A mouse model mimicking human first night effect for the evaluation of hypnotics

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Abstract

In humans, a first night effect (FNE) is characterized by increased sleep latency and decreased total sleep time in an unfamiliar environment, but the mechanism and treatment options for this universally experienced acute insomnia are unclear. We continuously recorded electroencephalography (EEG) and electromyogram (EMG) and measured plasma corticosterone levels to develop a mouse FNE model by inducing acute insomnia in mice that have been placed in unfamiliar cage environments. The sleep latency of mice 'moved to clean cages' (MCC) was longer than that for mice 'moved to dirty ones' (MDC). As compared to MDC mice, MCC mice showed stronger decreases in the amount of non-rapid eye movement (non-REM, NREM) and REM sleep, with a lower power density of NREM sleep, increased fragmentation and decreased stage transitions from NREM sleep to wake, and higher variation in plasma corticosterone levels. Treatment of MCC mice with zolpidem, diazepam, raclopride, pyrilamine, except SCH23390 shortened NREM sleep latency. In addition, zolpidem significantly increased NREM and REM sleep with the increase in slow wave activity (1.00–2.75 Hz), while raclopride significantly increased NREM and REM sleep without changing the EEG power density in MCC mice, whereas diazepam increased sleep with a drastic decrease in power density of the frequency band between 1.00 and 4.00 Hz, diazepam also increased the frequency band between 9.75 and 24.75 Hz during NREM sleep. These results indicate that a MCC mouse can mimic a FNE phenotype of humans and that zolpidem and raclopride may be useful drugs to prevent acute insomnia, including FNE.

1. Introduction

Insomnia is a highly prevalent condition ranging from inability to fall asleep promptly to a total lack of sleep. Acute insomnia occurs very frequently when people are in a new and unfamiliar environment where they experience significant difficulties with sleep initiation. First-night effect (FNE) is often observed in unfamiliar environments and considered to result from a person’s lack of adaptation to the new sleeping environment (Rechtschaffen and Verdone, 1964). In basic human sleep research, FNE has often been used to study acute insomnia (Kitaoka et al., 2009; Suetsugi et al., 2007). Although acute insomnia is usually transient, it can progress to long-term, chronic insomnia in one third of those affected (Riemann et al., 2009), and 10–15% of patients are eventually diagnosed with moderate to severe stages of the disorder (Morphy et al., 2007). Effective treatments for acute insomnia, including FNE, are still unclear, one reason being the lack of a suitable animal model for drug development and evaluation.

Laboratory animals routinely undergo cage cleaning as part of normal husbandry, an intervention that produces significant alterations in their behaviors, including sleep disturbances (Canò et al., 2008; Tang et al., 2005). In the present study, we sought to determine whether sleep loss in rodents after cage change, as a response induced by an unfamiliar environment and/or the result of transient stress, may provide an animal model for mimicking human FNE. We found that mice moved to a clean cage (MCC) increased sleep latency more than mice moved to a dirty cage (MDC). We then investigated the ability of hypnotics including zolpidem, diazepam, as well as other sleep-inducing substances, pyrilamine, SCH23390, and raclopride, to improve sleep disturbances in MCC mice. Our results indicated that zolpidem and raclopride are highly effective in preventing FNE in MCC mice.
2. Materials and methods

2.1. Animals

Male inbred C57BL/6J mice (Experimental Animal Center, Fudan University), 10 weeks old and weighing 24 ± 2 g, were used at the beginning of the experiments. Ambient room temperature was maintained at a constant temperature (23 ± 1 °C) and relative humidity (60 ± 5%) on an automatically controlled 12 h/12 h light/dark cycle (lights on at 08:00). Water and food were available ad libitum. All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Fudan University Committee on Animal Care and Use. Additionally, all efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.2. Drugs

Zolpidem, pyrilamine maleate, SCH-23390, and raclopride were purchased from Sigma-Aldrich (Saint Louis, MO, USA), and diazepam from Wako Pure Chemical Industries (Osaka, Japan). All drugs were freshly prepared prior to use, and an injection volume (20 ml/kg) was kept constant throughout the experiments. The dosage selections, route of drug administration, and injection time of different compounds were based on preliminary experiments and pharmacokinetic considerations. Zolpidem and diazepam were dissolved in saline containing 0.3% Tween 80 and all other drugs were dissolved in saline.

2.3. Polygraphic recording and behavioral state analysis

The implant surgery was performed 10 days after the mice arrived from the supplier and they were allowed a post-surgery recovery period of 10 days. Following the recovery period after the surgery, the mice were housed individually in transparent barrels and habituated to the recording cage for 4 days before polygraphic recordings. Then the polygraphic recordings were recorded continuously for 48 h in freely moving mice.

Cortical EEG and EMG signals were first amplified and filtered (EEG, 0.5–30 Hz; EMG, 20–200 Hz) and then digitized at a sampling rate of 128 Hz and recorded by using SleepSign® (Kissei Comtec, Japan) as described earlier (Huang et al., 2005, 2006). When completed, polygraphic recordings were automatically scored off-line by 4 s epochs as wakefulness, NREM, and REM sleep according to standard criteria (Huang et al., 2001; Yan et al., 2011). As a final step, defined sleep–wake stages were examined visually, and corrected, if necessary.

2.4. Cage change procedure and drug treatment

After 24 h uninterrupted baseline sleep recording, cage change was performed at 10:00 AM (2 h after lights on, when the sleep pressure is high) (Vyazovskiy et al., 2008) on the experimental day. Each mouse was moved either into a clean cage with fresh paper-chip bedding, or a dirty cage previously occupied by another mouse for 5 days to establish the FNE mouse model. In addition, mice that only received tail handling and put back to the recording cage immediately into a clean cage with fresh paper-chip bedding at 10:00 AM on the experimental day, following the baseline sleep recording. Afterwards, sleep recordings were obtained for another 24 h after the drug treatment and cage change. All mice were used only once.

2.5. Blood sampling and corticosterone assay

To test the degree to which the hypothalamic–pituitary–adrenal axis was involved in the insomnia observed after placing the mice in a new cage environment, we measured the levels of circulating corticosterone. Blood sampling was performed by cardiac puncture under deep anesthesia immediately after moving mice to another cage and at 30, and 120 min after the mice were placed in a clean or dirty cage. Every blood sampling was conducted within 2 min, which is rapid enough to ensure that the stress imposed in the blood-sampling procedure did not affect corticosterone levels in plasma (Riley, 1960). To obtain the basal corticosterone levels, mouse blood were collected from their home cages at the corresponding time point, where they were undisturbed. All the mice here were used only once and all of the blood samples were collected in the EDTA-coated tubes on ice and immediately centrifuged at 15,000 rpm for 5 min, 4 °C. Plasma samples were collected into the sterilized tubes and frozen at −80 °C until assay. Plasma corticosterone was measured with specific enzyme immunoassay kits (Enzo life science, USA), following manufacturers’ protocol.

2.6. Statistical analysis

All results were expressed as means ± SEM (n = 5–8). Time course changes in the amounts of sleep–wake, sleep latency, number and duration of sleep/wake bouts in light/dark phases were compared among groups by using a one-way ANOVA followed by the post hoc Tukey test or non-paired, two-tailed student’s t tests. In all cases, p < 0.05 was taken as the level of significance.

3. Results

3.1. Increased sleep latency in MCC and MDC mice

As shown in Fig. 1A–C, typical examples of EEG/EMG and hypnogram showed a long continued period of wakefulness when a mouse was moved to a clean or dirty cage, or after tail handling in its host cage. The latency to sleep onset, defined as the time from the mouse being moved into another cage to the appearance of the first NREM or REM sleep episode of 20 s or more (Jiu et al., 2012; Qiu et al., 2009), was significantly increased in MCC and MDC mice. As compared with the tail-handled control mice, the latency to NREM (F2, 15 = 130.63, p < 0.01) and REM (F2, 15 = 31.08, p < 0.01) sleep was increased in both MCC and MDC mice (Fig. 1D). In addition, MCC mice exhibited a longer latency to both NREM (91.8 ± 1.6 min vs. 69.4 ± 2.8 min, p < 0.01) and REM (113.5 ± 6.6 min vs. 91.6 ± 4.1 min, p < 0.01) sleep than MDC mice.

3.2. Severe sleep disturbance in MCC mice

Under basal conditions, all mice exhibited a clear circadian sleep–wake rhythm with more sleep during the light period than during the dark period (Fig. 2A). Tail handling at 10:00 (2 h after lights on) induced wakefulness that lasted for little more than 30 min. By contrast, MCC and MDC mice showed the absence of both NREM and REM sleep for more than 1 h after the cage change (Fig. 2B). Interestingly, when compared to MCC mice, MDC mice showed an increment in NREM (t1, 12 = 3.81, p = 0.02) and REM (t1, 12 = 2.83, p = 0.02) sleep during the fourth hour after cage change (Fig. 2B). The total amount of NREM (F3, 20 = 13.08, p < 0.01), and REM (F3, 20 = 31.15, p < 0.01, Fig. 2C) sleep decreased between the second hour after cage change, and the following 3 h, as compared to the baseline. When compared to tail handled mice, only the total amount of NREM sleep of the MCC mice significantly decreased (F3, 20 = 13.08, p = 0.02, Fig. 2C). The mean duration of NREM and REM sleep during 4 h increased 1.16-fold and 1.27-fold, respectively, in the MDC mice, while the mean duration of REM sleep decreased to 79.21% in MCC mice, as compared to tail-handled mice (Fig. 2D). The number of episodes of
The MCC mice had more severe sleep disturbances than MDC mice and therefore, we employed MCC mice as a model for the FNE to evaluate beneficial effects of hypnotics on FNE. As shown in Fig. 3A and B, MCC mice that received an intraperitoneal injection of zolpidem at a dose of 5 mg/kg immediately before the cage change, showed shortened sleep latency (average 4.8 min), while vehicle-treated MCC mice were awake for an average of 96.3 min. In addition, zolpidem-treated MCC mice spent more time in sleep than those treated with vehicle (Fig. 4D). Similar changes were observed in MCC mice treated with raclopride, diazepam, or pyrilamine, which decreased the latency to NREM sleep to 12.1, 20.2, and 43.7 min, respectively, and were significantly shorter than that of 96.3 min in vehicle-injected mice (Fig. 4C). However, there was no significant difference in REM sleep latency among MCC mice treated with hypnotics or vehicle (Fig. 3B). All MCC mice treated with SCH23390 showed no shortening latency to NREM or REM sleep as compared to the vehicle-treated MCC mice. These findings clearly indicate that zolpidem, raclopride, and diazepam suppressed cage change-induced wakefulness and accelerated the recovery of NREM sleep.

3.5. Hypnotics increased NREM and REM sleep in FNE mouse model

Fig. 4 summarizes the time-courses of the hourly amounts of NREM and REM sleep in MCC mice treated with zolpidem, raclopride, or diazepam (Fig. 4A–C) and their cumulative amounts of NREM and REM sleep for 4 h after cage change (Fig. 4D–F). As compared with the vehicle, zolpidem at 5 mg/kg markedly increased the amount of NREM sleep in the first hour (t1, 10 = 14.91, p < 0.01) and REM sleep in the third hour (t1, 10 = 3.23, p < 0.05) after the cage change. The increase in NREM sleep and decrease in wakefulness appeared 1 h after the i.p. injection (Fig. 4A). Similar changes were observed in MCC mice treated with raclopride (2 mg/kg) or diazepam (3 mg/kg) (Fig. 4B and C). However, during the dark period, the MCC mice treated with diazepam showed increases of NREM sleep, despite the fact that mice should have spent more time in wakefulness (Fig. 4C). This observation is similar to diazepam-caused daytime sleepiness in humans (Lader et al., 2009). These data indicate that zolpidem, raclopride, and diazepam may suppress FNE and promote sleep. Next, we calculated the total time spent in NREM and REM sleep during 4 h after the drug treatment and cage change. Zolpidem (F3, 22 = 41.23, p = 0.02) and raclopride (F3, 22 = 28.45, p = 0.04) increased NREM sleep dose-dependently, but raclopride decreased REM sleep time (F3, 22 = 18.05, p < 0.01).
after cage change (Fig. 4D and E). However, the MCC mice treated with pyrilamine at 5 mg/kg or SCH23390 at 30 μg/kg did not increase sleep after cage change (Fig. 4F). Therefore, we did not test lower dosages of pyrilamine and SCH23390, because the dosages used are sufficient for pharmacological effects of pyrilamine (Huang et al., 2006) or SCH23390 on wakefulness (Qu et al., 2008).

3.6. Hypnotics changed the number of episodes, mean duration, stage transition, and power density of NREM sleep in MCC mice

To address sleep efficiency of tested hypnotics in MCC mice, we determined the NREM and REM sleep bout distribution as a function of bout or episode between the second hour after cage change and the 3 h that follow, because vehicle-injected FNE mice were completely awake during the first hour after the injection. Compared with the vehicle control, zolpidem and raclopride did not change the number of NREM sleep episodes and the mean duration. Only diazepam-treated MCC mice showed sleep fragmentation with increased numbers of episodes and shorter mean duration (F3, 16 = 15.64, p = 0.01). On the other hand, the REM sleep episodes in raclopride-treated MCC mice decreased (F3, 16 = 20.00, p < 0.05) and in diazepam-treated MCC mice increased (F3, 16 = 20.00, p < 0.01) without alteration of their mean duration (Fig. 5A and B). In addition, zolpidem (5 mg/kg), raclopride (2 mg/kg), and diazepam (3 mg/kg) increased the number of stage transitions from wakefulness to NREM sleep and subsequently from NREM sleep to wakefulness during 3 h (11:00–14:00; Fig. 5C, p < 0.01). Neither a change in the number of transitions from NREM to REM sleep nor REM sleep to wakefulness was found in zolpidem-treated MCC mice. Raclopride decreased the number of stage transitions from NREM to REM sleep and REM sleep to wakefulness, whereas diazepam only increased stage transitions from REM sleep to wakefulness.

We then determined the EEG power spectra for NREM sleep for 3 h from 11:00 to 14:00, because mice in the vehicle control showed completely awake during the first hour after cage change, so that the...
power density of sleep could not be calculated. The power of each 0.25 Hz-bin was first averaged across the sleep stages individually and then normalized as a group by calculating the percentage of each bin from the total power (0–24.75 Hz) of the individual animal (Yan et al., 2011). As shown in Fig. 5D, compared to the baseline, vehicle and zolpidem-treated MCC mice exhibited a drastic increase of power density in the frequency ranges of 1.00–3.25 Hz (black line in Fig. 5D, $F_{4, 16} = 19.35$, $p < 0.05$, presumably because of the circadian drive and the increased homeostatic pressure caused by the sleep loss in the first hour after vehicle treatment) and 1.00–2.75 Hz (pink line in Fig. 5D, $F_{4, 16} = 16.17$, $p < 0.05$), respectively, while diazepam decreased NREM power density in the frequency ranges of 1.00–4.00 Hz (green line in Fig. 5D, $F_{4, 16} = 22.38$, $p < 0.05$) and increased it in the frequency range of 9.75–24.75 Hz ($F_{4, 16} = 12.69$, $p = 0.05$). However, raclopride did not change the power spectral (blue line) in MCC mice. These data indicated that zolpidem increased, whereas diazepam decreased the power density in the delta range, on the other hand, diazepam increased the fast range of EEG spectra drastically. Raclopride did not change the NREM sleep power density in FNE mice.

4. Discussion

We reported the development of a mouse model for FNE based on the exposure of a mouse to a cage that is different from its host cage during the sleep phase under two different conditions. In the new and unfamiliar environment, the mice showed longer insomnia in clean cages than in dirty ones. After a long duration of continued wakefulness, MDC mice went to sleep with less sleep fragmentation characterized by a decreased episode number and longer mean duration. By contrast, MCC and MDC mice showed decreased number of stage transitions and increased EEG power density in the low frequency bands, the latter of which may be due to the circadian drive or caused by increased homeostatic pressure due to sleep loss in the first 2 h after cage change. The MCC paradigm produced an initial period of acute sleep disturbance similar to the main characteristic of FNE in humans with longer sleep latency (Rechtschaffen and Verdone, 1964). This finding suggests that the MCC procedure represents a suitable animal model for the FNE. Moreover, the use of our MCC model to evaluate potential hypnotics may be an efficient tool to predict their use under clinical conditions, as widely used hypnotics, such as diazepam, given at a dose corresponding to human dosage, have no significant effect on NREM sleep in normal mice (Kopp et al., 2004).

In our study, mice that were moved to dirty cages occupied by other mice for more than 5 days showed shortened latency to sleep than mice that were moved to clean cages. A possible explanation for this finding may be that the smell of another mouse is not a stressor per se, because the mice are housed in adjacent cages in the animal isolators and continually exposed to each other’s smell. Mice use their sense of smell to detect food, predators, sexual receptivity, and many other aspects and to quickly familiarize with their environment (Beauchamp and Yamazaki, 2003), whereby mice are known to show prolonged activity in a novel environment without any social cues (Tang et al., 2005). In fact, for behavioral tests in neuroscience laboratories, many researchers wipe the facilities gently with a damp paper towel and/or only remove urine and feces, based on the assumption that a constant layer of olfactory cues will be less stressful for test animals than a very clean environment (Baumann, 2005). The mice employed in our study faced to two different stressors, i.e., clean and dirty cages, causing the circulating...
corticosterone level to increase. Short mild stress usually involves a sleep rebound, while the long and intense stress periods induce sleep disturbance (Marinesco et al., 1999). In the present study we found that the plasma corticosterone levels dramatically increased in clean or dirty cage conditions, suggesting that mice under both conditions have severe sleep disturbances. However, mice slept 2 h after the cage change, despite higher corticosterone level. These results indicated the corticosterone is not the primary cause of wakefulness in our experiments.

A previous study in rats showed that the dirty cage condition is more efficient to induce acute insomnia than the clean cage approach, as well as, after being moved to dirty cages, the rats developed two phases of sleep disturbance with an acute phase after the cage change and a second sleep disturbance phase 4–6 h later (Cano et al., 2008). We found, however, that mice showed dramatically longer sleep latency when moved to clean cages (91.8 ± 1.6 min), whereby the sleep latency was not only longer than for mice moved to a dirty cage (69.4 ± 2.8 min), but also significantly longer than rats moved to a dirty cage in the previous study (58.7 ± 7.4 min). We did not observe any second/delayed phase of sleep disturbances neither under the MCC nor MDC conditions, which may be due to species differences. For example, rats are known to be more territorial after exposure to olfactory and visual cues of a competitor (Whishaw et al., 2001), whereas mice seem to be more sensitive to a new environment or novel objects (Tang et al., 2004; Whishaw et al., 2001) and are usually more actively engaged in natural behaviors, like nest building (Tang et al., 2005). Our findings indicated that FNE/MCC mice are more suitable to evaluate the effects of hypnotic substances: sleep latencies of mice or rats after vehicle injections are around 40 min (Kopp et al., 2003; Utsu et al., 2007) so that it is difficult to observe a clear difference when the rat model is employed in pharmacological experiments due to the rats’ only slightly longer sleep latency when moved to another cage than the one after the vehicle injection (Cano et al., 2008).

Benzodiazepines (BZs) such as diazepam and non-BZ hypnotics such as zolpidem are first line drugs used for the therapy of insomnia, despite the fact that hypnotics such as diazepam only reduce sleep latency slightly (Pick et al., 2005; Winsky-Sommerer, 2009). We employed our new FNE model based on MCC mice to evaluate hypnotics and found that zolpidem significantly reduces sleep latency and increases total sleep time in a novel sleep environment, but lacks the typical daytime sleepiness of diazepam, the latter of which may cause clinically

**Fig. 4.** Hypnotics increased the sleep time of the mice after ‘moved to clean cages’ (MCC). Effects of zolpidem (A), raclopride (B), diazepam (C) on sleep profiles of MCC mice. Each circle represents the hourly mean amount of each stage. The horizontal open and filled bars on the y-axes indicate the 12 h light and 12 h dark periods, respectively. Zolpidem (D) and raclopride (E) increased total sleep time during 4 h in a dose-dependence manner. Pyrilamine and SCH23390 had no significant effect on sleep time (F). Drugs were given at 10:00, and followed by changing the mice’s cages immediately on the experimental day. Values are the means ± SEM (n = 6–8). *p < 0.05, **p < 0.01 compared with vehicle control, assessed by two-tailed unpaired student’s t test in (A–C), and one-way ANOVA followed by the Tukey test in (D–F).
significant daytime distress and functional impairment (Dundar et al., 2004; Winsky-Sommerer, 2009). Zolpidem has pharmacological profiles distinct from those of the classical BZs such as diazepam, in that it is highly selective for α1/γ2 receptors in the CNS, thereby producing a strong sedative and hypnotic profile that predominates over the anticonvulsant and anxiolytic activity of diazepam (Koester et al., 2013), which is mediated primarily by GABA receptors composed of α2βγ2 subunits (Low et al., 2000). Although at higher concentrations zolpidem will modulate receptors containing α2 or α3 subunits (Cope et al., 2004), we did not use so high dosage of zolpidem in the current study. Furthermore, zolpidem may shorten sleep latency in MCC mice in part of inhibiting the histamine neurons through its synaptic GABA<sub>A</sub> receptors (Zecharia et al., 2012). It was demonstrated that GABA receptors composed of α1γ2 receptors mediate the sedative, anterograde amnestic, defensive behavioral, and in part the anticonvulsant actions of diazepam (da Cruz et al., 2013; Koester et al., 2013; Olsen and Sieghart, 2009), since diazepam predominantly interacts with GABA receptors composed of α1βγ2, α2βγ2, α3βγ2, or α5βγ2, which mediate the different clinical effects through different receptor subtypes (Olsen and Sieghart, 2009).

In addition, we previously found that D<sub>2</sub>R knockout (KO) mice exhibited a significant decrease in wakefulness (Qu et al., 2010) and that L-stepholidine, the first compound known to have mixed dopamine D<sub>2</sub>R agonist/D<sub>1</sub>R antagonist properties, may potentially be used for the treatment of insomnia (Qu et al., 2009). Here, we evaluated the hypnotic effects of the D<sub>2</sub>R antagonist raclopride and the D<sub>1</sub>R antagonist SCH23390 in MCC mice, and found that only raclopride significantly reduces NREM sleep latency of MCC mice, resulting in longer sleep times and more transitions from wakefulness to NREM sleep. Mice treated with raclopride also showed a power density of NREM sleep that was similar to physiological sleep under basal conditions. Our results suggest that the dopamine/D<sub>2</sub>R system is involved in enhanced arousal in a novel environment. It is known that mice lacking dopamine (Qu et al., 2010) or histamine (Ohtsu and Watanabe, 2003) can sleep faster after a mild stress, including cage change. Dopaminergic neurons send excitatory projections to the histaminergic tuberomammillary nucleus, the nopepinephrinergic locus coeruleus, and other arousal regions (Huang et al., 2007; Parmentier et al., 2002), suggesting that dopaminergic and histaminergic systems are involved in arousal. In fact, H<sub>1</sub>R KO mice have previously been demonstrated to have fewer incidents of brief awakening under baseline conditions (Haas and Panula, 2003; Huang et al., 2006). However, mice under MCC conditions that were treated with the histamine H<sub>1</sub>R antagonist pyrilamine had a shortened NREM sleep latency, but lacked any effect on the mean duration, episode number of each stage and stage transition as compared to untreated MCC mice (data not shown). Pyrilamine used at the dosage as described above may not be strong enough to improve acute insomnia in MCC mice with a high degree of wakefulness for more than 90 min. Long half-lives and peripheral side effects, however, prevents the clinical usage of pyrilamine at higher dosages and thus, limit its use in controlling insomnia (Tiligada et al., 2011).

5. Conclusions

We developed a mouse model for FNE characterized by prolonged sleep latency when mice are moved to a clean cage. We found that zolpidem and raclopride almost completely antagonized the FNE.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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