Changes of Sleep Patterns in Rats with Chronic Constriction Injury under Aversive Conditions

Shin Tokunaga, Yasuhiro Takeda, Kazuaki Shinomiya, Wataru Yamamoto, Yoshiaki Utsu, Katsuo Toide, and Chiaki Kamei*

*Department of Medicinal Pharmacology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences; †Department of Pharmaceutical Care and Health Sciences, Faculty of Pharmaceutical Sciences, Okayama University; 1–1–1 Tsushima-naka, Okayama, 700–8530, Japan; and ‡Pfizer Global Research & Development, Nagoya Laboratories, Pfizer Japan Inc., 5–2 Taketoyo, Aichi 470–2393, Japan.

In the present study, we investigated the changes of sleep parameters in rats with chronic constriction injury (CCI) under aversive conditions. The electroencephalogram (EEG) in the frontal cortex of CCI rats and electromyogram (EMG) were measured over 6 h by placing rats on sandpaper as an aversive condition, to compare with rats placed on sawdust. Six days after CCI surgery, the rats exhibited significant mechanical allodynia, and also had neuropathic pain. When rats were placed on sawdust, no significant difference was observed between the CCI group and sham-operated control group in sleep latency, total waking time, total non-REM sleep time and total REM sleep time. On the other hand, when CCI rats were placed on sandpaper, a significant increase was observed in sleep latency and total waking time compared with the sham group; however, no significant difference was observed in the total non-REM sleep time and total REM sleep time between these two groups. These results indicate that an important factor of sleep disturbance in CCI rats is not only damage to the nerves but also being under aversive conditions. In addition, it was found that CCI rats placed on sandpaper as an aversive condition can serve as a new sleep disturbance model.

Key words  sleep disturbance model; neuropathic pain; chronic constriction injury; sandpaper; electroencephalogram pattern

It is well known that acute and unbearable pain are alarm reactions for the body; therefore, pain is necessary for life to protect the body. However, chronic pain is only an aversive feeling that produces various physical symptoms, such as decreased appetite, lower satisfaction with life, anxiety, mood disturbance, depression and poor sleep.1–4) These symptoms reduce the quality of life of patients. Of these symptoms induced by chronic pain, sleep disturbance is well known to influence the social life of patients. Patients with chronic pain, together with postherpetic neuralgia, diabetic peripheral neuropathy and complex regional pain syndromes, often experienced sleep disturbances, such as delayed sleep onset, and difficulty in maintaining sleep and remaining asleep4–11); therefore, an animal model of these conditions is needed to develop useful medicine for insomnia.

There have been a few papers about sleep patterns in chronic constriction injury (CCI) rats reported as a neuropathic pain model,12,13) however, conflicting findings are demonstrated, that is, one paper showed no significant alterations of sleep parameters, whereas another reported the opposite results. It seems difficult to assess the effects of hypnotics for sleep disturbance using only CCI model rats.

Therefore, in the present study, we investigated the changes of sleep patterns in rats with CCI under aversive conditions.

MATERIALS AND METHODS

Animals  Male Sprague-Dawley rats weighing 235—265 g (Charles River Japan, Yokohama, Japan) were used. All animals were maintained in an air-conditioned room with controlled temperature (24±2 °C) and humidity (55±15%). They were housed in aluminum cages with sawdust and kept under a light–dark cycle (lights on from 07:00 to 19:00). The animals were allowed free access to food and water, except during the experiments. All procedures involving animals were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center.

Surgery  The animals were anesthetized with pentobarbital sodium (Nembutal®, 35 mg/kg, i.p., Abbott Laboratories, North Chicago, IL, U.S.A.), and then placed in stereotaxic apparatus (SR-5N, Narishige, Tokyo, Japan). For EEG recording, a stainless steel screw electrode (200 μm) was chronically implanted into the right frontal cortex (A: 0.5, L: 1.5) according to the atlas of Paxinos and Watson.14) To record the electromyogram (EMG), stainless steel wire electrodes (200 μm) were implanted into the dorsal neck muscle. The electrodes were connected to a miniature receptacle and the whole assembly was fixed to the skull with dental cement. At least 7 d were allowed for recovery from surgery.

Surgery for Chronic Constriction Injury (CCI) Model  Surgery was performed according to the method of Bennett and Xie.15) Rats were anesthetized with pentobarbital sodium (Nembutal®, 35 mg/kg, i.p.). The left common sciatic nerve was exposed at the level of the mid-thigh. Proximal to the sciatic trifurcation, about 7 mm of nerve was freed of adhering tissue and 4 ligatures (4–0 silk) were tied loosely around it at about 1 mm spacing. Great care was taken to tie the ligatures such that the diameter of the nerve was seen to be just barely constricted when viewed with 40× magnification. The incision was closed in layers. The same procedure was performed without sciatic nerve ligation for the sham group.

Evaluation of Static AlloDyNia  Static alldynia was measured using Semmes-Weinstein von Frey hairs. The animals were habituated to wire mesh bottom cages prior to the
start of the experiment. Static allodynia was evaluated by the application of von Frey hairs in an ascending order of force (0.16, 0.4, 0.6, 1, 1.4, 2, 4, 6, 8, 10, 15, 26 g) to the plantar surface of the hind paw. Each von Frey hair was applied to the paw for 6 s, or until a withdrawal response occurred. Once a withdrawal response was established, the paw was re-tested, starting with the next descending von Frey hair until no response occurred. The highest force of 26 g lifted the paw, as well as eliciting a response, thus representing the cut-off point. Each animal had both hind paws tested in this manner. The lowest amount of force required to elicit a response was recorded as the paw withdrawal threshold (PWT) in grams. Static allodynia was defined as present if the animals responded to the previously innocuous 4 g von Frey hair or below.16)

**EEG and EMG Recordings** EEG and EMG were recorded with an electroencephalograph (Model EEG 4314, Nihon Kohden, Tokyo, Japan) for 6 h (10:00—16:00). The recording was carried out according to the method described previously.17—20) The signals were amplified and filtered (EEG, 0.5—30 Hz; EMG, 16—128 Hz), digitized at a sampling rate of 128 Hz and recorded using the data acquisition program SleepSign ver.2.0 (Kissei Comtec, Nagano, Japan). EEG and EMG of the rat were measured in a plastic cage (diameter, 26 cm; height, 31 cm) with its floor covered with sawdust or sandpaper, which seemed to cause a sense of aversive. When rats were placed on the floor covered with sandpaper, they immediately transferred to the floor covered with sawdust. The observation cage was placed in a sound-proof and electrically shielded box (70×60×60 cm). Behavior was also monitored by TV camera.

**Sleep-Wake State Analysis** The sleep-wake states were automatically classified into 10-s periods as awake, non-rapid eye movement (non-REM) or rapid eye movement (REM) sleep by SleepSign ver.2.0, according to the criteria previously described.14,21) As a final step, defined sleep-wake stages were examined visually, and corrected if necessary. Each state was characterized as follows: awake, low-amplitude EEG and high-voltage EMG activities; non-REM sleep, high-amplitude slow or spindle EEG and low-EMG activities; REM sleep, low-voltage EEG and high-EMG activities.

**Experimental Protocol** First, the electrodes for EEG and EMG recording were implanted; next, baseline EEG was measured 7 d after implantation of EEG and EMG electrodes; then, baseline PWT to von Frey hairs was determined. CCI surgery was then performed under pentobarbital anesthesia. Six days after CCI surgery, PWT was re-examined. Finally, EEG and EMG were measured on the sawdust (natural environment) or sandpaper (aversive condition) 7 d after CCI surgery.

**Data Analysis and Statistics** PWT values shown are the median and 1st and 3rd quartiles. Other values shown are the means±S.E.M. The Mann–Whitney U-test was used to compare PWT in sham-operated control rats and CCI rats. Student’s t-test was used for comparison of the sleep parameters in normal rats placed on sawdust and sandpaper, and for comparison of the sleep parameters in sham and CCI rats under sawdust or sandpaper conditions. Sleep latency was defined as the time from the start of the experiment up to the first 12 consecutive 10-s periods of sleep.

**RESULTS**

**Measurement of Static Allodynia in CCI Model** There was no significant difference in PWT between the CCI group and the sham group in pre-surgery; however, a significant decrease of PWT was observed in CCI rats at 6 d after surgery (p=0.00067) (Fig. 1). This effect lasted for at least 4 weeks after CCI surgery. During the application of von Frey hairs, rats showed a paw lifting and/or arousal state.

**Changes of Sleep Parameters in Sham and CCI Groups Placed on Sawdust** Sleep parameters were assessed over 7 d after surgery. Values are the percentages of the values of the pre-surgery level. Columns and vertical bars represent the means±S.E.M. (sham: n=6; CCI: n=8, Student’s t-test).

**DISCUSSION**

It was found in the present study that a significant decrease
in PWT was observed in the CCI group 6 d after surgery, and this effect lasted for 4 weeks after surgery, suggesting that this finding shows the development of neuropathic pain. Bennett and Xie\(^1\) also reported that PWT decreased within a week after CCI surgery and this decrease in PWT lasted for 2 months after surgery. Attal et al.\(^2\) and Wallas et al.\(^3\) demonstrated that escape responses, such as paw-lifting behavior, hyperalgesia and allodynia, were frequently observed after CCI surgery induced by mechanical stimulation. In our study, behavioral changes similar to those reported in these papers\(^4,5\) were also observed. Until now, there have been few reports about the effects on sleep patterns in CCI rats; moreover, conflicting findings have been demonstrated.\(^6,7\)

For instance, Kontinen et al.\(^8\) reported no significant difference in waking time, light slow-wave sleep time, deep slow-wave sleep time and REM sleep time between CCI rats and sham rats during the light or dark phase. On the other hand, Andersen and Tüfik\(^9\) demonstrated that CCI-induced sleep alterations such as reduced sleep efficacy and increased frequency of arousal, especially during the light period, and sleep patterns were most affected between day 2 and day 11. Another report noted that 30% of CCI rats showed a significant alteration in sleep parameters during both dark and light periods, 25% of CCI rats showed significant alteration in sleep parameters only during the light period, and 45% of CCI rats showed no alteration in sleep parameters\(^10\); that is to say, it seems likely that sleep alteration of CCI rats is inconsistent. In our present study, the EEG measuring was performed during the light period, because, from these reports, changes of sleep parameter might be easy to observe in the sleep phase of the rat. However, in the present results, when CCI rats were placed on sawdust, no significant changes were observed in sleep parameters during the light period; therefore, it can be assumed that only CCI surgery might be insufficient to develop a sleep disturbance model caused by neuropathic pain.

Shinomiya et al.\(^11\) reported that the sleep pattern was affected by the condition during EEG measurement; for instance, sleep latency was prolonged in rats placed on the grid compared with rats placed on sawdust under approximately normal conditions, that is, the experimental condition is an important factor closely linked to sleep alteration. From the above findings, we used sandpaper as an aversive condition and maintained the pain reaction of CCI rats during the sleep measurement. As shown in the results, CCI rats had a significant increase in sleep latency and total awake time compared with sham-operated rats. Recently, it has been reported that pain-induced anxiety-like behavior was observed in rats with CCI,\(^12\) suggesting that the rats experienced psychological stress. Clinically, it was also found that patients with chronic pain showed anxiety and depression, together with sleep disturbance.\(^3\) This psychological stress in rats may be closely related with the pain sensation in patients. As is clear from our present study, sandpaper may intensify the pain response of CCI rats, that is, psychological stress seems to be increased and maintained. As a result, clear sleep disturbance occurred in CCI rats.

In conclusion, these results indicate that an important factor of sleep disturbance in CCI rats is not only damage to the nerves but also the aversive condition. In addition, it was found that CCI rats placed on sandpaper as an aversive condition can serve as a new sleep disturbance model.

REFERENCES