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Effects of citalopram on jaw-closing muscle activity during sleep and wakefulness in mice

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ABSTRACT

In this study, we investigated the effects of chronic administration of the selective serotonin reuptake inhibitor (SSRI) citalopram on sleep/wake cycles and masseter (jaw-closing) muscle electromyogram (EMG) activity over a 24-h period. From the dark to the light period, the times of wakefulness decreased, while those of non-REM (NREM) and REM sleep increased. Citalopram did not induce major alterations in the temporal changes of sleep–wake distributions, except for leading to a decrease in the time of NREM sleep during the light period and an increase in the durations of REM sleep episodes. Moreover, citalopram did not modify mean masseter EMG activity during any of the vigilance states and did not affect the temporal changes related to the shifts between dark/light periods. However, citalopram increased the time engaged in masseter EMG activation during NREM sleep in the second and the first halves of the dark and light periods, respectively. These results suggest that chronic citalopram treatment does not affect the temporal changes of sleep–wake distributions, but has a limited facilitatory influence that fails to increase the number of epochs of high levels of masseter muscle activation.

1. Introduction

Selective serotonin (5-HT) reuptake inhibitors (SSRIs) inhibit the serotonin transporter and antagonize serotonin$_{1A}$ (5-HT$_{1A}$) somatodendritic autoreceptors in the raphe nucleus, which results in an earlier and significantly larger increase in extracellular 5-HT levels (Sanchez et al., 2015). Thus, SSRIs are often used as pharmacological agents for the treatment of chronic depression. Incidents of iatrogenic nocturnal bruxism have been reported in patients treated with SSRIs such as citalopram (Ak et al., 2009; Wise, 2001), paroxetine (Kishi, 2007; Romanelli et al., 1996), and fluvoxamine (Miyaoka et al., 2003). A 5-HT$_{2A}$ receptor single nucleotide polymorphism has been reported to increase the risk of sleep bruxism (Abe et al., 2012). Serotonin is involved in various functions including sleep–wake behavior, autonomic functions, and repetitive central pattern generator (CPG)-mediated behaviors, such as locomotion, respiration, and chewing (Jacobs et al., 2002; Monti, 2011). Moreover, tremor is considered to be the second-most common neurological adverse effect of SSRI use (Edwards and Anderson, 1999).

Sleep bruxism is classified as a sleep-related motor disorder. Sleep bruxism may lead to undesirable orofacial problems such as tooth destruction, breakage of dental prostheses, and orofacial pain (Kato et al., 2013; Lavigne et al., 2003, 2008). Sleep bruxism is considered to have a multifactorial etiology (Lobbezoo et al., 2006), although its underlying causes are not yet known. Several hypotheses regarding the etiology and pathophysiology of sleep bruxism suggest that neurochemicals such as adrenaline, noradrenaline and dopamine, and exogenous factors such as alcohol, caffeine, cigarette smoking, illicit drug use, and medication intake (including SSRI use) may be involved (Carra et al., 2012; Falisi et al., 2014; Lavigne et al., 2007). Previous studies suggest that SSRI intake may be a risk factor for sleep bruxism (Lobbezoo et al., 2001). However, the effects of SSRIs on sleep/wake cycles and the activity of the jaw-closing muscle (masseter muscle) have until now not been investigated.

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The aim of the present study was to examine the effects of the continuous administration of the SSRI citalopram for 6 days on the time distributions of wakefulness, non-rapid eye movement (NREM), and REM sleep, and on masseter muscle activity over a 24-h period. Our results suggest that citalopram does not influence the regulatory systems underlying the inter-day fluctuations of sleep–wake states, while it may have a modulatory influence on sleep-state-dependent changes in jaw motor activity in relation to the dark/light periods.

2. Materials and methods

All experiments followed the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (USA), and were approved by the International Animal Research Committee of Showa University in accordance with Japanese Government Law No. 105 for the care and use of laboratory animals (approval numbers 14012 and 15039). All experiments were conducted as double-blind trials to avoid subjective bias.

2.1. Animals

C57BL/6 mice (males, 7–10 weeks old, 18–23 g) were used for this study. We made every effort to minimize the number of animals used. Animals were housed individually under a 12-h light/dark cycle (lights on from 08:00 to 20:00) and standard temperature conditions. Food and water were available ad libitum.

2.2. Surgical implantation

Mice were prepared for long-term recordings from the masseter muscle during all sleep/wake stages using electroencephalography (EEG), electrooculography (EOG), and electromyography (EMG), as described previously (Katayama et al., 2015). Surgery was performed under anesthesia by ketamine hydrochloride (100 mg/kg, intraperitoneal [i.p.], Ketalar®, Daiichi Sankyo, Tokyo, Japan) and xylazine hydrochloride (10 mg/kg, i.p., Sigma–Aldrich, St. Louis, MO, USA). Three stainless steel screws (M1-2, Unique Medical, Tokyo, Japan) to which urethane-coated stainless steel wires (diameter: 0.12 mm, Unique Medical) were soldered before surgery, were implanted into the skull (two EEG screws were placed over the cortex and one was used as ground). Two electrodes for EOG recording were positioned subcutaneously on each side of the orbit. For EMG recordings, pairs of urethane-coated stainless steel wires (diameter: 0.12 mm, Unique Medical) were inserted into the neck (upper trapezius muscle) and the masseter muscles. The wires used for EMG were subcutaneously routed to the vertex of the skull. The wires for EEG, EOG, and EMG were soldered to a multiple-pin socket, which was used as a connector (Unique Medical). The connectors were firmly fixed to the skull using dental acrylic resin.

2.3. Recording procedures

Mice were housed individually in breeding racks during the recovery period for 5–7 days after the operation. For the subsequent 6 days, mice were transported from the vivarium to the testing room on alternate days during three separate 24-h periods beginning at 20:00. In the testing room, the mice were allowed to adapt to the recording cage and the connected recording cable (TY213-042, Unique Medical). Mice were given chow and water in the testing room. On the last day of the training sessions, a test recording session was started at 20:00 and lasted for 24 h (day 0). Because the plasma half-life of a single dose of citalopram is approximately 1.5 h in mice, which is fairly short compared to that in rats (3 h) and human (33 h) (Fredricson Overo, 1982), we continuously administered citalopram to mice using a subcutaneous osmotic minipump. After finishing the recording on day 0, an osmotic minipump (2002 ALZET, reservoir volume of 200 μL, 0.5 μL per hour for 6 days, DURECT Corporation, Cupertino, USA) was implanted subcutaneously in the back of the mouse under anesthesia using ketamine hydrochloride (100 mg/kg, i.p.) and xylazine hydrochloride (10 mg/kg, i.p.). Animals were randomly divided into three groups: Cit10 [administration of citalopram hydrobromide (Tokyo Chemical industry Co., Ltd., Tokyo, Japan), 10 mg/kg/day, $n = 9$], Cit100 (administration of citalopram hydrobromide, 100 mg/kg/day, $n = 7$), and Saline [administration of saline (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan), $n = 8$]. After implantation of the minipumps, mice were returned to their cages in breeding racks for 5 days and given ad libitum access to pellets and distilled water. Since fluoxetine, an SSRI, is known to lead to sustained significant increases in extracellular 5-HT levels beginning at day 7 in both the midbrain raphe nuclei and the hippocampus (Popa et al., 2010), we evaluated the effects of chronic treatment with citalopram using the data on day 6. The mice were transferred again in their cages to the recording apparatus at 19:40 on day 6. A recording session was started at 20:00 and lasted for 24 h. The electrical signals were amplified (FE135, AD Instruments Inc., Colorado Springs, CO, USA) to optimal bandwidths (EEG and EOG: 0.3–100 Hz; EMG 100–1000 Hz). EEG, EOG, and EMG data were digitized at 400 Hz, 400 Hz, and 4 kHz, respectively, using PowerLab 8/35 (PL3508, AD Instruments) and stored on a personal computer with Chart 7 software (AD Instruments). We recorded the animals' body weight on day 0 immediately after implantation of the minipump and after the recording session on day 6. Data from days 0 and 6 were used for later analysis.

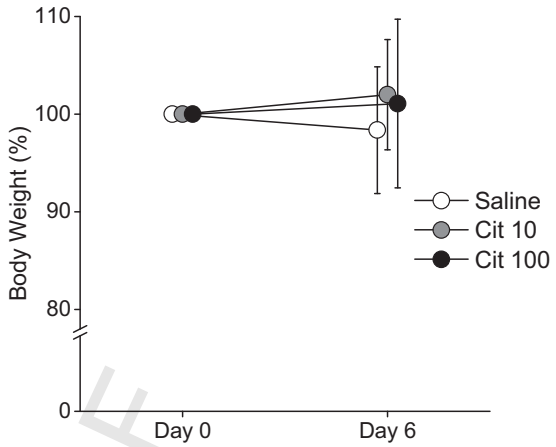

Fig. 1. Changes in body weights of mice before (day 0) and after chronic administration of drugs (day 6). Citalopram (10 mg/kg/day, Cit10, $n = 9$; or 100 mg/kg/day, Cit100, $n = 7$) or Saline ($n = 8$) were administered to mice for 6 days using a subcutaneous osmotic minipump. Each animal's body weight on day 0 was measured and the value was scaled to correspond to 100%. Each animal's body weight on day 6 was normalized using the value on day 0 and expressed as a percentage. Error bars represent S.D.

2.4. Data analysis

We determined three states of vigilance (wakefulness, NREM sleep, and REM sleep) in 10-s epochs based on the EEG, the neck muscle EMG, and EOG activities, as described previously (Katayama et al., 2015). EMG activity of the masseter muscle was first rectified and integrated for every 10-s epoch using SleepSign software (Kissei Comtec Co., Ltd., Nagano, Japan). The mean integrated value of the masseter EMG activity during wakefulness on day 0 was calculated in each animal and its mean value was scaled to correspond to 100%. The corresponding EMG levels in all three vigilance states

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