Neuroscience Letters 682 (2018) 74–78

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research article

Sleep patterns deteriorate over time in chronic corticosterone-treated rats

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ARTICLE INFO

Keywords:
Sleep
Depression
Corticosterone
Locus coeruleus

ABSTRACT

Repeated corticosterone (CORT) injections reliably produce depressive-like behavior in rodents. Our previous study showed that sleep parameters were altered in rats after daily injections of CORT for 7 days, and sleep disturbances appeared to be correlated with depressive-like behavior. The aim of the present study was to investigate time-dependent correlations between changes in sleep parameters and the formation of depressive-like behavior in rats after more prolonged treatment with CORT. Rats received daily injections of CORT (40 mg/kg, s.c.) for 7, 14, or 21 days. Electroencephalographic recordings were performed to study sleep parameters. The sucrose preference test and forced swim test were performed to evaluate depressive-like behavior. Western blot was used to detect protein levels. Our results showed that 7-day CORT treatment resulted in no significant depressive-like behavior or changes in rapid-eye-movement (REM) sleep. However, the duration of non-REM sleep significantly decreased, tyrosine hydroxylase (TH) levels significantly increased, and glucocorticoid receptor (GR) expression decreased in the locus coeruleus. Treatment with CORT for 14 and 21 days increased depressive-like behavior, enhanced REM sleep, shortened REM sleep latency, decreased TH and GR levels, and increased the levels of the chaperone FK506 binding protein 51 (FKBP51) in the locus coeruleus. These results indicate that the development of depression after chronic CORT treatment may be related to the formation of sleep disorders. Abnormalities of REM sleep may be a characteristic of sleep in models of depression that is induced by chronic CORT administration in rats. The noradrenergic system and GR pathway in the locus coeruleus may be involved in the formation of depression concomitant with sleep disturbances.

1. Introduction

Sleep disturbances are a common symptom in a majority of people who suffer from depression [1]. A large body of evidence suggests that sleep abnormalities often precede the onset of depression and constitute an independent risk factor for the development of depression in humans [2]. However, the underlying pathological mechanisms need to be elucidated.

The locus coeruleus (LC) is a brainstem nucleus that is a major source of norepinephrine (NE) projections throughout the central nervous system. The LC is involved in modulating the sleep-wake cycle [3]. The involvement of the LC-NE system in the depression patients [4] and animal models of depression [5,6] is still controversial, the activity of which has been reported to be either enhanced or attenuated. The LC has been found to be a highly stress-reactive nucleus that is capable of prolonged activation and modulating the central stress response [7]. Markey et al. indicated that noradrenergic neurons in LC might be target cells for glucocorticoids, meaning that the effect of glucocorticoids on tyrosine hydroxylase (TH) might be receptor-mediated [8]. Polymorphisms of the glucocorticoid receptor (GR) gene might play a role in the pathophysiology of major depression [9]. FKBP51 (FK506 binding protein 51) is an inhibitory co-chaperone of the GRs. When it binds to the GR, cortisol binds with lower affinity, and the nuclear translocation of the GRs is less efficient [10]. FKBP51 has been suggested to be a therapeutic target for stress-related mood [10] and sleep [11] disorders. In previous studies, we used a model to investigate sleep disorders that are induced by stress, in which we administered corticosterone (CORT) daily for 7 days. After 7-day CORT treatment, rats exhibited significant sleep disturbance, and the levels of GRs decreased in the LC. The activation of noradrenergic neurons in the LC was also observed, reflected by simultaneous elevations of NE and TH levels in the LC [7]. These results support the hypothesis that repeated CORT treatment may decrease GR levels and induce the activation of noradrenergic neurons in the LC, consequently result in sleep disorders. Additionally, sucrose preference (*i.e.*, a measure of anhedonia) was positively correlated with total sleep time and light sleep bouts, and it was negatively correlated with the rapid-eye-movement (REM) sleep time ratio [12]. However, unknown are the ways in which sleep

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https://doi.org/10.1016/j.neulet.2018.06.017
Received 24 May 2016; Received in revised form 7 June 2018; Accepted 8 June 2018
Available online 09 June 2018
0304-3940/

patterns are altered by continuous CORT treatment for more than 7 days or until rats exhibit depressive-like behavior. Thus, the present study characterized sleep patterns across the development of depression. We prolonged daily CORT treatment to 14 and 21 days. To confirm whether GRs, FKBP51 and TH in the LC participate in sleep disorders that are induced by chronic CORT administration, the levels of these proteins were detected in the LC.

2. Materials and methods

2.1. Animals

Male Wistar rats (Grade I, purchased from the Animal Center of Peking University, Beijing, China) were used. All of the experiments were conducted in accordance with European Community guidelines for the use of experimental animals and approved by the Peking University Committee on Animal Care and Use. The rats were individually housed in acrylic cages and had access to food and water *ad libitum* under a 12 h/12 h light/dark cycle (lights on at 9:00 AM). The average ambient temperature was 22 °C ± 2 °C, and the relative humidity was 50% ± 10%.

As shown in Fig. 1, the animals were randomly assigned to four groups: one control group (vehicle administration only) and three CORT groups (40 mg/kg CORT; TCI Development Co., Ltd, Shanghai, China; dissolved in 1 ml/kg of saline with 2% Tween 80) that were treated once daily at 9:00 AM for 7, 14, and 21 days, respectively. The dosage was chosen based on a pilot study (data not shown).

2.2. Surgery

Twenty-eight animals underwent standard surgical procedures [19]. Briefly, under chloral hydrate (300 mg/kg) anesthesia, two stainless-steel screws that were attached to an insulated wire were implanted in the skull over the frontal-parietal cortex for encephalographic (EEG) recording. One screw was placed approximately 2 mm anterior and 2 mm to the right of bregma, and another screw was placed approximately 3 mm posterior and 2 mm to the left of bregma. A ground electrode was placed between the two screws, 3 mm lateral to the midline. For electromyography (EMG), a pair of wire electrodes was threaded through the nuchal muscles. These electrodes were attached to a miniature connector, which was affixed to the skull with dental acrylic. After surgery, the rats were injected with antibiotics (penicillin) for 3 days and allowed to recover for 7 days before the experiments. For habituation, the animals were connected to the recording apparatus at least 1 day before the start of EEG recording.

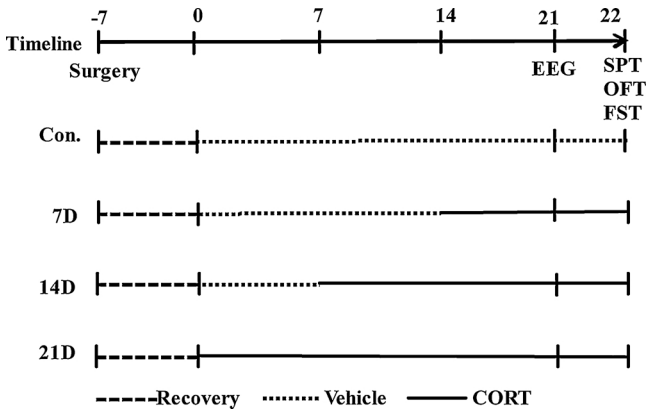

Fig. 1. A diagram of the experimental procedure: 21 days of corticosterone and vehicle injections, with subsequent sleep EEG recordings and behavioral tests.

2.3. EEG and EMG recordings and analysis

For the electrophysiological recordings, a lightweight shielded cable was plugged into the connector on the rat's head and attached to a counterbalanced swivel that allowed free movement of the animal. All rats were studied in an electrically shielded box and noise-attenuated environment that was free from interruptions. The signals were routed to an electroencephalograph (Model MP 150, BIOPAC Systems, Goleta, CA, USA). Recordings were performed for 24 h for each group, from 9:00 AM to 9:00 AM the next day. The signals were amplified, filtered (EEG, 0.5–30 Hz; EMG, 10–100 Hz), digitized at a sampling rate of 128 Hz, and recorded using AcqKnowledge software (BIOPAC Systems). The EEG and EMG recordings were analyzed in 10 s epochs using standard criteria in SleepSign 2.0 software (Kissei Comtec Co, Ltd, Nagano, Japan). Sleep-wake states were automatically classified as wakefulness, REM sleep, and non-REM (NREM) sleep. As a final step, the defined sleep/wake stages were manually examined and corrected according to visual observations of the animal's behavior, which was recorded using a video camera and then analyzed according to criteria in a previous report [7].

2.4. Behavioral assessment

Twenty-eight rats were used ($n = 7$). To avoid potential interference by water intake and wet fur in the forced swim test, the sucrose preference test was conducted first, followed by the open field test and then the forced swim test.

In the sucrose preference test, the rats were trained for 48 h to adapt to a 1% sucrose solution (w/v) at the beginning of the experiment in which two bottles with 1% sucrose solution were placed in each cage. At the end of sleep recording, the rats were deprived of water for 4 h and then underwent the test. The rats were housed in individual cages for 1 h and then exposed to two identical bottles, one filled with 1% sucrose solution and the other filled with water. At the end of the 1-h test, sucrose and water consumption was measured. Sucrose preference (%) was calculated as sucrose consumption / (sucrose consumption + water consumption).

In the open field test, locomotor activity was measured in clear Plexiglas chambers (40 cm × 40 cm × 65 cm) using an automated video tracking system (DigBehv-LM4, Shanghai Jiliang Software Technology, Shanghai, China). The videos were analyzed using DigBehv analysis software. Anxiety-like behavior was reflected by a reduction of the distance traveled in the central zone. Locomotor activity, expressed as the total distance traveled in 15 min, was also measured.

In the forced swim test, each rat was individually placed for 5 min into a 25 cm diameter ×60 cm height Plexiglas cylinder that was filled with 23–25 °C water to a depth of 40 cm. Behavior was recorded with two video cameras that were mounted on the top and side of the tank. Passive behavior (*i.e.*, immobility) and active behavior (*i.e.*, swimming and climbing) were scored from videotapes by a researcher who was blind to each rat's treatment condition [13]. The water in the tank was changed for each rat.

2.5. Western blot

Tissues from the LC were collected and processed according to a previous study [7]. Briefly, LC tissue (with some surrounding tissue) was punched (2 mm diameter) bilaterally using a brain matrix, guided by the Paxinos and Watson (1998) rat brain atlas [14], and pooled for each group. Proteins from the LC were extracted and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). The membranes were blocked with 5% skim milk for 1 h at room temperature and incubated with primary antibodies in TBS-T buffer (Tris-buffered saline and 10.1% Tween-20) at 4 °C overnight. The