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The relationship between anxiety and sleep-wake behavior after stressor exposure in the rat

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Abstract

Disturbed sleep is a common subjective complaint among individuals diagnosed with anxiety disorders. In rodents, sleep is often recorded after exposure to various foot-shock paradigms designed to induce an anxiety state. Although differences in sleep-wake architecture are noted, the relationship to specific level of anxiety is often assumed or absent. Utilizing the elevated plus maze (EPM) after exposure to escapable shock (ES), inescapable shock (IS) or fear conditioning (FC), resulting differences in sleep architecture were compared to an objective measure of anxiety. Male Wistar rats were implanted with EEG, EMG and hippocampal theta electrodes to record sleep-wake behavior. After recovery and recording of baseline sleep, rats were exposed to one of five manipulations: ES, IS, FC, or control (CES or CIS; utilizing either chamber with no shock exposure). Shortly after experimental manipulation, the EPM was employed to quantify traditional and ethological measures of anxiety and polygraphic signs of sleep-wake behavior were recorded continuously for 6h. Although no significance was observed in EPM measurements across groups, profound differences in sleep architecture were present. Individual correlation analysis revealed no differences in anxiety level and total percentage of time spent in sleep-wake states. These results indicate that differences in sleep architecture after foot-shock exposure may not be simply due to increased anxiety. Rather, individual anxiety may be exacerbated by disrupted sleep. To fully understand the relationship between anxiety and sleep-wake behavior, a more objective analysis of anxiety after stressor exposure is mandated.

Keywords

stress; anxiety; sleep; wake; elevated-plus maze; fear conditioning

1. Introduction

Difficulties with sleep have been linked with a host of psychological disorders including depression, schizophrenia, post-traumatic stress disorder and anxiety (Costa e Silva, 2006; Gottesmann and Gottesman, 2007; Lavie, 2001; Tan et al., 1984). In patients with psychiatric disorders—most notably anxiety—insomnia is the most commonly reported sleep disturbance (Szelenberger and Soldatos, 2005; Tan et al., 1984). Clinical studies of anxiety patients, however, indicate the prevalence of both hypersomnia and insomnia (23.9% and 27.6%, respectively) (Ford and Kamerow, 1989). These clinical studies utilize self reporting measures to indicate disturbed sleep. Thus, the disrupted sleep is a subjective complaint that

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is only representative of insufficient or nonrestorative sleep. Objective clinical studies of sleep disturbances and anxiety have focused primarily on long-term effects of anxiety (Lavie, 2001). Such trials have noted an increase in sleep latency, smaller percentage of deep sleep and decreased rapid eye movement (REM) sleep density (Fuller et al., 1997). The immediate effects on sleep architecture occurring in anxious individuals are considerably more elusive.

To study immediate effects of anxiety, many researchers utilize the rodent model as a convenient way to analyze behavioral and pharmacological responses to stress. The elevated plus-maze (EPM) is the most extensively used behavioral paradigm to measure anxiety in the rodent model (Carobrez and Bertoglio, 2005; Hogg, 1996). Anxiety-related behavior reflects a conflict between the rodent's desire to explore a novel environment and innate preference to protected areas (Walf and Frye, 2007). The open arm of the EPM is considered anxiogenic, or anxiety inducing; thus, rats exhibiting greater anxiety-related behavior will enter and spend less time in the open arms of the maze (Bertoglio and Carobrez, 2000; Pellow et al., 1985). The percentage of time spent on the open arm is increased and decreased with anxiolytic and anxiogenic substances, respectively (Bertoglio and Carobrez, 2000; Pellow et al., 1985; Rodgers et al., 1992). When used appropriately, the EPM is the most effective and popular animal model to observe and quantify anxiety within the rodent model (Rodgers and Dalvi, 1997).

The rodent model is also frequently employed to measure the changes in sleep-wake architecture after experimental manipulation (Ho et al., 2002; Jha et al., 2005; Tang et al., 2005). Classical fear conditioning, the most common paradigm, utilizes an unconditioned foot-shock presented with a conditioned stimulus to measure the acquisition, expression and/or extinction of fear (Jha et al., 2005; Pawlyk et al., 2005; Tang et al., 2005). Neuroimaging techniques, such as fMRI, have afforded researchers the opportunity to examine the similarities between structures involved in the acquisition and expression of fear in humans and rodents (Delgado et al., 2006). A number of studies have demonstrated that homologous structures in the human and rat are involved in fear acquisition, an important aspect of anxiety behavior (Buchel et al., 1998). Numerous other paradigms employ a range of stressors including immobilization, corticosterone administration and open field exposure (Tang et al., 2004; Tang et al., 2007; Vazquez-Palacios and Velazquez-Moctezuma, 2000). All of these techniques and manipulations have significant effects on sleep-wake behavior, but the degree of behavioral stress is often assumed or unknown.

The aim of the present study was to establish an effective link between sleep-wake behavior and an objective measure of anxiety in the rodent model. By subjecting rats to different foot-shock paradigms, we hoped to gauge the degree to which each paradigm is anxiogenic and the corresponding effect on sleep architecture. In doing so, innovative techniques and new diagnostic criteria to study and treat anxiety disorders and subsequent changes in sleep-wake behavior can be investigated.

2. Results

2.1 Anxiety resulting from stressor exposure

Figure 1 displays the traditional and ethological variables of EPM behavior after each manipulation. Although a trend seems apparent, no significant treatment effect was present in the two most common EPM measures of anxiety: percentage of time spent on open arm [One-way ANOVA; $F(3,22)=0.713$; $p=0.55$] and number of open arm entries [$F(3,22)=0.851$; $p=0.48$]. The absence of a significant effect could be due to a diverse range of EPM measurement variables within each treatment group. Thus, this data shows that stressor controllability (ES versus IS) as well as stressor exposure (controls versus experimental treatment) were not significant factors in traditional EPM measures of anxiety. Likewise, there was no significant main effect between the number of closed arm entries [$F(3,22)=1.009$;

$p=0.41$]. One-way ANOVA performed on other ethological variables also failed to show significant differences between treatments. Number of scanning episodes [$F(3,22)=0.246$; $p=0.863$], risk assessment episodes [$F(3,22)=0.875$; $p=0.469$], grooming behavior (not shown; [$F(3,22)=0.504$; $p=0.6835$]) and end exploration (not shown; [$F(3,22)=0.764$; $p=0.53$]) did not vary statistically across treatment groups. A significant difference in rearing, however, was present (not shown; [$F(3,22)=4.43$; $p=0.01$]). Scheffe F-test post-hoc analysis revealed significance only between the control and IS group ($p<0.05$).

2.2 Sleep differences after stressor exposure

Figure 2 shows the effect of stressor exposure on total percentages of sleep-wake stages over the six hour recording period. One-way ANOVA revealed that there was a significant treatment variation in percentage of time spent in wakefulness (W) [$F(3,22)=3.92$; $p=0.022$]. Compared to control group, post-hoc Scheffe F-test revealed that the total percentage of wakefulness was significantly higher in the ES ($p<0.05$), FC ($p<0.05$) and IS ($p<0.05$) groups (Fig. 2). One-way ANOVA showed the percentage of time spent in slow-wave sleep (SWS) was slightly significant between groups [$F(3,22)=3.11$; $p=0.047$]. Post-hoc Scheffe F-tests revealed the percentage of time spent in slow-wave sleep was significantly reduced in the ES ($p<0.05$) and FC ($p<0.05$) groups, but did not differ statistically in the IS group. One-way ANOVA revealed a significant effect in the total percentage of time spent in REM sleep over the six hour recording [$F(3,22)=9.35$; $p=0.0004$]. Post-hoc Scheffe F-tests revealed a significant reduction in total percentage of REM sleep between controls and ES ($p<0.01$), FC ($p<0.001$) and IS ($p<0.001$).

To examine various group specific differences in SWS and REM sleep, the stages were further analyzed to evaluate latency of onset and number of episodes. Figure 3 illustrates SWS and REM sleep latencies, total number of REM sleep episodes and a breakdown of REM sleep by episode length. One-way ANOVA revealed no significant treatment variation in SWS [$F(3,22)=0.835$; $p=0.489$] or REM sleep [$F(3,22)=0.976$; $p=0.421$] latencies. The total number of REM sleep episodes, however, were significantly different across groups [$F(3,22)=3.54$; $p=0.031$]. Compared to control, post-hoc Scheffe F-tests revealed significantly fewer REM sleep episodes were present in ES ($p<0.05$) and IS ($p<0.01$) groups. One-way ANOVA failed to show significant differences between the number of REM sleep episodes over the six hour recording with durations (RD) of 0-30 [$F(3,22)=1.28$; $p=0.307$], 35-60 [$F(3,22)=0.488$; $p=0.694$] or 65-120 seconds [$F(3,22)=0.875$; $p=0.469$]. One-way ANOVA demonstrated the number of REM sleep episodes with durations greater than 120 seconds was significantly lower in all three treatment groups [$F(3,22)=5.00$; $p=0.009$]. Post-hoc Scheffe F-test revealed significantly fewer REM sleep episodes greater than 120 seconds in ES ($p<0.05$), FC ($p<0.001$) and IS ($p<0.01$) groups.

2.3 Relationship between level of anxiety and percentage of time spent in wakefulness, slow-wave sleep and REM sleep

After evaluating differences in total percentage of sleep-wake stages across the three stressor exposure groups, the differences in total percentage of wakefulness, SWS and REM sleep were correlated with the percentage of time spent in the open arm of the plus maze. A higher percentage of time spent on the open arm was indicative of a lower level of anxiety; while a lower percentage of time spent on the open arm was indicative of a higher level of anxiety (Pellow et al., 1985). Pearson product-moment correlation analysis was performed to illustrate any relationship between anxiety level (represented by percentage of time in the open arm) and the total percentage of time spent in sleep-wake stages. No significant correlation (Pearson product-moment) was observed between anxiety level and total percentages of wakefulness ($r=-0.047$, $F=0.05$, $p=0.819$), SWS ($r=0.13$, $F=0.44$, $p=0.515$) or REM sleep ($r=0.13$, $F=0.39$, $p=0.538$) (Fig. 4). These results indicate that there is no linear relationship between varying levels of individual anxiety and percentage of time spent in W, SWS and/or REM sleep.

2.4 Relationship between level of anxiety and electroencephalographic spectral power of Delta and Theta frequency waves

Figure 5 shows the comparison between the EEG spectral power analysis and percent of time spent on the open arm of the EPM. Cortical EEG power was obtained from FFT analysis (SleepSign for Animal; Kissei Comtec Co., LTD, Tokyo, Japan) and divided into Delta (0.2-4Hz) and Theta (5-10Hz) ranges. The percent of total power was defined as the individual Delta or Theta range divided by the total power across all Hertz ranges (0.2-40Hz). No statistically significant correlation (Pearson product-moment) was observed between anxiety level and Delta power ($r = -0.181$, $F = 0.72$, $p = 0.407$) or Theta power ($r = 0.199$, $F = 0.87$, $p = 0.362$). Individual anxiety levels were represented by the percentage of time spent on the open arm. These results indicate there is no linear relationship between Delta or Theta power and individual anxiety level after experimental manipulation.

3. Discussion

In humans, disrupted sleep has been reported in a variety of psychological disorders, notably anxiety, PTSD and depression (Ford and Kamerow, 1989; Gale and Davidson, 2007; Szelenberger and Soldatos, 2005; Tan et al., 1984). Similarly, normal sleep is also critical for psychological and emotional well-being (reviewed in Datta and Maclean, 2007). Thus, evaluating changes in sleep-wake behavior in after stressor exposure could elucidate diagnostic criteria and symptoms for specific disorders. In the animal model, various well-established techniques are used to induce stress and subsequent differences in sleep architecture are often assessed. As a result, to compare rodent and human behavior, common foot-shock paradigms are used in the current study to evaluate resulting anxiety and sleep-wake behavior.

The results of the present study demonstrate that stressor exposure does not reliably result in an immediate increase in behavioral anxiety. Neither stressor controllability nor stressor exposure results in differences between traditional and ethological EPM measures of anxiety. This finding is important because it demonstrates the potential for a range of anxiety levels within each stressor exposure group. It also indicates that simply presenting an aversive stimulus to induce stress may not result in a consistent level of anxiety. Some rats react strongly to foot-shock presentation and record a heightened level of anxiety; other rats are never exposed to a stressor and still record a high level of anxiety. The same is true for low anxiety and exposure, or no exposure, to foot-shock. Similar to humans, it appears that rats also differ in their behavioral response patterns to stressor exposure. Thus, at least in studies using a foot-shock as a stressor, any subsequent change in sleep architecture cannot simply be attributed to an increase in individual anxiety.

A common criticism of the EPM is the wide range of measurements reported following behavioral or pharmacological manipulation both within and between laboratories (Hogg, 1996; Silvestri, 2005). A host of methodological and physical variables including rodent strain, lighting levels, maze construction, behavioral scoring, handling and experimental manipulation can have significant effects on EPM measurements. Thus, the discrepancies in plus-maze results between labs has led to the development of more sensitive ethological behavioral measurements (Carobrez and Bertoglio, 2005; Cruz et al., 1994). The use of ethological measurements assess anxiety has been validated by the behaviorally selective, anti-anxiety effects of muscimol, a GABA_A receptor agonist (Rodgers and Dalvi, 1997). Recent studies also suggest that measuring EPM behavior after stressor exposure results in a more effective measure of individual anxiety. This type of paradigm has been termed “fear-potentiated plus-maze behavior” (Mechiel Korte and De Boer, 2003). Supporters claim that inducing a state of anxiety with various aversive stimuli (e.g. foot-shock, restraint, cat odor, etc.) measures an enhanced anxiety state, or allostatic state, instead of an innate fear of open

spaces. This method reflects a true measurement of behavioral anxiety and pharmacological manipulations (Mechiel Korte et al., 1999).

In the present study, the range of anxiety measurements within each group does not fully support the notion that traditional and ethological EPM measurements after stressor exposure are consistent between rats. Given the variability of plus-maze measurements across laboratories, however, our results can not refute fear-potentiated plus-maze behavior as a valuable tool in studying anxiety in the rodent model. All ethological EPM behaviors, with the exception of rearing, did not show any significance or trends across groups. Increased rearing has been suggested to be a sign of decreased anxiety (Cruz et al., 1994); however, a more recent study showed a decrease in rearing was observed after 1.0 mg/kg injection of muscimol, a known anxiolytic substance (Rodgers and Dalvi, 1997). Thus, the interpretation of the significant difference between the IS group and controls with regard to rearing is unclear. It is possible the increased rearing could be indicative of locomotion, but there were no significant differences in arm entries that would be expected to accompany increased locomotion. As a result, rearing was not considered a reliable ethological measure of anxiety in this study.

In the present study, although there were no significant differences in plus-maze behavior across groups, profound differences were evident in sleep-wake behavior. Previous studies using similar experimental paradigms have reported congruent results with regard to sleep-wake behavior after stressor exposure (Jha et al., 2005; Kant et al., 1995; Pawlyk et al., 2005). Numerous recent studies have shown decreased REM sleep episodes after fear conditioning paradigms and inescapable shock exposure (Jha et al., 2005; Liu et al., 2003; Pawlyk et al., 2005; Tang et al., 2005). Additionally, after re-exposure to training context, one study observed an increase and decrease in the total percentage of wakefulness and REM sleep, respectively (Jha et al., 2005). The reductions in total percentage of REM sleep were primarily due to decreased episode length and number of episodes (Jha et al., 2005; Pawlyk et al., 2005; Tang et al., 2005). Reductions in NREM sleep present after a second shock training episode and re-exposure to context have also been observed (Tang et al., 2005). Studies measuring the immediate effect of foot-shock exposure on sleep architecture have also found decreases in the total percentage of REM sleep (Kant et al., 1995; Vazquez-Palacios and Velazquez-Moctezuma, 2000). Another study used a passive avoidance paradigm to allow the rodent to escape a shock by returning to or remaining on a platform (Mavanji et al., 2003). Sleep-wake data from this study reported a marked decrease in REM sleep following 10 trials of passive avoidance. All of the above studies agree with our observation of a significant decrease in REM sleep and alterations in other sleep-wake states after varying shock exposure paradigms.

From the present study and previous literature, it is clear that exposure to a stressor does have a significant effect on sleep-wake behavior. However, based on the present study, the immediate anxiety level after stressor exposure is not as clear. In humans, anxiety is individual-specific. That is, certain stressors will produce a greater level of anxiety in certain people, but not in others. To evaluate the connection between anxiety level and sleep, the individual anxiety level must be taken into account. Accordingly, the individual anxiety level of each rodent, represented by the percentage of time spent on the open arm, was compared to the total percentages of each sleep-wake stage. Based on the EPM measurements, a higher percentage of time in the open arm was indicative of a lower percentage of anxiety; while a lower percentage of time in the open arm indicated a higher level of individual anxiety. These results demonstrate that significant changes in sleep-wake behavior are dependent upon stressor exposure, but not necessarily level of anxiety. Although a trend between stressor exposure and percentage of time in open arm and number of open arm entries seems apparent (see Fig. 1), differences in sleep-wake behavior are already clearly defined (see Fig. 2). These results put into question the degree to which changes in sleep-wake architecture after stressor exposure represent an increase in individual anxiety or are simply a physiological reaction to foot-shock.

A correlation analysis was also performed on EEG spectral power analysis compared to anxiety level. Again, no significant correlation was discovered between level of anxiety and EEG delta or theta power. A previous study analyzing sleep and EEG power after presentation of foot-shock demonstrated an immediate decrease in REM sleep but no difference in NREM and REM EEG power during the first eight hours of sleep recordings (Tang et al., 2007). However, a significant difference was observed during wakefulness (4.5-9Hz) between 5-8 hours. Since the present study was primarily focused on sleep occurring during the light period, other significant differences in Tang et al. (2007) over entire 20 hour recordings are not comparable. It is possible the same differences could be present in the current study; however, only total power of all sleep-wake states over the six hour recording was analyzed.

Taken together, these results indicate that changes in sleep architecture following stressor exposure may not be attributable to a heightened anxiety. Instead, existing anxiety could be exacerbated by disrupted sleep. Rather than an indicator of individual anxiety, the alterations in sleep architecture resulting from stressor exposure may be a function of the specific manipulation. Another possible explanation is the integration of anxiety after acute stressor exposure is not immediate. Instead, the changes in sleep-wake behavior present after foot-shock are an indicator of this delayed integration and expression of behavioral anxiety. Individual anxiety level could gradually increase as sleep becomes more disorganized. Indeed, in clinical studies, sleep deprivation has been an effective form of treatment for major depressive disorders in humans (Clark et al., 2001; Giedke et al., 2003; Gillin et al., 2001); but deprivation has shown little or negative results in patients diagnosed with anxiety disorders (Dinges et al., 1997; Lobbate et al., 1998). Results from recent REM and total sleep deprivation studies in rodents demonstrate the anxiogenic effect of disrupted sleep (Silva et al., 2004; Suchecki et al., 2002a; Suchecki et al., 2002b). The deprivation techniques used, however, also have been shown to induce a considerable amount of stress (Suchecki et al., 1998). In order for immediate anxiety to be measured after REM or total sleep deprivation, the deprivation technique employed must not be a significant source of stress for the subject.

Varying stressor manipulation (e.g. restraint, social isolation, diet restriction, open field exposure) may also result in different changes in sleep architecture (Da Silva et al., 1996; Tang et al., 2004; Vazquez-Palacios and Velazquez-Moctezuma, 2000). The present study attempted to induce an anxiety-state using only various foot-shock paradigms. The immediate effect of different stressors on anxiety may vary and could potentially induce a more consistent level of anxiety in the rodent model. It should be noted the current study only examined the immediate effects of stressor exposure on level of anxiety and sleep architecture. Since anxiety disorders affect an individual at every hour of the day, evaluating changes in sleep architecture for 24 hours after stressor exposure could be a more accurate assessment of individual anxiety and sleep architecture resulting from increased anxiety. Examining level of anxiety and sleep architecture a few days after stressor exposure may also provide a better assessment of individual anxiety and sleep-wake behavior. Additionally, the individual anxiety profiles of rats prior to stressor exposure may give more insight into anxiety level and predict behavior in multiple stressor manipulations (Ho et al., 2002).

Understanding the mechanisms underlying anxiety and sleep disturbances is critical to advancement in treatment strategies and diagnostic criteria. To effectively measure anxiety and sleep-wake states in the rodent model, researchers must first establish a reliable method of inducing anxiety. The results of the current study demonstrate that stressor exposure results in significant changes in sleep architecture before differences in anxiety level can be reliably established. These results clearly illustrate the need for further investigation into the relationship between anxiety and disrupted sleep.

4. Experimental Procedure

4.1 Animals

Experiments were performed on 23 adult male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing between 300 and 375 g. The rats were housed individually at 24°C with *ad libitum* access to food and water. Lights were on from 7:00 am to 7:00 pm (light cycle) and off from 7:00 pm to 7:00 am (dark cycle). The principles for the care and use of laboratory animals in research, as outlined by the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*, were strictly followed. All experimental manipulations took place at 09:00 am.

4.2 Surgical procedures for electrode implantation

All surgical procedures were performed stereotaxically under aseptic conditions and were in accordance with the guidelines approved by the institutional animal care and use committee (protocol AN-14084). Animals were anesthetized with pentobarbital (40 mg/kg, i.p.; Abbott Laboratories, Chicago, IL), placed in the stereotaxic apparatus, and secured using blunt rodent ear bars as described previously (Paxinos and Watson, 1997). The appropriate depth of anesthesia was judged by the absence of palpebral reflexes and a response to tail pinch. Core body temperature was maintained at $35 \pm 2^\circ\text{C}$ with a thermostatic heating pad and a rectal thermister probe. The scalp was cleaned and painted with povidone iodine. A scalp incision was made, and the skin was retracted. The skull surface was cleaned in preparation for electrode implantation. After completion of the surgical procedure, ampicillin (50 mg per rat, s.c., Bristol-Myers Squibb, Princeton, NJ) was administered to control any potential postsurgical infection. Potential postoperative pain was controlled with buprenorphine (0.03 mg/kg, s.c.; Abbott Laboratories).

To record the behavioral states of vigilance, cortical electroencephalogram (EEG), dorsal neck muscle electromyogram (EMG), hippocampal EEG (to record theta wave) recording electrodes were chronically implanted as described previously (Datta, 2000; Datta, 2002; Datta and Siwek, 2002). All electrodes were secured to the skull with dental acrylic. Electrodes were crimped to miniconnector pins and brought together in a plastic connector. Immediately after surgery, animals were placed in recovery cages and monitored for successful recovery from anesthesia and surgery. Successful recovery was gauged by the return of normal postures, voluntary movement, and grooming. After surgery, each animal was continuously handled for fifteen minutes daily (between 09:00 am and 10:00 am) for at least 7 days prior to manipulation to avoid any potential stress and/or anxiety from experimenter contact.

4.3 Elevated plus-maze (EPM)

The elevated plus-maze was constructed of polyurethaned wood and consisted of two opposed open arms (50 × 10 cm) and two opposed enclosed arms (50 × 10 × 40 cm) with open roof (Pellow et al., 1985). To prevent the rats from falling off the maze, a rim of Plexiglass 2.5 cm high surrounded the edge of the open arms. The maze was elevated 50 cm from the ground. At the end of experimental manipulation, the rat was placed back into its home cage for ten minutes; after which individual anxiety was assessed using the EPM. The ten minute lapse between stressor exposure and EPM test was designed to reduce the disruption of risk assessment behaviors by acute stress (Quartermain et al., 1996). Animals were placed into the maze, facing an enclosed arm, immediately after experimental manipulation. Each rat was tested only once for a duration of 5 minutes. Prior to each individual test, elevated plus-maze was thoroughly sanitized. Tests were digitally recorded and hand-scored in one minute intervals. Traditional measurements for EPM behavior included percentage of time spent on open arm, open arm entries and closed arm entries. An entry into any compartment was defined as all four paws being placed on the arm. Additionally, other ethological variables were

recorded including: 1) scanning: protruding head over edge of open arm and scrutinizing in any direction; 2) risk assessment: two forepaws placed on open arm and investigating surroundings (this behavior usually was accompanied by body stretching); 3) grooming behavior; 4) end exploration: reaching the end of an open arm; and 5) rearing: rising on hind limbs.

4.4 Escapable shock (ES)

The escapable shock apparatus is an automated two-way shuttle scan box ($45.7 \times 20.3 \times 30.5$ cm) made of high grade acrylic and a metal grid floor (Shuttle-flex test chamber, SFII; Accuscan Instruments, Columbus, OH). A doorway divided the box into two distinct and equal compartments. For escapable shock exposure, naïve rats were placed in the shuttle box and were given ten minutes of adaptation prior to shock trials. During adaptation, the rat could move freely between both compartments of the shuttle box. After adaptation, rats were subjected to 10 trials of escapable shock exposure. The conditioned stimulus (CS), tone and pulsatile light, was paired with an unconditioned stimulus (UCS), 1.0 mA scrambled foot-shock. Each trial consisted of a 5 second presentation of the CS and UCS. To escape from the foot-shock, the animal had to move to the opposite compartment. If the animal did not move to the opposite compartment, the scrambled foot-shock continued for a maximum of 5 seconds. While receiving the UCS, if the rat escaped to the opposite compartment, both the CS and UCS terminated immediately. The inter-trial interval was variable with a mean of 60 seconds. Individual anxiety was assessed using the EPM and sleep was recorded for 6 h (10:00am to 4:00pm). Procedure for control group (CES) was identical, except no CS or UCS was administered.

4.5 Inescapable shock (IS)

The inescapable shock apparatus is a freezing behavior testing chamber ($30.5 \times 24.1 \times 21.0$ cm) with a metal grid floor located inside of a lighted, enclosed cubicle (Standard modular test chamber, ENV-008; Sound attenuating cubicle, ENV-022MD; Med Associates, Inc, St. Albans, Vermont). Prior to beginning of inescapable shock exposure, naïve rats are given ten minutes to adapt to the chamber. After adaptation, rats were subjected to 10 trials of shock exposure. Each trial consists of an unavoidable 1 second scrambled foot-shock (1.0 mA) with a one minute inter-trial interval. Behavior was recorded by a video camera ("Video Freeze" software) located inside the cubicle. Individual anxiety was assessed using the EPM and sleep was recorded for 6 h (10:00am to 4:00pm). Procedure for control group (CIS) was identical, except no shock was administered.

4.6 Fear conditioning (FC)

The procedure for the fear conditioning group is identical to the inescapable shock group except individual anxiety level was measured after re-exposure to fear conditioning context 24 h later. After fear conditioning shock exposure, sleep was recorded for 6 h. The next morning, at 09:30am, the rat was placed in the freezing behavior chamber for a 5 minute testing session. Freezing behavior was defined as the absence of movement with the exception of respiration. Behavior was recorded by a video camera ("Video Freeze" software) located inside the cubicle. Individual anxiety was assessed using the EPM and sleep was recorded for 6 h (10:00am to 4:00pm).

4.7 Determination of behavioral states and cortical EEG spectral analysis

For the purpose of determining possible effects on sleep and wakefulness, polygraphic data were captured on-line with a computer using Gamma software (Grass product group; Astro-Med, West Warwick, RI). From this captured data, three behavioral states were distinguished and scored visually using Rodent Sleep Stager software (Grass product group). These three

states were W, SWS, and REM sleep. The physiological criteria for the identification of these wake—sleep states were described in detail previously (Datta et al., 2004; Datta and Siwek, 2002). The behavioral states of W, SWS and REM sleep were scored in successive 5 second epochs.

For power spectral analysis, cortical EEG signals from test day were amplified and bandpass filtered (0.2–100Hz) with a polygraph and Rodent Sleep Stager software (Grass, Quincy, MA). The amplified and filtered data were digitized at a sampling frequency of 200Hz and subjected to a fast Fourier transformation (SleepSign for Animal; Kissei Comtec Co., LTD, Tokyo, Japan) to calculate the cortical EEG power during the total 6 hour recording. Analysis focused on two frequency ranges: delta (0.2–4.0Hz) and theta (5–10Hz). The power of each frequency band was averaged and expressed as a percent of the total power within the frequency range of 0.2–40Hz.

4.8 Statistics

After random placement in one of five experimental groups (CES, CIS, ES, IS, FC), each rat was subjected to a manipulation, anxiety was measured using the EPM and subsequent sleep was recorded for six hours. For statistical comparisons, the level of anxiety was assessed using the percentage of time spent in the open arm of the EPM. A high percentage of time on the open arm indicated a low level of anxiety; correspondingly, a low percentage of time on the open arm indicated a high level of anxiety. Since the controls from both the ES and IS manipulation received no stressor (shock), and the corresponding anxiety and sleep data did not differ statistically, the sleep and EPM measurements were combined for analyses. The main effects of stressor group on all measures of plus-maze variables were evaluated using a one way analysis of variance (ANOVA) followed by post-hoc Scheffe F test for comparing treatments with control group. To determine the relationship between level of anxiety and sleep-wake behavior, a regression analysis on the percentage of time spent in the open arm and total percentages of W, SWS and REM sleep was performed. A regression analysis on Delta and Theta EEG spectral power and level of anxiety was also performed. For both correlation analyses, a Pearson product-moment correlation was performed to evaluate any relationship between variables. Significance for all statistical measures was assessed at alpha equal to 0.05.

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References

- Bertoglio LJ, Carobrez AP. Previous maze experience required to increase open arms avoidance in rats submitted to the elevated plus-maze model of anxiety. *Behav Brain Res* 2000;108:197–203. [PubMed: 10701663]
- Buchel C, Morris J, Dolan RJ, Friston KJ. Brain systems mediating aversive conditioning: an event-related fMRI study. *Neuron* 1998;20:947–957. [PubMed: 9620699]
- Carobrez AP, Bertoglio LJ. Ethological and temporal analyses of anxiety-like behavior: the elevated plus-maze model 20 years on. *Neurosci Biobehav Rev* 2005;29:1193–1205. [PubMed: 16084592]
- Clark CP, Frank LR, Brown GG. Sleep deprivation, EEG, and functional MRI in depression: preliminary results. *Neuropsychopharmacology* 2001;25:S79–84. [PubMed: 11682279]
- Costa e Silva JA. Sleep disorders in psychiatry. *Metabolism* 2006;55:S40–44. [PubMed: 16979426]
- Cruz AP, Frei F, Graeff FG. Ethopharmacological analysis of rat behavior on the elevated plus-maze. *Pharmacol Biochem Behav* 1994;49:171–176. [PubMed: 7816869]
- Da Silva NL, Ferreira VM, Carobrez Ade P, Morato GS. Individual housing from rearing modifies the performance of young rats on the elevated plus-maze apparatus. *Physiol Behav* 1996;60:1391–1396. [PubMed: 8946480]

- Datta S. Avoidance task training potentiates phasic pontine-wave density in the rat: A mechanism for sleep-dependent plasticity. *J Neurosci* 2000;20:8607–8613. [PubMed: 11069969]
- Datta S. Evidence that REM sleep is controlled by the activation of brain stem pedunculopontine tegmental kainate receptor. *J Neurophysiol* 2002;87:1790–1798. [PubMed: 11929900]
- Datta S, Maclean RR. Neurobiological mechanisms for the regulation of mammalian sleep-wake behavior: Reinterpretation of historical evidence and inclusion of contemporary cellular and molecular evidence. *Neurosci Biobehav Rev* 2007;31:775–824. [PubMed: 17445891]
- Datta S, Mavanji V, Ulloor J, Patterson EH. Activation of phasic pontine-wave generator prevents rapid eye movement sleep deprivation-induced learning impairment in the rat: a mechanism for sleep-dependent plasticity. *J Neurosci* 2004;24:1416–1427. [PubMed: 14960614]
- Datta S, Siwek DF. Single cell activity patterns of pedunculopontine tegmentum neurons across the sleep-wake cycle in the freely moving rats. *J Neurosci Res* 2002;70:611–621. [PubMed: 12404515]
- Delgado MR, Olsson A, Phelps EA. Extending animal models of fear conditioning to humans. *Biol Psychol* 2006;73:39–48. [PubMed: 16472906]
- Dinges DF, Pack F, Williams K, Gillen KA, Powell JW, Ott GE, Aptowicz C, Pack AI. Cumulative sleepiness, mood disturbance, and psychomotor vigilance performance decrements during a week of sleep restricted to 4-5 hours per night. *Sleep* 1997;20:267–277. [PubMed: 9231952]
- Ford DE, Kamerow DB. Epidemiologic study of sleep disturbances and psychiatric disorders. An opportunity for prevention? *Jama* 1989;262:1479–1484. [PubMed: 2769898]
- Fuller KH, Waters WF, Binks PG, Anderson T. Generalized anxiety and sleep architecture: a polysomnographic investigation. *Sleep* 1997;20:370–376. [PubMed: 9381061]
- Gale C, Davidson O. Generalised anxiety disorder. *Bmj* 2007;334:579–581. [PubMed: 17363830]
- Giedke H, Klingberg S, Schwarzler F, Schweinsberg M. Direct comparison of total sleep deprivation and late partial sleep deprivation in the treatment of major depression. *J Affect Disord* 2003;76:85–93. [PubMed: 12943937]
- Gillin JC, Buchsbaum M, Wu J, Clark C, Bunney W Jr. Sleep deprivation as a model experimental antidepressant treatment: findings from functional brain imaging. *Depress Anxiety* 2001;14:37–49. [PubMed: 11568981]
- Gottesmann C, Gottesman I. The neurobiological characteristics of rapid eye movement (REM) sleep are candidate endophenotypes of depression, schizophrenia, mental retardation and dementia. *Prog Neurobiol* 2007;81:237–250. [PubMed: 17350744]
- Ho YJ, Eichendorff J, Schwarting RK. Individual response profiles of male Wistar rats in animal models for anxiety and depression. *Behav Brain Res* 2002;136:1–12. [PubMed: 12385785]
- Hogg S. A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacol Biochem Behav* 1996;54:21–30. [PubMed: 8728535]
- Jha SK, Brennan FX, Pawlyk AC, Ross RJ, Morrison AR. REM sleep: a sensitive index of fear conditioning in rats. *Eur J Neurosci* 2005;21:1077–1080. [PubMed: 15787712]
- Kant GJ, Pastel RH, Bauman RA, Meininger GR, Maughan KR, Robinson TN 3rd, Wright WL, Covington PS. Effects of chronic stress on sleep in rats. *Physiol Behav* 1995;57:359–365. [PubMed: 7716216]
- Labbate LA, Johnson MR, Lydiard RB, Brawman-Mintzer O, Emmanuel N, Crawford M, Kapp R, Ballenger JC. Sleep Deprivation in social phobia and generalized anxiety disorder. *Biol Psychiatry* 1998;43:840–842. [PubMed: 9611674]
- Lavie P. Sleep disturbances in the wake of traumatic events. *N Engl J Med* 2001;345:1825–1832. [PubMed: 11752360]
- Liu X, Tang X, Sanford LD. Fear-conditioned suppression of REM sleep: relationship to Fos expression patterns in limbic and brainstem regions in BALB/cJ mice. *Brain Res* 2003;991:1–17. [PubMed: 14575871]
- Mavanji V, Siwek DF, Patterson EH, Spoley EE, Datta S. Effects of passive-avoidance training on sleep-wake state-specific activity in the basolateral and central nuclei of the amygdala. *Behav Neurosci* 2003;117:751–759. [PubMed: 12931960]
- Mechiel Korte S, De Boer SF. A robust animal model of state anxiety: fear-potentiated behaviour in the elevated plus-maze. *Eur J Pharmacol* 2003;463:163–175. [PubMed: 12600708]

- Mechiel Korte S, De Boer SF, Bohus B. Fear-potential in the elevated plus-maze test depends on stressor controllability and fear conditioning. *Stress (Luxembourg)* 1999;3:27–40.
- Pawlyk AC, Jha SK, Brennan FX, Morrison AR, Ross RJ. A rodent model of sleep disturbances in posttraumatic stress disorder: the role of context after fear conditioning. *Biol Psychiatry* 2005;57:268–277. [PubMed: 15691528]
- Paxinos, G.; Watson, C. The rat brain in stereotaxic coordinates. Academic; San Diego: 1997.
- Pellow S, Chopin P, File SE, Briley M. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* 1985;14:149–167. [PubMed: 2864480]
- Quartermain D, Stone EA, Charbonneau G. Acute stress disrupts risk assessment behavior in mice. *Physiol Behav* 1996;59:937–940. [PubMed: 8778890]
- Rodgers RJ, Dalvi A. Anxiety, defence and the elevated plus-maze. *Neurosci Biobehav Rev* 1997;21:801–810. [PubMed: 9415905]
- Rodgers RJ, Lee C, Shepherd JK. Effects of diazepam on behavioural and antinociceptive responses to the elevated plus-maze in male mice depend upon treatment regimen and prior maze experience. *Psychopharmacology (Berl)* 1992;106:102–110. [PubMed: 1738787]
- Silva RH, Kameda SR, Carvalho RC, Takatsu-Coleman AL, Niigaki ST, Abilio VC, Tufik S, Frussa-Filho R. Anxiogenic effect of sleep deprivation in the elevated plus-maze test in mice. *Psychopharmacology (Berl)* 2004;176:115–122. [PubMed: 15160262]
- Silvestri AJ. REM sleep deprivation affects extinction of cued but not contextual fear conditioning. *Physiol Behav* 2005;84:343–349. [PubMed: 15763570]
- Suchecki D, Lobo LL, Hipolide DC, Tufik S. Increased ACTH and corticosterone secretion induced by different methods of paradoxical sleep deprivation. *J Sleep Res* 1998;7:276–281. [PubMed: 9844854]
- Suchecki D, Tiba PA, Tufik S. Paradoxical sleep deprivation facilitates subsequent corticosterone response to a mild stressor in rats. *Neurosci Lett* 2002a;320:45–48. [PubMed: 11849760]
- Suchecki D, Tiba PA, Tufik S. Hormonal and behavioural responses of paradoxical sleep-deprived rats to the elevated plus maze. *J Neuroendocrinol* 2002b;14:549–554. [PubMed: 12121491]
- Szelenberger W, Soldatos C. Sleep disorders in psychiatric practice. *World Psychiatry* 2005;4:186–190. [PubMed: 16633547]
- Tan TL, Kales JD, Kales A, Soldatos CR, Bixler EO. Biopsychobehavioral correlates of insomnia. IV: Diagnosis based on DSM-III. *Am J Psychiatry* 1984;141:357–362. [PubMed: 6703098]
- Tang X, Xiao J, Liu X, Sanford LD. Strain differences in the influence of open field exposure on sleep in mice. *Behav Brain Res* 2004;154:137–147. [PubMed: 15302119]
- Tang X, Yang L, Sanford LD. Rat strain differences in freezing and sleep alterations associated with contextual fear. *Sleep* 2005;28:1235–1244. [PubMed: 16295208]
- Tang X, Yang L, Sanford LD. Interactions between brief restraint, novelty and footshock stress on subsequent sleep and EEG power in rats. *Brain Res* 2007;1142:110–118. [PubMed: 17300767]
- Vazquez-Palacios G, Velazquez-Moctezuma J. Effect of electric foot shocks, immobilization, and corticosterone administration on the sleep-wake pattern in the rat. *Physiol Behav* 2000;71:23–28. [PubMed: 11134681]
- Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. *Nat Protoc* 2007;2:322–328. [PubMed: 17406592]

Fig 1. Effects of different stressor exposure on the traditional and ethological elevated plus-maze measurements of anxiety

EPM measurements (mean $\pm$ SEM) in all three experimental groups, escapable shock (ES), inescapable shock (IS), fear conditioning (FC), were compared to rats that received no shock (CONT). Although a trend seems apparent, no significant differences were shown between traditional EPM measures of anxiety: percentage of time spent on the open arm and number of open arm entries. Other ethological measures including closed arm entries, scanning episodes and risk assessment episodes, also failed to reveal any significance across groups.

Fig 2. Effects of different stressor exposure on the percentages (means $\pm$ SEM) of total recording time (6h) spent in wakefulness, slow-wave sleep (SWS) and rapid eye movement (REM) sleep Compared to controls (CONT), a significant increase in wakefulness was present in escapable shock (ES), inescapable shock (IS) and fear conditioning (FC) groups. A slight decrease in total percentage of SWS was also present ES and FC compared to control group. The total percentages of REM sleep were significantly reduced in all stressor exposure groups when compared to controls. *: $p < 0.05$; ***: $p < 0.001$

Fig 3. Effects of different stressor exposure on sleep and rapid eye movement (REM) sleep latencies and a breakdown of REM sleep episode counts and duration

No significant differences existed across all groups between sleep or REM sleep latencies. There was a significant difference between ES and IS with regard to total number of REM sleep episodes compared to controls throughout the 6h recording. To further evaluate the group specific differences in REM sleep, each episode was separated by duration (RD). These durations were 0-30s, 35-60s, 65-120s and greater than 120s. No significant differences existed between the first three divisions; however, a significant difference was present in episodes greater than 120s. Compared to controls, all three groups had a decreased number of REM sleep episodes greater than 120s. ES: escapable shock; IS: inescapable shock; FC: fear conditioning; CONT: controls; *: $p < 0.05$; **: $p < 0.01$.

Fig 4. The relationships between levels of anxiety and individual percentages of total recording time (6h) spent in wakefulness, slow-wave sleep (SWS) and rapid eye movement (REM) sleep
To compare individual anxiety levels of escapable shock (ES), inescapable shock (IS), fear conditioning (FC) and control (CONT) rats with corresponding sleep architecture, the percentage of time spent in the open arm was correlated to the percentage of time spent in each sleep-wake state. Heightened anxiety was represented by a lower percentage of time spent in the open arm; while a higher percentage of time spent in the open arm indicated a lower level of anxiety. No significant correlation (Pearson product-moment correlation) was present when anxiety level was compared to wakefulness, SWS or REM sleep.

Fig 5. The relationships between levels of anxiety and cortical EEG power spectra analysis of total recording time (6h)

After fast Fourier transformation (FFT), total power from frequencies ranges 0.2-4Hz (Delta) and 5-10Hz (Theta) were calculated. Power from Delta and Theta were then divided by the total power across all frequency ranges (0.2-40Hz) during the six hour recording. The resulting number represented the Delta and Theta power as a percent of the total power. To compare Delta and Theta power with individual anxiety level, the percentage of time spent in the open arm was correlated to the corresponding Delta and Theta power of the rat. Heightened anxiety was represented by a lower percentage of time spent in the open arm; while a higher percentage of time spent in the open arm indicated a lower level of anxiety. No significant correlation

(Pearson product-moment correlation) was present when anxiety level was compared to Delta or Theta power.