during 30 min of CS: Mice in all three groups spent most of their time in wakefulness during the 30 min of CS exposure (Table 2). ***Sleep–wakefulness during subsequent 90 min of CS + US:*** One-way ANOVA revealed a significant difference in wakefulness [$F(2, 14) = 9.3$; $p = .004$] and NREM sleep [$F(2, 14) = 8.5$; $p = .005$] between three groups (NOC, NPOC, and POT) during 90 min of CS + US exposure (Figure 1A). The amount of time spent in REM sleep [$F(2, 14) = 1.9$; $p = .2$] remained unchanged. Subsequent post hoc analysis revealed that, compared with mice in the NOC group, mice in POT group displayed a significant ($p < .01$; Dunnett's test) increase in the amount of time spent in wakefulness and a significant ($p < .01$) reduction in the amount of time spent in NREM sleep. No such change was observed in NPOC mice. ***Spectral analysis during 90 min of CS + US:*** Hippocampal θ and γ activities are implicated in memory encoding [29, 30]. Therefore, wake θ and γ activities were also examined. One-way ANOVA revealed a significant difference between three groups in wake θ [$F(2, 14) = 7.7$; $p = .007$; Figure 1B] and γ [$F(2, 14) = 13.4$; $p = .001$; Figure 1C] activities. Post hoc analysis (Dunnett's test) revealed a significant increase in wake θ ($p < .01$) and γ ($p < .01$) activities in mice exposed to POT (POT group) compared with NOC control. θ and γ activities in NPOC and NOC groups were comparable.

Figure 1.



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Mice exposed to POT displayed electrophysiological indicators of memory acquisition during FMA training. (A) Mice exposed to POT spent significantly more time in wakefulness and less time in NREM sleep compared with mice in the control (NOC) group during FMA training. No such change was observed in NPOC group. REM sleep values were comparable in all three groups. (B) Compared with controls, mice exposed to POT displayed an increase in hippocampal θ power during FMA training. Increase in hippocampal θ power is indicative of memory acquisition. Mice in NPOC group did not show such an increase. (C) During FMA training, mice exposed to POT displayed an increase in hippocampal γ power compared with controls, indicative of memory acquisition. Mice in NOC and NPOC groups displayed comparable values. $^{**}p < .01$.

Table 2.

Sleep–wakefulness during 30 min of contextual cage exposure on day 1

	Wakefulness	NREM	REM
NOC	99.9 ± 1.0	1.0 ± 1.0	0.0 ± 0.0
NPOC	97.8 ± 2.2	2.2 ± 2.2	0.0 ± 0.0
POT	100 ± 0.0	0.0 ± 0.0	0.0 ± 0.0

NOC = no odor control; NPOC = nonpredator odor control; POT = predator odor trauma.

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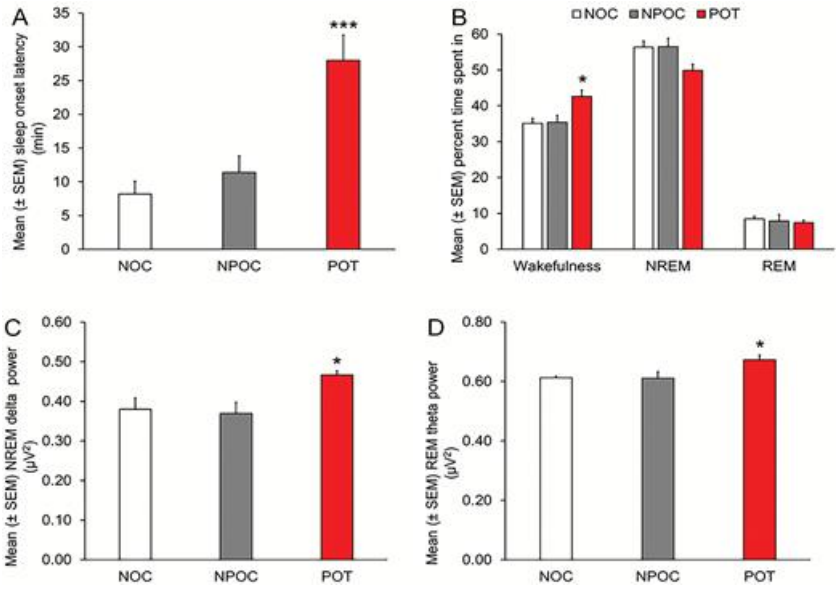
Changes in sleep–wakefulness during remaining 9 hr of light period post-FMA

NREM sleep latency: One-way ANOVA suggested a significant [$F(2, 14) = 14.4$; $p = .001$] change in NREM latency between three groups (Figure 2A). NREM latency was comparable in NOC and NPOC groups. However, compared with NOC group, mice in the POT group displayed a significant ($p < .001$; Dunnett's test) increase in NREM latency. ***REM sleep latency***: REM sleep latency (min) values [$F(2, 14) = 0.6$; $p = .6$] were comparable in all three groups [(Mean ± SEM): NOC = 47.4 ± 3.6; NPOC = 43.6 ± 5.6; POT = 50.6 ± 4.6]. ***Time spent in sleep–wakefulness:*** During the remaining light period, post-FMA, amount of time spent in wakefulness [$F(2, 14) = 6.2$; $p = .01$] and NREM sleep [$F(2, 14) = 3.8$; $p = .05$] showed a significant change. REM sleep [$F(2, 14) = 0.2$; $p = .8$] was unaffected. Post hoc analysis suggested that mice exposed to POT spent significantly ($p < .05$; Dunnett's test) more time in wakefulness compared with mice in NOC group. Wakefulness values in NOC and NPOC groups were comparable. Interestingly, NREM sleep values were comparable between NOC and NPOC, as well as, between NOC and POT groups (Figure 2B). ***Bout frequency:*** No significant effect was observed between groups on bout frequency for all three states of behavior [wakefulness = $F(2, 14) = 0.4$; $p = .7$; NREM = $F(2, 14) = 1.4$; $p = .28$; REM = $F(2, 14) = 1.3$; $p = .29$; Table 3]. ***Bout duration:*** A significant main effect was observed on wake [$F(2, 14) = 6.2$; $p = .01$ and NREM [$F(2, 14) = 4.9$; $p = .028$] bout duration. Post hoc analysis revealed that compared with mice in NOC group, while mice in POT group showed an increase in wake bout duration, NREM bout duration was comparable between NOC and POT.

Wake and NREM bout duration values were comparable in NOC and NPOC groups (Table 3). ***Spectral analysis:*** Since NREM δ and REM θ are implicated in memory consolidation [31–33], spectral analysis was performed to examine NREM δ and REM θ activities during 9 hr of light period post-FMA. One-way ANOVA suggested a significant change in NREM δ [$F(2, 14) = 5.1$; $p = .02$] and REM θ [$F(2, 14) = 5.1$; $p = .02$] during 9 hr of light period post-FMA. Although NREM δ and REM θ values were comparable between NOC and NPOC groups, mice in the POT group displayed a significant ($p < .05$; Dunnett's test) increase in NREM δ and REM θ compared with NOC group (Figure 2C and D).

Figure 2.



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Mice exposed to POT displayed sleep changes and electrophysiological indicators of memory consolidation during light period post-FMA. (A) Post-FMA training, mice exposed to POT displayed an increase in NREM latency compared with mice in the NOC group. Mice in NPOC group displayed comparable values to mice in NOC group. (B) During the remaining light (sleep) period, post-FMA training, mice exposed to POT spent significantly more time in wakefulness compared with NOC controls. NOC and NPOC groups did not show any difference in wakefulness, NREM, and REM sleep values. (C) Compared with NOC controls, mice exposed to POT group displayed a significant increase in NREM δ power during remaining light (sleep) period post-FMA training. Increase in NREM δ power is an indicator of memory consolidation. However, no such increase was observed when NOC group was compared with NPOC group. (D) During the remaining 9 hr of light (sleep) period, post-FMA training, mice in the POT group displayed a significant increase in REM-θ power indicative of memory consolidation compared with controls. REM-θ power was comparable between NOC and NPOC groups. ***$p < .001$; *$p < .05$.

Table 3.

Bout frequency and duration of sleep–wakefulness during light period on day 1 post-FMA

	Wakefulness		NREM		REM	
	Frequency	Duration (s)	Frequency	Duration (s)	Frequency	Duration (s)
NOC	207.8 ± 4.8	55.6 ± 0.6	208.2 ± 4.2	83.6 ± 1.9	43.0 ± 3.9	60.1 ± 1.3
NPOC	200.2 ± 19.0	56.6 ± 5.9	203.4 ± 18.1	87.0 ± 6.2	35.8 ± 9.2	72.4 ± 5.3
POT	185.0 ± 22.8	87.4 ± 11.1*	231.8 ± 11.9	68.1 ± 4.4	28.8 ± 3.5	73.3 ± 5.9

NOC = no odor control; NPOC = nonpredator odor control; POT = predator odor trauma.

* $p < .05$.

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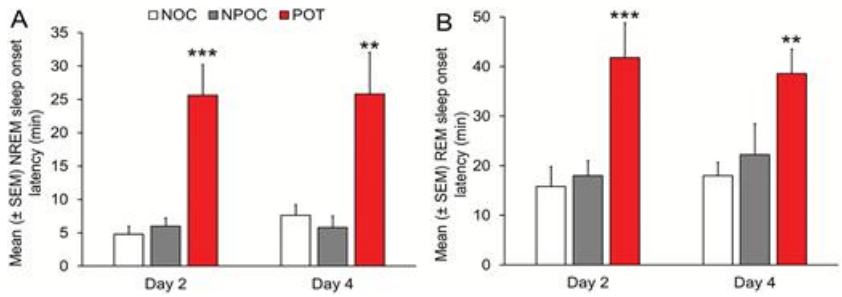
Changes in sleep–wakefulness during light period on days 2 and 4 post-FMA

NREM sleep latency: Two-way ANOVA suggested a significant main effect of treatment [$F(2, 12) = 40.0$; $p = .0001$] on NREM latency. Time [$F(1, 12) = 0.07$; $p = .8$] and interaction were not significant [$F(2, 12) = 0.08$; $p = .9$]. Subsequent post hoc analysis suggested that the POT group showed a significant increase in NREM latency on days 2 ($p < .001$) and 4 ($p < .01$) compared with NOC group. NREM latency values were comparable in NOC and NPOC groups on both days 2 and 4 (Figure 3A). ***REM sleep latency:*** A significant main effect of treatment [$F(2, 12) = 17.9$; $p = .0003$] was observed on REM latency. Time [$F(1, 12) = 0.06$; $p = .8$] and interaction [$F(2, 12) = 0.24$; $p = .79$] did not show any significant effects. Bonferroni's post hoc analysis suggested that compared with NOC group, mice in the POT group displayed a significant increase in REM latency on days 2 ($p < .001$) and 4 ($p < .01$). REM latency values were comparable in NOC and NPOC groups on both days 2 and 4 (Figure 3B). ***Time spent in wakefulness:*** Two-way ANOVA suggested a significant effect of treatment on wakefulness [$F(2, 12) = 16.8$; $p = .0003$]. No such significance was observed with time [$F(1, 12) = 0.7$; $p = .4$] and interaction [$F(2, 12) = 0.01$; $p = .9$]. Post hoc analysis suggested that compared with NOC group, mice in POT group spent significantly more time in wakefulness on both days 2 ($p < .05$) and 4 ($p < .05$). However, mice in NOC and NPOC group spent comparable time in wakefulness on both days 2 and 4 (Figure 4A). ***Time spent in NREM sleep:*** A significant main effect of treatment was observed on NREM sleep [$F(2, 12) = 13.9$; $p = .0008$]. The effect of time [$F(1, 12) = 1.7$; $p = .7$] and interaction [$F(2, 12) = 0.002$; $p = .9$] remained unaffected. Compared with NOC group, mice in POT group spent significantly less time in NREM sleep on both days 2 ($p < .05$) and 4 ($p < .05$). On both days 2 and 4, mice in NOC and NPOC groups spent comparable amount of time in NREM sleep (Figure 4B). ***Time spent in REM sleep:*** There was no effect of treatment [$F(2, 12) = 0.5$; $p = .6$], time [$F(1, 12) = 2.5$; $p = .1$], or interaction [$F(2, 12) = 0.68$; $p = .5$] on REM sleep suggesting that REM sleep values were comparable in all three groups on days 2 and 4 (Figure 4C). ***Bout frequency:*** A significant effect of treatment was observed on wake [$F(2, 12) = 17.2$; $p = .0003$] and NREM [$F(2, 12) = 20.1$; $p = .0001$] bout frequencies. Time [wake: $F(1, 12) = 0.6$; $p = .45$; NREM: $F(1, 12) = 1.2$; $p = .3$] and interaction [wake: $F(2, 12) = 0.11$; $p = .9$; NREM: $F(2, 12) = 0.06$; $p = .9$] remained unchanged. Wake and NREM bout frequency values were comparable between NOC and NPOC groups. However, compared with NOC group, mice in POT group showed a significant increase in wake (day 2: $p < .01$; day 4: $p < .05$) and NREM bout frequencies (day 2: $p < .01$; day 4: $p < .05$). No significant effect of treatment [$F(2, 12) = 0.3$; $p = .8$], time [$F(1, 12) = 0.1$; $p = .8$], and interaction [$F(2, 12) = 1.7$; $p = .2$] was observed on REM sleep bout frequency (Table 4). ***Bout duration:*** REM sleep bout duration did not show any significant change [treatment: = $F(2, 12) = 0.6$; $p = .6$; time: $F(1, 12) = 2.1$; $p = .2$; and interaction: $F(2, 12) = 1.0$; $p = .4$. However, two-way ANOVA analysis suggested a significant main effect of treatment on wake [$F(2, 12) = 4.1$; $p = .04$] and NREM [$F(2, 12) = 17.7$; $p = .0003$] bout duration. Time [wake: $F(1, 12) = 0.0$; $p = .9$; NREM: $F(1, 12) = 0.2$; $p = .7$] and interaction [wake: $F(2, 12) = 0.03$; $p = .9$; NREM: $F(2, 12) = 0.09$; $p = .9$] did not show any significance. Post hoc analysis revealed that wake and NREM sleep bout duration values were comparable in NOC and NPOC group on both days 2 and 4. However, compared with NOC, mice in the POT group showed a significant decrease ($p < .05$) in NREM sleep bout duration on day 4. NOC and POT groups had comparable values of wake and NREM bout duration on day 2 (Table 4). ***NREM δ activity:*** Although time [$F(1, 12) = 0.1$; $p = .7$] and interaction [$F(2, 12) = 0.3$; $p = .7$] were unaffected, a significant main effect of treatment [$F(2, 12) = 5.9$; $p = .02$] was observed on NREM δ activity. Post hoc analysis revealed that compared with mice in NOC group, mice in the POT group had a significant ($p < .05$) reduction in NREM δ power during the light period of day 2. NREM δ power values were comparable on day 4. NOC and NPOC had comparable NREM δ power on days 2 and 4 (Figure 5A). ***NREM β activity:*** Although time [$F(1, 12) = 0.04$; $p = .8$] and interaction [$F(2, 12) = 3.1$; $p = .07$] remained unaffected, a significant main effect of treatment [$F(2, 12) = 4.3$; $p = .04$] was observed on NREM β power. Post hoc analysis revealed that, while mice in NOC and NPOC groups had comparable NREM β power on days 2 and 4, mice in POT group had a significant ($p < .05$) increase in NREM β power on day 2 compared with mice in NOC group. Mice in the POT and NOC groups had comparable NREM β power on day 4 (Figure 5B).

Figure 3.



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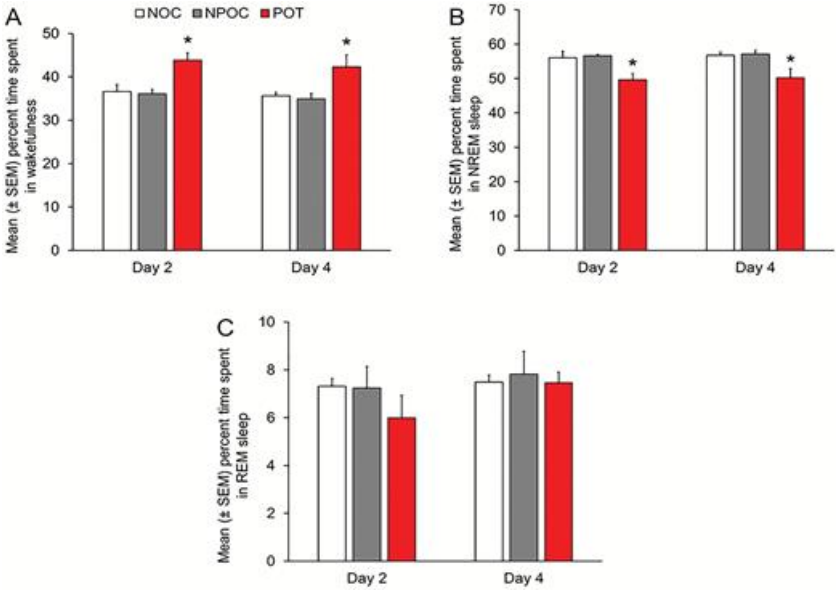
Mice exposed to POT displayed difficulty in initiating sleep during normal sleep (light) periods for 4 days post-FMA. (A) Compared with NOC, mice in the POT group took significantly more time to fall asleep as evident by an increase in NREM latency on days 2 and 4. NREM sleep latency values were comparable in NOC and NPOC groups on days 2 and 4. (B) Mice in the POT group displayed a significant increase in REM sleep latency on both days 2 and 4, compared with mice in the NOC group. No such increase was observed when NPOC group was compared with NOC group. ***$p < .001$; **$p < .01$.

Figure 4.



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Mice exposed to POT displayed protracted sleep disruptions post-FMA. (A) The amount of time spent in wakefulness, on days 2 and 4, was comparable between NOC and NPOC groups. However, compared with NOC group, mice in the POT group displayed a significant increase in wakefulness on days 2 and 4. (B) Compared with NOC, mice in the POT group spent significantly less time in NREM sleep during the light period on days 2 and 4. NREM sleep values were comparable in NOC and NPOC groups on days 2 and 4. (C) The amount of time spent in REM sleep, on days 2 and 4, was comparable between all three groups: NOC, POT, and NPOC. *$p < .05$.

Table 4.

Bout frequency and duration of sleep–wakefulness during light period

		Wakefulness		NREM		REM	
		Frequency	Duration (s)	Frequency	Duration (s)	Frequency	Duration (s)
Day 2	NOC	162.0 ± 11.6	98.5 ± 5.8	165.2 ± 11.2	150 ± 13.4	49.8 ± 3.4	63.8 ± 2.9
	NPOC	170.2 ± 27.5	103.7 ± 21.1	169.8 ± 28	164.7 ± 34.4	51.4 ± 9.0	63.4 ± 4.0
	POT	253.2 ± 23.7**	76.7 ± 5.9	259 ± 20.8**	85.6 ± 8.8	37.4 ± 5.1	65.7 ± 6.2
Day 4	NOC	159.6 ± 7.0	97.5 ± 5.9	152 ± 12.7	165.1 ± 13.6	46.0 ± 2.4	68.7 ± 3.1
	NPOC	155.8 ± 14.0	100.2 ± 11.7	156.0 ± 14.1	163.0 ± 12.5	46.2 ± 8.5	75.1 ± 4.0
	POT	232.8 ± 13.9*	80.1 ± 9.6	233.6 ± 14.7*	94.0 ± 5.9*	50.6 ± 5.2	65.0 ± 3.9

NOC = no odor control; NPOC = nonpredator odor control; POT = predator odor trauma.

* $p < .05$.

** $p < .01$.

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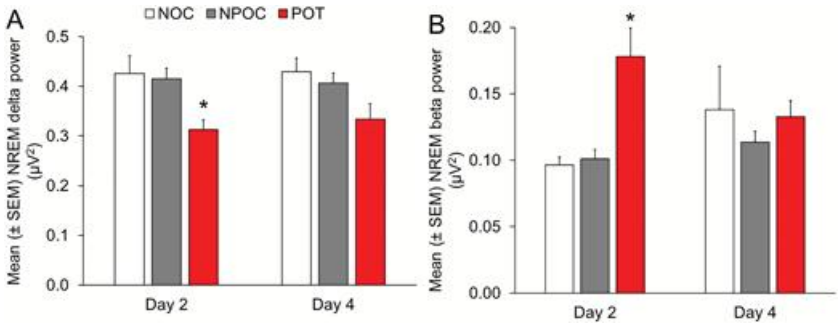
Figure 5.



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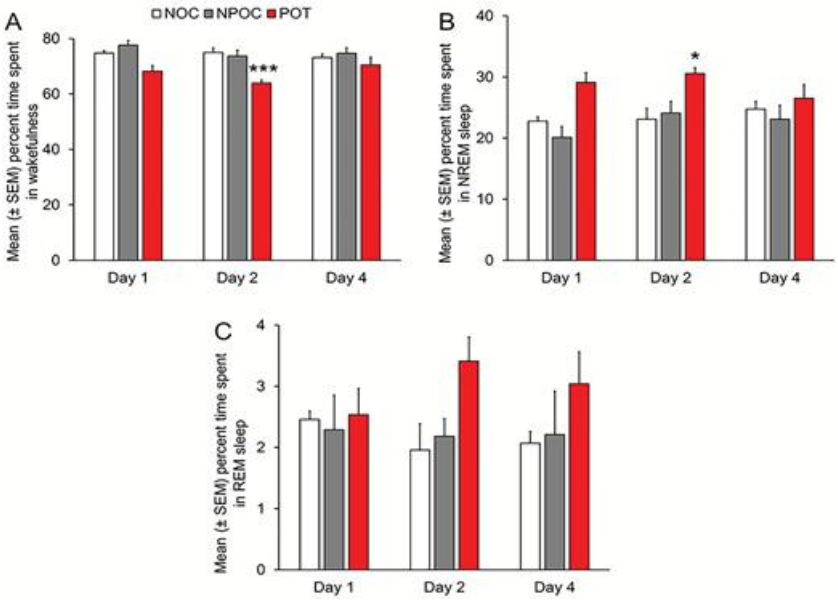
Mice exposed to POT displayed reduced quality of sleep post-FMA. (A) Although NREM δ power values were comparable between NOC and NPOC groups, a significant decrease in NREM δ power, an indicator of NREM sleep quality, was observed in mice exposed to POT compared with NOC controls, during light period on day 2. All three groups had comparable NREM δ activity values on day 4. (B) Compared with NOC controls, a significant increase in NREM β power was noted in mice exposed to POT during the light period on day 2. No such increase in NREM β power was observed in mice in the NPOC group. NREM β power values were comparable in all three groups during light period on day 4. *$p < .05$.

Changes in sleep–wakefulness during dark period on days 1, 2, and 4 post-FMA

Time spent in wakefulness: Two-way ANOVA suggested a significant main effect of treatment on wakefulness [$F(2, 24) = 9.6$; $p = .003$]. No such significance was observed with time [$F(2, 24) = 2.8$; $p = .08$] and interaction [$F(4, 24) = 2.5$; $p = .07$]. Post hoc analysis suggested that compared with NOC group, mice in POT group spent significantly less time in wakefulness on only on day 2 ($p < .001$), NOC and POT had comparable values on days 1 and 4. Mice in NOC and NPOC groups spent comparable time in wakefulness on days 1, 2, and 4 (Figure 6A). ***Time spent in NREM sleep:*** A significant main effect of treatment was observed on NREM sleep [$F(2, 24) = 6.7$; $p = .01$]. The effect of time [$F(2, 24) = 1.7$; $p = .2$] and interaction [$F(4, 24) = 2.05$; $p = .1$] remained unaffected. NREM sleep was significantly increased in POT group compared with NOC group only on day 2 ($p < .05$). NREM was on days 1 and 4 was comparable in NOC and POT groups. NREM sleep values were comparable between mice in NOC and NPOC groups on all 3 days (days 1, 2, and 4; Figure 6B). ***Time spent in REM sleep:***There was no effect of treatment [$F(2, 24) = 1.6$; $p = .2$], time [$F(2, 24) = 0.06$; $p = .9$], or interaction [$F(4, 24) = 1.2$; $p = .3$] on REM sleep suggesting that REM sleep values were comparable in all three groups on days 1, 2, and 4 (Figure 6C). ***Bout frequency:*** A significant effect of treatment [wake: $F(2, 24) = 4.33$; $p = .038$; NREM: $F(2, 24) = 6.20$; $p = .014$], time [wake: $F(2, 24) = 32.09$; $p < .0001$; NREM: $F(2, 24) = 24.25$; $p < .0001$], and interaction [wake: $F(4, 24) = 13.87$; $p < .0001$; NREM: $F(2, 24) = 4.10$; $p = .011$] was observed on wake and NREM bout frequency. Post hoc analysis revealed that wake and NREM sleep bout frequencies values were comparable for all 3 days (days 1, 2, and 4) in NOC and NPOC groups. However, compared with NOC, mice in POT group showed a reduction in wake ($p < .0001$) and NREM ($p < .001$) bout frequency only on day 2, but not on days 1 and 4. No significant effect of treatment [$F(2, 24) = 0.9$; $p = .4$], time [$F(2, 24) = 0.4$; $p = .7$], and interaction [$F(4, 24) = 1.6$; $p = .2$] was observed on REM sleep bout frequency (Table 5). ***Bout duration:*** Treatment [wake: $F(2, 24) = 7.83$, $p = .007$; NREM: $F(2, 24) = 36.77$; $p < .0001$], time [wake: $F(2, 24) = 48.61$, $p < .0001$; NREM: $F(2, 24) = 35.93$; $p < .0001$], and interaction [wake: $F(4, 24) = 27.72$, $p < .0001$; NREM: $F(4, 24) = 21.74$; $p < .0001$] showed significant effects on duration of wake and NREM bouts. Although wake and NREM bout duration values were comparable for all 3 days (days 1, 2, and 4) in NOC and NPOC groups, post hoc analysis revealed compared with NOC, mice in the POT group displayed a significant ($p < .0001$) increase in wake and NREM bout duration only on day 2. On days 1 and 4, wake and NREM bout duration values were comparable between POT and NOC groups. No significant effect of treatment [$F(2, 24) = 2.06$; $p = .2$], time [$F(2, 24) = 0.8$; $p = .5$], and interaction [$F(4, 24) = 0.7$; $p = .6$] was observed on duration of REM sleep bouts (Table 5).

Figure 6.



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Mice exposed to POT displayed an increase in NREM sleep coupled with reduction in wakefulness during the active (dark) period. (A) Although wakefulness values between all three groups were comparable during the active period on days 1 and 4, mice in the POT group spent significantly more time in NREM sleep on day 2 compared with NOC controls. In contrast, wakefulness values in NOC and NPOC groups were comparable on day 2. (B) A significant increase in NREM sleep was observed in POT group, during active period on day 2 compared with NOC controls. No such increase was observed when NPOC group was compared with NOC group. NREM values between all three groups were comparable during the active period on days 1 and 4. (C) REM sleep remained unchanged in all three groups (NPO, NPOC, and POT) on all 3 days: days 1, 2, and 4. ***$p < .001$; *$p < .05$.

Table 5.

Bout frequency and duration of sleep–wakefulness during dark period

		Wakefulness		NREM		REM	
		Frequency	Duration (s)	Bouts	Duration (s)	Bouts	Duration (s)
Day 1	NOC	102.4 ± 4.7	295.9 ± 16.3	122.4 ± 8.3	75.2 ± 3.2	14.8 ± 1.7	72.3 ± 10.2
	NPOC	109.4 ± 6.0	282.9 ± 11.0	129.8 ± 16.0	63.3 ± 5.0	15.4 ± 4.4	66.5 ± 7.3
	POT	124.0 ± 6.0	222.9 ± 14.7	104.0 ± 12.1	119.2 ± 11.0	13.4 ± 2.2	77.8 ± 2.6
Day 2	NOC	100.0 ± 9.5	337.9 ± 39.4	101.0 ± 10.6	100.8 ± 11.3	12.8 ± 2.1	56.9 ± 9.2
	NPOC	86.6 ± 4.5	372.9 ± 20.5	87.0 ± 7.8	126.0 ± 16.8	15.0 ± 2.4	61.6 ± 6.5
	POT	38.6 ± 1.9***	722.3 ± 33.5***	37.8 ± 1.2***	350.5 ± 15.4***	20.0 ± 3.1	78.3 ± 8.1
Day 4	NOC	75.6 ± 9.7	444.9 ± 52.8	70 ± 9.9	169.9 ± 30.7	12.6 ± 2.0	67.1 ± 3.9
	NPOC	87.2 ± 4.2	373.0 ± 23.5	88.2 ± 4.3	114.6 ± 12.2	12.8 ± 3.1	64.6 ± 9.0
	POT	78.8 ± 4.8	391.4 ± 25.9	75.2 ± 6.9	156.3 ± 19.3	19.2 ± 3.7	64.8 ± 3.1

NOC = no odor control; NPOC = nonpredator odor control; POT = predator odor trauma.

*** $p < .001$.

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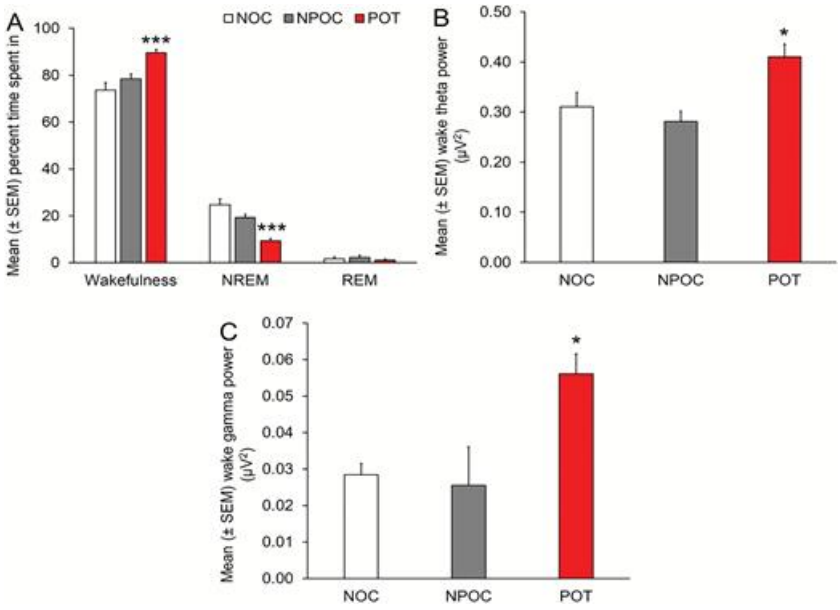
Electrophysiological indicators of memory retrieval observed during FMR

Time spent in sleep–wakefulness: FMR was performed by exposing the animals to CS, without US, for 2 hr. One-Way ANOVA suggested a significant difference in wakefulness [$F(2, 14) = 12.5$; $p = .001$] and NREM sleep [$F(2, 14) = 19.4$; $p = .0001$] between three groups. The amount of time spent in REM sleep remained unaffected [$F(2, 14) = 0.6$; $p = .6$]. Post hoc analysis suggests that mice in NOC and NPOC groups spent comparable time in wakefulness and NREM sleep. However, compared with NOC groups, mice in POT group displayed a significant increase in wakefulness ($p < .001$) and a significant reduction in NREM sleep ($p < .001$; Figure 7A). ***Spectral analysis performed during FMR:*** One-way ANOVA suggested significant differences in wake θ [$F(2, 14) = 7.1$; $p = .009$] and γ [$F(2, 14) = 5.4$; $p = .02$] activities between three groups. Although θ and γ values were comparable in NOC and NPOC groups, post hoc analysis revealed a significant increase in θ ($p < .05$) and γ ($p < .05$) activities in POT group compared with NOC group (Figure 7B and C).

Figure 7.



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Mice exposed to POT display indicators of fear memory during FMR testing on day 5. (A) Compared with NOC controls, mice in the POT group when exposed to objective reminders of trauma (contextual cage) spent significantly more time in wakefulness and significantly less time in NREM sleep during 2 hr of FMR testing. The NPOC controls did not show such a change. REM sleep remained unchanged. (B) Compared with NOC controls, hippocampal θ power was significantly increased in mice exposed to POT. No such change was observed in NPOC group. Increase in hippocampal θ power indicates memory recall. (C) During FMR, mice in the POT group displayed a significant increase in hippocampal γ power compared with NOC controls. This change was not observed in NPOC group. ***$p < .001$; *$p < .05$.

Discussion

In this study, we performed contextual conditioning, using POT as the US, and examined hippocampal field potentials and sleep–wakefulness. Major findings of our study suggest that mice exposed to POT displayed as follows: (1) Contextual conditioning, as evident by a state of hyperarousal coupled with memory acquisition and retrieval (significant increase in the amount of time spent in wakefulness, hippocampal θ, and γ power) observed during FMA training and FMR testing [34–36]. (2) Memory consolidation following FMA training, as evident by an increase in NREM δ and REM θ power during sleep period post-FMA training [22, 24, 36–38]. (3) Severe and protracted sleep disruptions as evident by difficulty in falling asleep (increase in NREM and REM latency) and maintaining quantity (increased wakefulness, reduced NREM and REM sleep, increased wake and NREM bout frequencies, and reduced NREM sleep duration) and quality of sleep (reduced NREM δ power; increase in NREM β power) during normal sleep (light) periods along with symptoms of daytime sleepiness as evident by increased NREM sleep during active period [10, 39–42].

Our experiment design is logical. Inbred C57BL/6J mice were used to control for genetic variability. Compared with other strains of mice, C57BL/6J mice display a significant increase in anxiety and startle response following a single exposure of predator odor [43]. Mice prefer darkness and light enhances fear, especially learned fear [44]. Therefore, to enhance fear and stress, all fear conditioning experiments were performed during the light period.

We used POT as the US and performed contextual conditioning. Recently, several studies have begun to use predator odor to examine fear and

anxiety responses due to their potential relevance in animal models of stress and anxiety disorders including PTSD (reviewed in Refs. 12, 45–50). Use of predator odor as US for studying contextual fear offers several advantages: (1) Most physical stressor models, including the most extensively used "inescapable footshock model," involve physical pain or discomfort. In contrast, exposure of rodents to predator odor does not involve pain rather; it is fear provoking, stressful, and produces protracted behavioral and physiological responses [12, 16, 17, 46, 51–54]. (2) Rodents have innate hard-wired (genetic) fear for predator odor and even laboratory rat and mice, which have never experienced (or exposed to) a cat or cat odor, and display fear and stress when exposed to cat odor. Thus, predator odor conditioning can act as a biologically relevant model for innate as well as learned fear [12, 16, 46, 50, 55–57]. (3) Olfaction is the primary sensory system used by rodents for majority of survival-related behaviors [58–61]. (4) Odors are strong sensory stimuli for cuing emotional memories [62]. (5) Similar to the extensively used "inescapable footshock model," the amygdala is the central site, and a dose-dependent relationship exists between US and conditional response, and US and secretion of stress hormones in the predator odor model [63].

Two controls were used in this study: (1) NOC group exposed to the same amount of clean/fresh/unused cat litter; (2) The NPOC group control exposed to the same amount of "mouse used cat litter" or cat litter used (as a bedding) by a different C57BL/6J mouse for 2 days. This control provided a significant, yet nonpredator odor. In order to have a robust development of contextual conditioning, mice were allowed to explore contextual cage (CS) for 30 min followed by exposure to soiled cat litter (US) for 90 min during FMA training. Subsequently, mice were left undisturbed (except for sleep recordings) until tested on day 5. FMR testing was performed by exposing the animals to objective reminders of trauma: contextual cage.

Electrophysiological measures were used to examine contextual conditioning and changes in sleep–wakefulness. Mice in the POT group displayed a state of hyperarousal, increased wakefulness along with increased hippocampal θ and γ activities, during FMA training and during FMR testing. Increased hippocampal θ and γ activities are indicators of memory encoding and retrieval [34, 64–66]. This was followed by a significant increase in NREM δ and REM θ activities post-FMA training, suggesting memory consolidation during subsequent sleep period [22, 24].

In our study, mice exposed to POT showed a robust and persistent increase in wakefulness (day 2 = 19.7% increase; day 4 = 18.6 %), mainly due to the increase in the frequency of wakefulness bouts, during the normal sleep (light) period that lasted for 4 days. Concomitantly mice exposed to POT had persistent difficulty in falling asleep (increased NREM and REM sleep latency) and maintaining NREM sleep during the normal sleep (light period) as evident by an increase in NREM bout frequency on both days 2 and 4. In addition, mice exposed to POT displayed reduced quality of NREM sleep (reduced δ and increased β activities), especially on day 2, during the normal sleep period post-POT exposure. In contrast, reduced wakefulness and increased NREM sleep were observed during the active (dark) period especially on day 2. These findings are congruent with what is observed in human PTSD; majority of human PTSD studies suggest severe and protracted insomnia, nightmares, reduction in quality and quantity of NREM sleep along with excessive daytime sleepiness in PTSD [39, 67–71].

Some human PTSD studies have observed REM sleep changes (increased REM sleep with chronic PTSD; reduced REM sleep proximate to trauma exposure) [68, 72–74]. In our study, we did not observe any major quantitative changes in REM sleep on days 2 and 4. This may be a limitation of our model.

Fear is an emotional feeling of disquiet that appears rapidly in the presence of threat or danger and dissipates quickly once the threat or danger is removed. Fear can be innate or learned. Innate fear may be genetic and responses are activated by intrinsically threatening stimuli. Learned fear is acquired and experience dependent and can develop across the lifespan. Convincing evidence exist to suggest that distinct neural circuits are involved in the control of innate and learned fear [75, 76]. Thus, delineating fear circuitry involved in innate and learned fear and examination of its interactions with circuits regulating sleep–wakefulness will help us understand and develop efficacious treatment strategies for fear and anxiety disorders such as PTSD.

In summary, we have used POT as the US and performed contextual fear conditioning in C57BL/6J mice. The results of our study suggest, for the first time, that animals exposed to POT display severe and protracted sleep disturbances similar to sleep disturbance observed in human PTSD patients.

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Notes

Conflict of interest statement. None declared.

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References

1. Association AP. *Diagnostic and Statistical Manual of Mental Disorders* . 5th (Revised) ed. Washington D.C.: American Psychiatric Press; 2013.

2. Neylan TCet al. Sleep disturbances in the Vietnam generation: findings from a nationally representative sample of male Vietnam veterans. *Am J Psychiatry* . 1998; 155(7): 929–933.
Google Scholar Crossref PubMed

3. Ohayon MMet al. Sleep disturbances and psychiatric disorders associated with posttraumatic stress disorder in the general population. *Compr Psychiatry* . 2000; 41(6): 469–478.
Google Scholar Crossref PubMed

4. Harvey AGet al. Sleep and posttraumatic stress disorder: a review. *Clin Psychol Rev* . 2003; 23(3): 377–407.
Google Scholar Crossref PubMed

5. Sturm Aet al. Effects of unconditioned stimulus intensity and fear extinction on subsequent sleep architecture in an afternoon nap. *J Sleep Res* . 2013; 22(6): 648–655.
Google Scholar Crossref PubMed

6. Spoormaker VIet al. Effects of rapid eye movement sleep deprivation on fear extinction recall and prediction error signaling. *Hum Brain Mapp* . 2012; 33(10): 2362–2376.
Google Scholar Crossref PubMed

7. Spoormaker VIet al. The neural correlates and temporal sequence of the relationship between shock exposure, disturbed sleep and impaired consolidation of fear extinction. *J Psychiatr Res* . 2010; 44(16): 1121–1128.
Google Scholar Crossref PubMed

8. Spoormaker VIet al. Disturbed sleep in post-traumatic stress disorder: secondary symptom or core feature?*Sleep Med Rev* . 2008; 12(3): 169–184.
Google Scholar Crossref PubMed

9. Ross RJet al. Sleep disturbance as the hallmark of posttraumatic stress disorder. *Am J Psychiatry* . 1989; 146(6): 697–707.
Google Scholar Crossref PubMed

10. Germain A. Sleep disturbances as the hallmark of PTSD: where are we now?*Am J Psychiatry* . 2013; 170(4): 372–382.
Google Scholar Crossref PubMed

11. Germain Aet al. Sleep-specific mechanisms underlying posttraumatic stress disorder: integrative review and neurobiological hypotheses. *Sleep Med Rev* . 2008; 12(3): 185–195.
Google Scholar Crossref PubMed

12. Goswami Set al. Animal models of post-traumatic stress disorder: face validity. *Front Neurosci* . 2013; 7: 89.
Google Scholar Crossref PubMed

13. Cohen Het al. Animal model for PTSD: from clinical concept to translational research. *Neuropharmacology* . 2012; 62(2): 715–724.
Google Scholar Crossref PubMed

14. Borghans Bet al. Animal models for posttraumatic stress disorder: An overview of what is used in research. *World J Psychiatry* . 2015; 5(4): 387–396.
Google Scholar Crossref PubMed

15. Matar MAet al. Translationally relevant modeling of PTSD in rodents. *Cell Tissue Res* . 2013; 354(1): 127–139.
Google Scholar Crossref PubMed

16. Dielenberg RAet al. Defensive behavior in rats towards predatory odors: a review. *Neurosci Biobehav Rev* . 2001; 25(7-8): 597–609.
Google Scholar Crossref PubMed

17. Blanchard DCet al. Ethoexperimental approaches to the biology of emotion. *Annu Rev Psychol* . 1988; 39: 43–68.
Google Scholar Crossref PubMed

18. Adamec REet al. Lasting effects on rodent anxiety of a single exposure to a cat. *Physiol Behav* . 1993; 54(1): 101–109.
Google Scholar Crossref PubMed

19. Faul Fet al. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* . 2007; 39(2): 175–191.
Google Scholar Crossref PubMed

20. Franklin KBet al. *The Mouse Brain in Stereotaxic Coordinates* . 3rd ed. New York, NY: Academic Press; 2008.

21. Gervasoni Det al. Global forebrain dynamics predict rat behavioral states and their transitions. *J Neurosci* . 2004; 24(49): 11137–11147.
Google Scholar Crossref PubMed

22. Ognjanovski Net al. CA1 hippocampal network activity changes during sleep-dependent memory consolidation. *Front Syst Neurosci* . 2014; 8: 61.
Google Scholar Crossref PubMed

23. Lima GZDSet al. Predictability of arousal in mouse slow wave sleep by accelerometer data. *PLoS One* . 2017; 12(5): e0176761.
Google Scholar Crossref PubMed

24. Ognjanovski Net al. Parvalbumin-expressing interneurons coordinate hippocampal network dynamics required for memory consolidation. *Nat Commun* . 2017; 8: 15039.
Google Scholar Crossref PubMed

25. Ribeiro Set al. Long-lasting novelty-induced neuronal reverberation during slow-wave sleep in multiple forebrain areas. *PLoS Biol* . 2004; 2(1): E24.
Google Scholar Crossref PubMed

26. Emrick JJet al. Different simultaneous sleep states in the hippocampus and neocortex. *Sleep* . 2016; 39(12): 2201–2209.
Google Scholar Crossref PubMed

27. Sharma Ret al. Rapid tolerance development to the NREM sleep promoting effect of alcohol. *Sleep* . 2014; 37(4): 821–824.
Google Scholar Crossref PubMed

28. Sharma Ret al. Lesion of the basal forebrain cholinergic neurons attenuates sleepiness and adenosine after alcohol consumption. *J Neurochem* . 2017; 142(5): 710–720.
Google Scholar Crossref PubMed

29. Colgin LLet al. Gamma oscillations in the hippocampus. *Physiology (Bethesda)* . 2010; 25(5): 319–329.
Google Scholar PubMed

30. Hasselmo MEet al. Theta rhythm and the encoding and retrieval of space and time. *Neuroimage* . 2014; 85: 656–666.
Google Scholar Crossref PubMed

31. Mölle Met al. Slow oscillations orchestrating fast oscillations and memory consolidation. *Prog Brain Res* . 2011; 193: 93–110.
Google Scholar Crossref PubMed

32. Born J. Slow-wave sleep and the consolidation of long-term memory. *World J Biol Psychiatry* . 2010; 11: 16–21.
Google Scholar Crossref PubMed

33. Boyce Ret al. Causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation. *Science* . 2016; 352(6287): 812–816.
Google Scholar Crossref PubMed

34. Osipova Det al. Theta and gamma oscillations predict encoding and retrieval of declarative memory. *J Neurosci* . 2006; 26(28): 7523–7531.
Google Scholar Crossref PubMed

35. Ehlers CLet al. Electrophysiological responses to affective stimuli in American Indians experiencing trauma with and without PTSD. *Ann N Y Acad Sci* . 2006; 1071: 125–136.
Google Scholar Crossref PubMed

36. Headley DBet al. Common oscillatory mechanisms across multiple memory systems. *NPJ Sci Learn* 2017; 2: 1.
Google Scholar Crossref

37. Abel Tet al. Sleep, plasticity and memory from molecules to whole-brain networks. *Curr Biol* . 2013; 23(17): R774–R788.
Google Scholar Crossref PubMed

38. Prince TMet al. Sleep deprivation during a specific 3-hour time window post-training impairs hippocampal synaptic

plasticity and memory. *Neurobiol Learn Mem* . 2014; 109: 122–130.
Google Scholar Crossref PubMed

39. Kobayashi Iet al. Polysomnographically measured sleep abnormalities in PTSD: a meta-analytic review. *Psychophysiology* . 2007; 44(4): 660–669.
Google Scholar Crossref PubMed

40. Moldofsky Het al. Disturbed EEG sleep, paranoid cognition and somatic symptoms identify veterans with post-traumatic stress disorder. *BJPsych Open* . 2016; 2(6): 359–365.
Google Scholar Crossref PubMed

41. Spoormaker VIet al. Disturbed sleep in post-traumatic stress disorder: secondary symptom or core feature?*Sleep Med Rev* . 2008; 12(3): 169–184.
Google Scholar Crossref PubMed

42. Mellman TA. Sleep and post-traumatic stress disorder: a roadmap for clinicians and researchers. *Sleep Med Rev* . 2008; 12(3): 165–167.
Google Scholar Crossref PubMed

43. Cohen Het al. Post-traumatic stress behavioural responses in inbred mouse strains: can genetic predisposition explain phenotypic vulnerability?*Int J Neuropsychopharmacol* . 2008; 11(3): 331–349.
Google Scholar Crossref PubMed

44. Warthen DMet al. Light enhances learned fear. *Proc Natl Acad Sci U S A* . 2011; 108(33): 13788–13793.
Google Scholar Crossref PubMed

45. Pitman RKet al. Biological studies of post-traumatic stress disorder. *Nat Rev Neurosci* . 2012; 13(11): 769–787.
Google Scholar Crossref PubMed

46. Matar MAet al. Translationally relevant modeling of PTSD in rodents. *Cell Tissue Res* . 2013; 354(1): 127–139.
Google Scholar Crossref PubMed

47. Cohen Het al. Animal models of post-traumatic stress disorder. *Curr Protoc Neurosci* . 2013; Chapter 9: Unit 9.45.
Google Scholar PubMed

48. Daskalakis NPet al. Animal models in translational studies of PTSD. *Psychoneuroendocrinology* . 2013; 38(9): 1895–1911.
Google Scholar Crossref PubMed

49. Zovkic IBet al. Interindividual variability in stress susceptibility: a role for epigenetic mechanisms in PTSD. *Front Psychiatry* . 2013; 4: 60.
Google Scholar Crossref PubMed

50. Rosen JB. The neurobiology of conditioned and unconditioned fear: a neurobehavioral system analysis of the amygdala. *Behav Cogn Neurosci Rev* . 2004; 3(1): 23–41.
Google Scholar Crossref PubMed

51. Blanchard RJet al. The characterization and modelling of antipredator defensive behavior. *Neurosci Biobehav Rev* . 1990; 14(4): 463–472.
Google Scholar Crossref PubMed

52. Adamec REet al. Neural plasticity and stress induced changes in defense in the rat. *Neurosci Biobehav Rev* . 2001; 25(7–8): 721–744.
Google Scholar Crossref PubMed

53. Nanda SAet al. Predator stress induces behavioral inhibition and amygdala somatostatin receptor 2 gene expression. *Genes Brain Behav* . 2008; 7(6): 639–648.
Google Scholar Crossref PubMed

54. Seligman MEet al. Failure to escape traumatic shock. *J Exp Psychol* . 1967; 74(1): 1–9.
Google Scholar Crossref PubMed

55. Apfelbach Ret al. The effects of predator odors in mammalian prey species: a review of field and laboratory studies. *Neurosci Biobehav Rev* . 2005; 29(8): 1123–1144.
Google Scholar Crossref PubMed

56. Ferrero DMet al. Detection and avoidance of a carnivore odor by prey. *Proc Natl Acad Sci U S A* . 2011; 108(27): 11235–11240.
Google Scholar Crossref PubMed

57. Takahashi LKet al. The smell of danger: a behavioral and neural analysis of predator odor-induced fear. *Neurosci Biobehav Rev* . 2005; 29(8): 1157–1167.
Google Scholar Crossref PubMed

58. Restrepo Det al. Emerging views on the distinct but related roles of the main and accessory olfactory systems in responsiveness to chemosensory signals in mice. *Horm Behav* . 2004; 46(3): 247–256.
Google Scholar Crossref PubMed

59. Luo Met al. Encoding pheromonal signals in the accessory olfactory bulb of behaving mice. *Science* . 2003; 299(5610): 1196–1201.
Google Scholar Crossref PubMed

60. Brennan PAet al. Neural mechanisms of mammalian olfactory learning. *Prog Neurobiol* . 1997; 51(4): 457–481.
Google Scholar Crossref PubMed

61. Wang MEet al. Differential roles of the dorsal and ventral hippocampus in predator odor contextual fear conditioning. *Hippocampus* . 2013; 23(6): 451–466.
Google Scholar Crossref PubMed

62. Herz RSet al. The emotional distinctiveness of odor-evoked memories. *Chem Senses* . 1995; 20(5): 517–528.
Google Scholar Crossref PubMed

63. Takahashi LKet al. Predator odor-induced conditioned fear involves the basolateral and medial amygdala. *Behav Neurosci* . 2007; 121(1): 100–110.
Google Scholar Crossref PubMed

64. Tort ABet al. Theta-gamma coupling increases during the learning of item-context associations. *Proc Natl Acad Sci U S A* . 2009; 106(49): 20942–20947.
Google Scholar Crossref PubMed

65. Sederberg PBet al. Theta and gamma oscillations during encoding predict subsequent recall. *J Neurosci* . 2003;

23(34): 10809–10814.
Google Scholar PubMed

66. Siegle JHet al. Enhancement of encoding and retrieval functions through theta phase-specific manipulation of hippocampus. *Elife* . 2014; 3: e03061.
Google Scholar Crossref PubMed

67. Neylan TCet al. Delta sleep response to metyrapone in post-traumatic stress disorder. *Neuropsychopharmacology* . 2003; 28(9): 1666–1676.
Google Scholar Crossref PubMed

68. Mellman TAet al. A relationship between REM sleep measures and the duration of posttraumatic stress disorder in a young adult urban minority population. *Sleep* . 2014; 37(8): 1321–1326.
Google Scholar Crossref PubMed

69. Miller KEet al. Sleep and dreaming in posttraumatic stress disorder. *Curr Psychiatry Rep* . 2017; 19(10): 71.
Google Scholar Crossref PubMed

70. Sinha SS. Trauma-induced insomnia: a novel model for trauma and sleep research. *Sleep Med Rev* . 2016; 25: 74–83.
Google Scholar Crossref PubMed

71. Koffel Eet al. Sleep disturbances in posttraumatic stress disorder: updated review and implications for treatment. *Psychiatr Ann* . 2016; 46(3): 173–176.
Google Scholar Crossref PubMed

72. Mellman TAet al. REM sleep and the early development of posttraumatic stress disorder. *Am J Psychiatry* . 2002; 159(10): 1696–1701.
Google Scholar Crossref PubMed

73. Breslau Net al. Sleep in lifetime posttraumatic stress disorder: a community-based polysomnographic study. *Arch Gen Psychiatry* . 2004; 61(5): 508–516.
Google Scholar Crossref PubMed

74. Ross RJet al. Rapid eye movement sleep disturbance in posttraumatic stress disorder. *Biol Psychiatry* . 1994; 35(3): 195–202.
Google Scholar Crossref PubMed

75. Gross CTet al. The many paths to fear. *Nat Rev Neurosci* . 2012; 13(9): 651–658.
Google Scholar Crossref PubMed

76. Rosén Jet al. Social, proximal and conditioned threat. *Neurobiol Learn Mem* . 2017; 142(Pt B): 236–243.
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