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Research report

Acute inescapable stress exposure induces long-term sleep disturbances and avoidance behavior: A mouse model of post-traumatic stress disorder (PTSD)

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ABSTRACT

The experience of traumatic stress often leads to long-lasting alteration in sleep quality and behavior. The objective of the present experiment was to investigate the short- and long-term effects of acute inescapable stress (i.e. two electric foot-shocks of 1.5 mA; 2 s) on sleep/wakefulness parameters and behavior in Swiss mice using electroencephalographic (EEG) analysis. Baseline EEG recording was performed in the home cage for 6 h prior to the application of the foot-shocks in the presence of an object (i.e. a plastic prism). One, 7, 14 or 21 days later, a second 6 h EEG recording session was performed after mice had been exposed or not to the same object for 5 min in their home cage. Results showed that at day 1, 7, 14 and 21 post-stress, shocked mice displayed sleep fragmentation as shown by an increase in the number of sleep episodes, regardless the presence of the object or not. In animals exposed to the object, the total duration of wakefulness over 6 h was significantly increased at days 7, 14 and 21 post-stress, and rapid eye movement (REM) sleep was significantly decreased at day 14 post-shock. Moreover, in the behavioral experiment, conditioned avoidance to a shock-paired object, which appeared as soon as 24 h after shock application, turned into generalized avoidance towards an unknown object 21 days after stress. These findings demonstrate that an acute inescapable stress exposure may cause long-lasting alterations in sleep patterns and behavior. Such modifications may be reminiscent of the profound changes observed in patients suffering from post-traumatic stress disorder.

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1. Introduction

Stress can have a significant negative impact on sleep and traumatic life events may produce at least temporary sleep disturbances that may include insomnia or subjective sleep problems [1]. Persistence of sleep disturbances after a traumatic event may represent a core feature of several psychiatric illnesses, including post-traumatic stress disorder (PTSD) [26], which is characterised by persistence of reexperiencing, avoidance and hyperarousal symptoms, following the traumatic event. Hyperarousal symptoms include difficulty falling or staying asleep, irritability or outburst of anger, difficulty concentrating, hypervigilance and exaggerated startled response [23]. The presence of sleep alteration following a stressful event has been suggested to be predictive of future development of stress-related disorders such as PTSD [1,2]. For instance, the occurrence of insomnia and nightmares within one month after experiencing a motor accident may predict development of PTSD in the upcoming six months [3]. This example and many others

suggest that disturbed sleep represents a risk factor for the development of PTSD and is one of its core symptoms.

Several studies in animals have investigated the effects of electric shocks on the sleep/wakefulness cycle. They showed that the application of foot-shocks induced changes in sleep architecture when animals were exposed to situational reminders of the training context at least up to 48 h later [4]. Moreover, using a fear conditioning paradigm in rats, Jha and colleagues (2005) showed that percentage of Rapid Eye Movement (REM) sleep was reduced 24 h following shock. Although these studies clearly showed alterations in sleep parameters following stress exposure, they did not study the long-term effects of electric shocks on sleep as they limited their investigation to 48 h post-stress. One study by Sandford et al. [5] reported that reminders of multiple shock training was able to impact REM sleep for at least month in mice. However, they did not find long-lasting effects on sleep when mice were trained to one tone-shock pairing, a result which they explained by a weak association between the cue and the shock or by an habituation due to the repeated presentation of the cue. Madan et al. [8] examined the long-term effects of fear conditioning on sleep architecture in rats focusing on REM sleep. The authors showed that conditioned fear significantly altered REM sleep microarchitecture as evidenced by a reduction in the number of sequential REMS episodes (i.e.

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REMS episodes separated by ≤ 3 min intervals, which appear in clusters), and an increased in single REMS episodes (>3 min episode separation) up to 14 days post-stress. Similarly, DaSilva and colleagues reported in a recent study [24] long-term alteration on REMS microarchitecture induced by fear conditioning in rats.

In addition, experiencing traumatic events in human can induce persistent avoidance of stimuli associated with the trauma. This symptom cluster (criterion C of PTSD symptoms, DSM-IV-TR) is considered as the most specific for identification of PTSD [9]. For example, 94% of bombing survivors from the terrorist attack in Oklahoma City who met group C criteria fulfilled the PTSD diagnosis, compared to none of those not fulfilling group C criteria [28]. Several studies in animals have addressed this issue. For example, Sawamura et al. [10] exposed rats to the same chamber in which they received electrical foot-shocks two weeks before and observed a dramatic increase in the number of avoidance events (i.e. moving to an other chamber) following an anxiogenic-like light signal. In a more recent study, Pamplona et al. [11] demonstrated conditioned avoidance to shock-paired odour, which turned into generalized avoidance 28 days later.

In this context, the aim of the present study was to investigate potential short- and long-term changes in sleep and behavior in stressed mice. A fear conditioning procedure was used in which animals were exposed to two unavoidable electric foot-shocks in the presence of an object (i.e. a plastic prism). This stress paradigm has similarities with the one described by Siegmund and Wotjak [12,13] which produced long-lasting conditioned, sensitized fear and depression-like behaviors. The short- and long-term consequences of traumatic stress exposure on sleep/wakefulness in mice were investigated by using electroencephalographic (EEG) recording, which was performed 1, 7, 14 or 21 days after stress in the presence or not of the object present during shock exposure, in order to determine if a cue reminder may alter sleep patterns further. Moreover, separate groups of animals were tested in the same conditioning procedure to determine if the alterations in sleep/wakefulness are accompanied by changes in behavioral responses when animals are subsequently exposed to the cue.

2. Material and methods

2.1. Animals

Swiss male mice (Janvier, Le Genest St-Isle, France) weighing 20–22 g at the start of the experiment were used. They were housed individually in plastic cages (30 cm \times 18 cm \times 18 cm) with free access to food and water *ad libitum*. They were maintained at a constant temperature of 21 ± 1 °C, humidity at $50 \pm 10\%$ and under a 12:12 light/dark cycle (light on at 7:00 a.m.). Experiments were conducted in accordance with the “Guide and Care and Use of Laboratory Animals” (National Institute of Health) and were approved by the in-house Animal Ethics Committee.

2.2. Shock application

Animals were placed into the shock chamber for a 190 s habituation period following which two electric foot-shocks (1.5 mA; for 2 s; 6 s apart) were delivered through the metal grid floor. This protocol is based on that described by Siegmund and Wotjak [13] who showed that it produced long-term (i.e. up to one month) PTSD-like symptoms when applied to mice. Animals remained in the shock chamber for another 60 s period with or without an object (i.e. a plastic prism used as a cue reminder) before they were returned to their home cage. For the avoidance test, control animals were exposed to the same procedure, but without receiving foot-shocks.

2.3. Sleep/wakefulness analysis

2.3.1. Surgery

Mice were anesthetized with Zoletil[®]50 (Tiletamine, Zolazepam, 60 mg/kg, i.p.), mounted in the stereotaxic apparatus and secured using blunt rodent ear bars. A scalp incision was made after local anesthesia with lidocaine 2% and the skin was retracted. The skull surface was cleaned to implant small stainless steel screw electrodes (0.9 mm in diameter). Three cortical electrodes were screwed into the bone over the sensorimotor cortex (1.5 mm lateral to the median and 1.5 mm behind the frontoparietal sutures), the visual cortex (1.5 mm lateral to the median and 1.5 mm in front of the parieto-occipital sutures) and over the cerebellum. They were attached to a connector (Winchester[®], 5-led) and fixed with dental cement (3M[®] ESPE) to the cranium. Animals were allowed to recover from surgery in their individual cage for two weeks prior recordings.

2.3.2. Recording procedure

Mice were habituated in their home cage to the recording cable and room for one day prior to each EEG recording session (Fig. 1). On the recording day, they were connected to the cable at 9:45 a.m. Recording sessions took place in the home cage between 10.00 a.m. and 04.00 p.m. and lasted 6 h. Baseline EEG parameters were recorded before the stress procedure. A second EEG recording session was performed 1, 7, 14 or 21 days following shock application. Prior to the second 6 h EEG recording session, the object which was present during shock exposure (i.e. the plastic prism) was placed into the home cage for 5 min. Separate groups of 4–5 mice were used for each recording time period to avoid habituation to the cue. In addition, a group of 5 animals was used to investigate the effects of foot-shocks alone on sleep/wakefulness architecture without exposure to the cue reminder. Finally, two additional non-stressed mice were used to control the stability of the EEG baseline over a 21-day period.

2.3.3. Signal processing and sleep parameters

Implanted mice were connected to an EEG recording system (2 Grass, 12 tracks, 79D model) by a flexible cable with a rotating collector (APCL 12 channels, Air precision), which allowed mice to move freely. EEG signals were filtered at 1 and 100 Hz (6 dB/octave). They were then acquired and digitized at 256 Hz using the software Coherence 32 (Deltamed). Activities in the sensorimotor and visual cortices were recorded over the 6 h recording period by comparison with the reference electrode placed over the cerebellar cortex. Three sleep/wakefulness states were considered: (1) wakefulness was characterized by low voltage EEG signal and fast frequency (theta rhythm: within the 6–9 Hz range) on both cortical derivations; (2) non rapid eye movements sleep (NREMS) were characterized by high voltage with slow wave (delta rhythm: within the 1–4 Hz range) with bursts of sleep spindles (sigma rhythm: within the 10–15 Hz range) on the sensorimotor derivation; (3) rapid eye movement sleep (REMS) by hypersynchronization of the theta rhythm (within the 4–9 Hz range) in the visual area. Analysis of the EEG signal was performed automatically by a computerized system discriminating between the various phases and visual control was also performed. The parameters examined were: (1) total wakefulness-time, (2) total NREMS-time, (3) total REMS-time, number and mean duration (4, 5) wakefulness and (6, 7) sleep episodes (NREMS + REMS) over the 6 h recording sessions. Statistical analyses were performed for each parameter using two-way ANOVA (*object exposure* and *post-stress delay*) with repeated measures on factor *stress*, so that each mouse was its own control. The two-way ANOVA was followed by post-hoc Winer analyses to examine the role of the cue reminder.

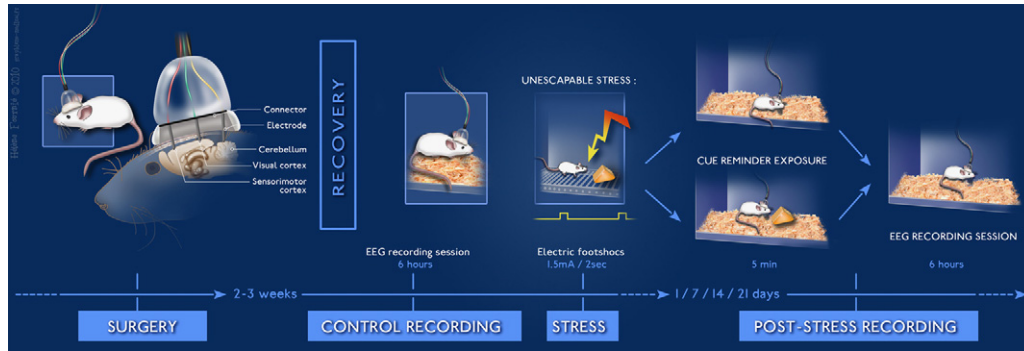


Fig. 1. Experimental design.

2.4. Conditioned object avoidance test

One, 7, 14, 21 or 28 days following stress exposure, a familiar (i.e. a plastic prism paired with shock application) or an unknown (i.e. a plastic cube) object was placed into the home cage for 5 min during which exploratory behavior was recorded. Diameter of both objects was about 4 cm. During the test, the upper grid of the home cage was replaced with a transparent plastic plate to allow mice to be observed from the top. Light intensity was 20 lx. The Behaviors scored were: context exploration (locomotion and/or sniffing movements directed towards the floor, walls), object burying (covering the object with sawdust) and object exploration (i.e. being in contact with the object such as sniffing or gnawing behaviors). Note that separate groups of 6–9 mice were used for each time point to avoid a possible habituation to the objects. Statistical analyses were performed using two-way ANOVAs (using *post-stress delay* and *mice group* as variables).

3. Results

3.1. Sleep architecture following shock application

A decrease in sleep episode mean duration was observed in stressed mice not previously exposed to the object 24 h following stress exposure (Fig. 2: $F_{(1,27)}=6.047, p=0.0206$), an effect which lasted 7 (Fig. 2: $F_{(1,27)}=8.916, p=0.0059$), 14 (Fig. 2: $F_{(1,27)}=10.292, p=0.0034$) and 21 days after stress (Fig. 2:

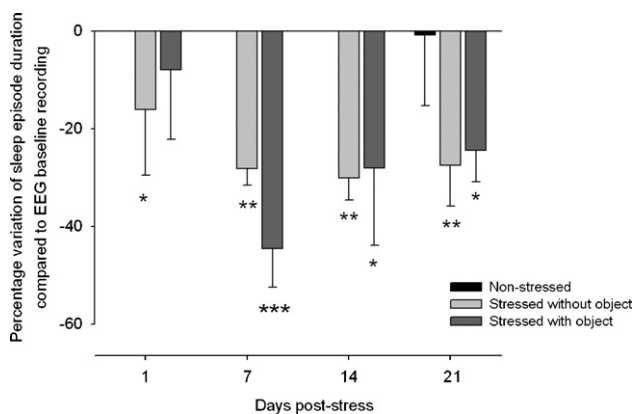


Fig. 2. Acute inescapable stress exposure leads to long-lasting decrease of sleep period duration. Data were recorded for 6 h during light period and are expressed in percentage variation of sleep period mean duration recorded before stress (control) and recorded at different time points after stress or 21 days after EEG control recording for the non-stressed group. Bars represent variations of sleep period mean duration (\pm s.e.m.), $n=2-5$ mice per group. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ post-stress vs control EEG recording.

$F_{(1,27)}=11.584, p=0.0021$). A significant effect was found for the variables *stress* ($F_{(1,27)}=54.61, p<0.0001$) and *mice group* ($F_{(1,27)}=4.77, p=0.0014$) without significant interaction between these parameters. Conversely, the number of sleep episodes was significantly increased one (Fig. 3: $F_{(1,27)}=5.048, p=0.0330$), 7 (Fig. 3: $F_{(1,27)}=5.167, p=0.0312$) and 14 days following stress exposure (Fig. 3: $F_{(1,27)}=6.833, p=0.0145$). Effects of main factors *stress* and *mice group* were significant ($F_{(1,27)}=14.97, p=0.0014$ and $F_{(1,27)}=7.11, p<0.0001$ respectively).

3.2. Role of a cue reminder in stress-induced sleep disturbances

Exposure to the object paired with shock significantly increased total wakefulness duration at day 7 post stress (Fig. 4 and Table 1: $F_{(1,27)}=28.030, p<0.0001$ vs baseline recording). This effect persisted 14 and 21 days post stress (Fig. 4 and Table 1: $F_{(1,27)}=10.534, p=0.0031$ and $F_{(1,27)}=4.541, p=0.0423$, respectively vs baseline recording). The interaction *stress* \times *mice group* was significant for this parameter ($F_{(1,27)}=2.487, p=0.0416$). A decrease in sleep episode mean duration was observed at day 7, 14 and 21 after stress exposure (Fig. 2: $F_{(1,27)}=15.324, p=0.0006$, $F_{(1,27)}=5.446, p=0.0273$ and $F_{(1,27)}=4.694, p=0.0393$ at 7, 14 and 21 days, respectively), with no change in sleep episode number (Fig. 3). Conversely, total NREMS duration was significantly reduced in mice groups exposed to the cue reminder 7, 14 and 21 days post-stress (Table 1: $F_{(1,27)}=15.244, p<0.0001$, $F_{(1,27)}=9.985, p=0.0039$ and $F_{(1,27)}=6.300, p=0.0184$ respectively vs baseline recording). Finally, a tendency to a decrease in time spent in REMS was observed throughout the recording period when mice were exposed to the object at day 1 or 7 post-stress (Table 1:

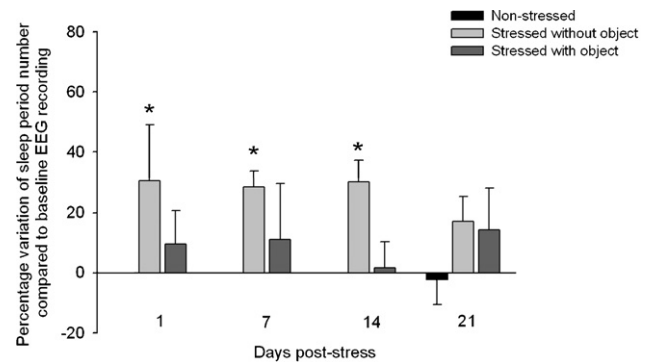


Fig. 3. Acute inescapable stress exposure increases long-lastingly sleep episode frequency. Data were recorded for 6 h during the light period. They are expressed as variation percentage of sleep episode number recorded before stress (control) and recorded at different time points after stress or 21 days after EEG control recording for the non-stressed group. Bars represent variations of sleep episode number (\pm s.e.m.), $n=2-5$ mice per group. * $p<0.05$, post-stress vs control EEG recording.

Table 1
Short- and longer-term wakefulness, NREMS and REMS total duration variations following exposure to a cue reminder paired with acute inescapable stress.

| | Post-stress delay (days) | Total wakefulness time (min) | | Total NREMS time (min) | | Total REMS time (min) | |
|---|--------------------------|------------------------------|-----------------------------|------------------------|----------------------------|-----------------------|-------------------------|
| | | Control | Test | Control | Test | Control | Test |
| Baseline EEG recording | 21 | 145.3 ± 14.3 | 162.1 ± 22.5 | 206.2 ± 14.4 | 190.8 ± 23.5 | 8.4 ± 0.1 | 7.1 ± 1.3 |
| EEG recording without object exposure | 1 | 199.8 ± 14.7 | 205.8 ± 6.3 | 151.9 ± 13.5 | 148.9 ± 6.2 | 8.3 ± 1.6 | 5.3 ± 1.6 |
| | 7 | 202.9 ± 16.0 | 217.8 ± 6.4 | 141.4 ± 14.1 | 124.6 ± 6.3 | 8.5 ± 2.0 | 6.5 ± 2.0 |
| | 14 | 199.8 ± 14.7 | 219.0 ± 15.6 | 151.9 ± 13.5 | 136.0 ± 14.1 | 8.3 ± 1.6 | 5 ± 2.2 |
| | 21 | 199.8 ± 14.7 | 219.0 ± 16.5 | 151.9 ± 13.5 | 134.9 ± 16.5 | 8.3 ± 1.6 | 6.1 ± 1.0 |
| EEG recording after 5 min object exposure | 1 | 129.4 ± 12.0 | 151.4 ± 7.2 _§ | 219.3 ± 10.6 | 201.4 ± 8.0 | 11.2 ± 2.6 | 7.1 ± 2.0 _§ |
| | 7 | 178.1 ± 23.9 | 243.9 ± 25.2 ^{***} | 162.5 ± 21.7 | 99.1 ± 24.7 ^{***} | 11.0 ± 3.8 | 6.7 ± 3.8 _§ |
| | 14 | 157.6 ± 6.9 | 207.9 ± 18.1 ^{**} | 171.2 ± 8.3 | 127.8 ± 18.1 ^{**} | 12.5 ± 2.6 | 6.4 ± 0.06 [*] |
| | 21 | 169.5 ± 13.1 | 199.1 ± 20.1 [*] | 171.4 ± 13.2 | 148.8 ± 18.6 [*] | 8.9 ± 1.0 | 6.4 ± 1.6 |

Data were recorded during light period before stress (control) and at different time points after stress exposure or 21 days after EEG control recording for the non-stressed group and are expressed as total wakefulness, total NREMS and total REMS means (±s.e.m), §*p* < 0.1, **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 vs control EEG recording.

$F_{(1,27)} = 2.821, p = 0.1046, F_{(1,27)} = 3.067, p = 0.0912$ respectively vs baseline recording). This effect reached statistical significance when mice are exposed to the object on the 14th day post stress ($F_{(1,27)} = 5.358, p = 0.0285$).

3.3. Avoidance test following acute inescapable stress exposure

Re-exposure to the object paired with shock led to a significant reduction in object exploration in stressed mice compared to controls as soon as one day following stress exposure (Fig. 5A: $F_{(5,186)} = 3.324, p = 0.0293$), an effect that lasted up to 28 days post-stress (Fig. 5A: $F_{(5,186)} = 4.399, p = 0.0055, F_{(5,186)} = 7.766, p < 0.0001$ and $F_{(5,186)} = 7.809, p = 0.0046$ respectively for day 14, 21 and 28 following stress). Unknown object exploration time was not significantly different between stressed and control mice one day after stress, but a non-significant trend to a decrease was observed at day 7 and 14 in stressed mice. This effect reached statistical significance at day 21 (Fig. 5B: $F_{(5,186)} = 7.766, p = 0.0147$) and 28 post-stress (Fig. 5B: $F_{(5,186)} = 7.809, p = 0.0003$). A significant interaction between post-stress delay and mice group was found for object exploration time ($F_{(20,186)} = 2.167, p = 0.0040$). No significant differences of context exploration time and object burying time between groups were observed (Table 2).

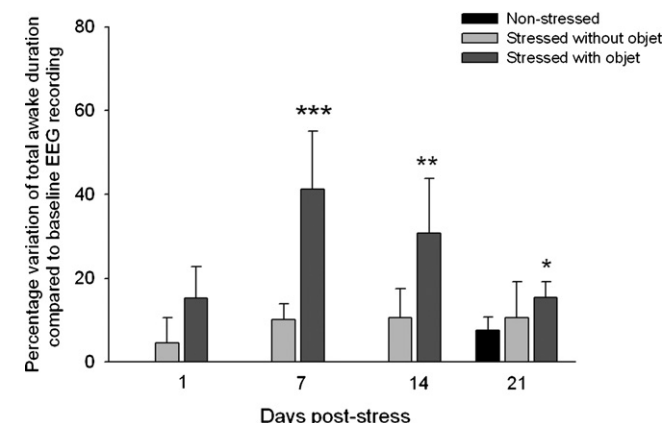


Fig. 4. Exposure to a cue reminder paired with acute inescapable stress increases wakefulness duration over the following 6 h. Data were recorded during light period and are expressed in percentage variation of the wakefulness time recorded before stress (control) and recorded at different dates after stress or 21 days after EEG control recording for the non-stressed group. Bars represent variation of wakefulness total time at different time points after stress compared to baseline recording (±s.e.m), *n* = 2–5 mice per group. **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 post-stress vs control EEG recording.

4. Discussion

The present study demonstrates that exposure to brief electric foot-shocks induced long-term disturbance of sleep patterns and time-dependent generalized avoidance. These data are an important extension of previous findings on the effects of an exposure to electric foot-shocks and development of PTSD-like symptoms.

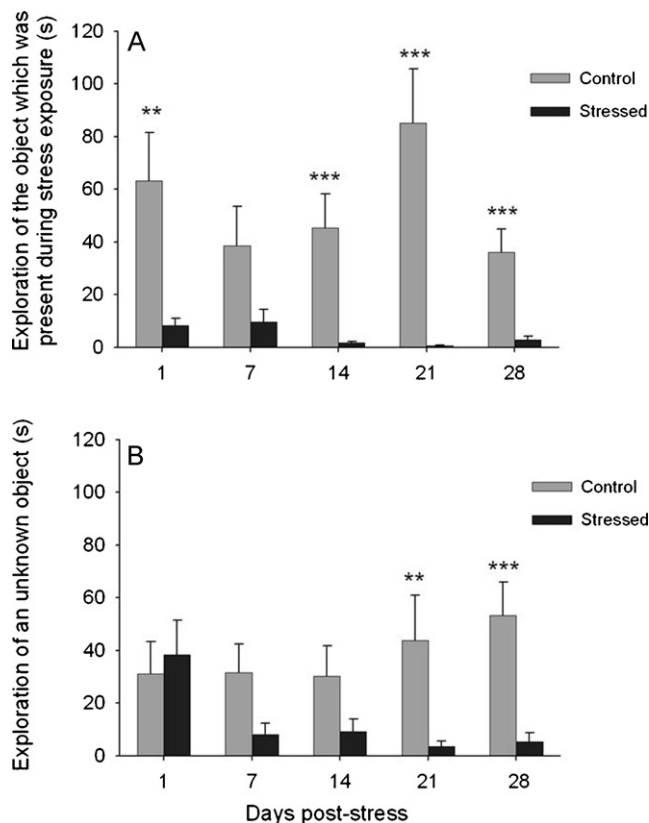


Fig. 5. Time-course of object avoidance in response to acute inescapable stress in mice. Mice were exposed to electric foot-shocks (1.5 mA) in the presence of a plastic prism. This object paired with shocks (A) or another unknown object, a plastic cube (B), was placed into the home cage of mice at different delays from stress. Data are expressed as means (±s.e.m.) of object exploration time. Context exploration and object burying time expressed as means (±s.e.m.). **p* < 0.05, ***p* < 0.01 and ****p* < 0.001, stressed vs controls.

Table 2
Context exploration and object burying time during avoidance test. The table presented sample size and the corresponding data as means (\pm s.e.m.).

| Group | Delay post stress (days) | Cue reminder placed into home cage | | | Unknown object placed into home cage | | |
|---------|--------------------------|------------------------------------|--------------------------------|---------------------------|--------------------------------------|--------------------------------|---------------------------|
| | | Sample size | Context exploration (secondes) | Object burying (secondes) | Sample size | Context exploration (secondes) | Object burying (secondes) |
| Control | 1 | 6 | 18.1 \pm 2.1 | 12.6 \pm 7.4 | 6 | 13.9 \pm 1.8 | 16.1 \pm 5.3 |
| | | 7 | 17.01 \pm 2.9 | 17.2 \pm 2.3 | 7 | 12.8 \pm 2.4 | 4.4 \pm 7.4 |
| Control | 7 | 7 | 17.9 \pm 4.4 | 6.6 \pm 3.9 | 6 | 24.2 \pm 5.2 | 9.4 \pm 1.6 |
| | | 7 | 17.47 \pm 2.9 | 11.1 \pm 7.1 | 8 | 15.2 \pm 2.7 | 7.6 \pm 3.6 |
| Control | 14 | 7 | 20.1 \pm 2.6 | 19.6 \pm 5.7 | 7 | 19.1 \pm 2.0 | 13.7 \pm 6.3 |
| | | 7 | 14.29 \pm 1.1* | 23 \pm 5.9 | 7 | 23.5 \pm 5.0 | 19.2 \pm 5.7 |
| Control | 21 | 7 | 14.8 \pm 1.7 | 6.4 \pm 3.1 | 7 | 16.8 \pm 2.9 | 20.2 \pm 6.1 |
| | | 6 | 16.21 \pm 3.5 | 21.9 \pm 6.0* | 7 | 14.27 \pm 4.2 | 33.0 \pm 3.1 |
| Control | 28 | 9 | 17.1 \pm 1.3 | 13.9 \pm 2.9 | 7 | 16.9 \pm 2.6 | 20.0 \pm 6.1 |
| | | 9 | 12.57 \pm 0.9 | 22.3 \pm 4.4 | 8 | 14.2 \pm 1.4 | 19.1 \pm 4.1 |

* $p < 0.05$, stressed vs controls.

4.1. Sleep/wakefulness profile after shock application

The application of two electric foot-shocks of 1.5 mA enhanced sleep fragmentation as shown by an increase in the number of sleep episodes one, 7, 14 and 21 days following stress. This finding fills a gap in the literature as long-term effects of shocks on sleep were poorly studied earlier, indeed, several studies have investigated the effects of stress (i.e. electric foot-shocks) on sleep patterns in rats or mice, but they mostly focused on the short-term consequences of stress. They reported changes in the vigilance state characterized predominantly by a decrease in REM sleep [4,6,7,14]. Some studies reported that fear conditioning has some long-term effects on sleep. Sanford et al. [5] demonstrated that reminders of an aversive event can impact long-lastingly sleep and particularly REMS. Similarly, conditioned fear was shown by Madan et al. [8] to alter REM sleep microarchitecture up to 14 days following stress. However, none of these studies described long-term residual effects of shock stress alone. Indeed, Sanford et al. [5] focused on total REMS and total NREMS, and they did not observe long-term effects of the initial training when mice were not exposed to the fearful cue. Likewise, Madan et al. [8] did not observe any change in sleep architecture in rats exposed to foot-shocks alone. Here, we demonstrate for the first time that stress exposure induced an increase in sleep fragmentation, which persists over time and which does not require the presence of a cue reminder. This difference between previous studies and the current findings is unclear, but may be attributed to differences in stress levels, to the use of different species and/or strains [6,7,24].

Our finding in mice of an increase in sleep fragmentation following exposure to foot-shocks fits with clinical observations as complaints about sleep quality and nightmares are not uncommon during the aftermath of a traumatic event. Subjective reports indicate that 70–91% of patients with PTSD experience sleep disruption [15]. However, findings from laboratory studies of PTSD have been inconsistent in terms of documenting objective impaired sleep maintenance [16]. One possible reason for the discrepancy between subjective and objective findings of sleep may be that patients with PTSD sleep more soundly in environments perceived to be safe, such as a laboratory [15].

4.2. Role of cue reminder in stress-induced sleep disturbances

Most studies that investigated modifications of sleep architecture following a traumatic event used classical fear conditioning procedures. They are usually based on pairing a tone or a light with a foot-shock. The former are later used as a cue reminder of the

stressful event generally in a different context. Here we used an object, which was present during shock exposure and later placed in the well-habituated home cage for 5 min just prior EEG recording. We tested the effects of both shock alone and shock plus cue on sleep changes. Our results showed that object exposure prior EEG recording produced a significant increase of total awake duration in stressed mice from day 7 to 21 as compared to baseline levels. Interestingly, the time-course analysis of this effect revealed that the increase in awakening duration was maintained throughout the entire recording session. Moreover, while the duration of sleep episodes was significantly and similarly decreased in both groups as compared to baseline levels, the number of sleep periods was only increased in the shock alone group. Altogether, these observations indicate that while both groups displayed fragmented sleep, only shocked mice exposed to the object displayed in addition a global increase in wakefulness.

Several studies report a decrease in total REMS in animals when exposed to cue or situational reminder associated with inescapable stress exposure [4,17]. In this experiment, exposure to the cue reminder elicited only a decreased tendency of total REMS one and 7 days after stress. However, the reduction in total REMS time became significant 14 days after stress. This is consistent with findings of Madan et al. [8], who observed significant effects on REMS when the stimulus was presented 14 days foot-shocks.

Because we aimed to investigate the effects of stress on sleep parameters, this study focused on the light period during which mice are mostly asleep. However, in humans, disturbed sleep is often associated with an increase in day-time fatigue, irritability and difficulties in concentrating [18,19]. It could therefore be interesting to investigate in a future study, sleep/wakefulness parameters during the activity phase as well (i.e. the dark period).

A weakness of this study was that the potential effects of an unfamiliar object or the cue alone were not investigated on sleep patterns. It cannot be totally excluded that exposure to an unfamiliar object may have impacted sleep parameters in shocked mice, especially when considering the behavioral findings, which showed that shocked mice avoided the unfamiliar object. Regarding a possible cue effect by itself, Pawlyk et al. [4] reported that exposure to a cue (i.e. a light) did not disturb sleep architecture of control rats. In another study, Tang et al. [20] investigated effects of environmental novelty (i.e. cage change or exposure to an object) on sleep time in mice and only observed a slight decrease in total sleep during the initial few hours of the recording period. It can therefore be assumed in the current study that an exposure to the object alone would not have modified noticeably sleep in non-stressed mice because of its potential anxiogenic-like nature.

4.3. Generalised avoidance behavior

As the object was hypothesized to be a stimulus that arouses recollection of the trauma, we suggest that the significant reduction of object exploration induced by acute inescapable stress exposure models persistent avoidance (criterion C of PTSD symptoms, [23]) observed in patients suffering from PTSD. It is striking that avoidance behavior was not specific to the object present during shock exposure as an unfamiliar object produced similar long-lasting avoidance responses in stressed mice. Noteworthy, the significant reduction in object exploration was independent of context exploratory behavior. These observations are consistent with those of Pamplona et al. [11], who demonstrated in mice that conditioned avoidance to shock-paired odour turned into generalized avoidance 28 days later. Moreover, they corroborate findings of Mikics et al. [21], which showed no discrimination between an object present during foot-shocks and a different one in stressed rats 28 days following stress, suggesting a generalization of fear to unknown objects. A comparison of our results with those of Mikics et al. [21] indicates that the two species, mice and rats, are similar as for their avoidance of unfamiliar objects. However, burying behavior, identified by Mikics et al. as hypervigilance, was only observed in shocked rats. In our experiment, control and stressed mice spent about the same amount of time burying the object. We suggest that, in mice, this behavior does not necessarily reflect an anxiety-like response, but could also be attributed to digging, a mouse-typical behavior [22].

In conclusion, this study reports for the first time long-lasting alterations in sleep/wakefulness accompanied by time-dependent generalized avoidance behavior after the application of brief electric foot-shocks in mice. These effects may be reminiscent of certain aspects of PTSD symptoms such as poor sleep quality, avoidance and hypervigilance. As such, our data extend previous findings on the effects of exposure to electric foot-shocks and reinforce the face validity of this procedure as a mouse model of PTSD, which may be used for identification of potential drug treatments.

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