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Original Article

# New pathways and data on rapid eye movement sleep behaviour disorder in a rat model

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ABSTRACT

*Objective:* An abnormality in auditory evoked responses localised to the inferior colliculus (IC) has been reported in rapid eye movement (REM) sleep behaviour disorder (RBD) patients. The external cortex of the inferior colliculus (ICX) has been demonstrated not only to be involved in auditory processing, but also to participate in the modulation of motor activity.
*Methods:* Rats were surgically implanted with electrodes for electroencephalography (EEG) and electromyography (EMG) recording and guide cannulae aimed at the ICX for drug infusions. Drug infusions were conducted after the animals recovered from surgery. Polysomnographic recordings with video were analysed to detect normal and abnormal sleep states.
*Results:* Baclofen, a gamma-aminobutyric acid B ($GABA_B$) receptor agonist, infused into the ICX increased phasic motor activity in slow-wave sleep (SWS) and REM sleep and tonic muscle activity in REM sleep; it also elicited RBD-like activity during the infusion and post-infusion period. In contrast, saclofen, a $GABA_B$ receptor antagonist, did not produce significant changes in motor activities in sleep. Baclofen infusions in ICX also significantly increased REM sleep during the post-infusion period, while saclofen infusions did not change the amount of any sleep–waking states.
*Conclusions:* This study suggests that $GABA_B$ receptor mechanisms in the ICX may be implicated in the pathology of RBD.



## 1. Introduction

One distinguishing feature of rapid eye movement (REM) sleep is muscle atonia, a loss of skeletal muscle tone. The failure of this motor suppression results in REM sleep without atonia, a phenomenon seen in REM sleep behaviour disorder (RBD) patients. RBD is characterised by vigorous movements accompanying vivid dreams during REM sleep, as well as by an increase in phasic motor activity in slow-wave sleep (SWS) [1,2]. The enactment of dream contents during REM sleep and motor hyperactivity during SWS and REM sleep, may not only result from an impairment of active inhibition systems, but may also result from a dysfunction of facilitatory systems [3–5]. The role of the pontine inhibitory area (PIA) and medial medulla in the control of muscle atonia during REM sleep has been well studied in animals [6–13]. Although lesions of the PIA and medial medulla elicit REM sleep without atonia, motor hyperactivity in SWS was not observed in the lesioned animals [14–16]. Furthermore, lesions of the PIA and medial medulla decrease sleep. This is contrast to RBD patients, who show either unchanged or increased sleep [17–19].

Our previous work using electrical stimulation in the decerebrate animals found that the brainstem inhibitory system extends from the pontomedullary reticular formation to the rostral pons and caudal midbrain, the ventral mesopontine junction (VMPJ) [8]. We found that acute lesions in the VMPJ in these decerebrate animals elicit spontaneous rhythmic activity and tactile stimulation-induced phasic muscle activity [20]. Our recent study showed that chronic lesions in the caudal part of the VMPJ (C-VMPJ) do not change the sleep pattern but cause an increase in motor activity in SWS and REM sleep and RBD-like activity in the cat [21]. These results suggest that dysfunction of C-VMPJ may be implicated in pathological phasic and tonic motor activity in sleep. Anatomically, an extensive reciprocal innervation between C-VMPJ and external cortex of the inferior colliculus (ICX) has been reported [22–25]. The ICX has been known to take part in the auditory and somatosensory responses [26–28]. An abnormal auditory brainstem-evoked potential wave V was reported in RBD patients [29], as well as in spinocerebellar ataxia patients [30]. These patients also have RBD [31]. These clues have led to our hypothesis on the role of ICX in movement disorders during sleep.

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## 2. Methods

### 2.1. Subjects

Seven male Sprague–Dawley rats (Charles River Laboratory, San Diego, CA, USA) weighing 300–600 g were used in this study. All procedures were approved by the Animal Research Committee of the VA Greater Los Angeles Healthcare System, and the study was conducted in compliance with National Institutes of Health (NIH) guidelines.

### 2.2. Implant surgery

Under general isoflurane anaesthesia, animals were implanted with screw electrodes for electroencephalography (EEG) and flexible wires (786000, A-M Systems, Carlsborg, WA, USA) for nuchal and hindlimb electromyography (EMG), and a guide cannula (CMA11, CMA Microdialysis Inc., North Chelmsford, MA, USA) aiming at the ICX unilaterally or bilaterally, 1 mm above the target infusion site (8.8 mm posterior and 6 mm ventral to the Bregma point and 2.7 mm from the midline). The EEG electrodes made contact to the dura via bone screws on the skull at the frontal and parietal areas. The EMG electrodes were implanted in the muscles of dorsal neck and tibialis anterior of hind limbs, and all the leads were routed subcutaneously to the Amphenol strip connector, which was then cemented to the skull. Animals were allowed to recover for 2 weeks before being subjected to the experiments.

### 2.3. Polygraphic and video recordings

Animals were individually housed in soundproof chambers in a 12–12 light–dark cycle (on at 7 AM and off at 7 PM), and were allowed to adapt to the Raturn rodent bowl (MD-1404, BioAnalytical System, West Lafayette, IN, USA). This system rotates in the opposite direction of the animal's circular locomotion to prevent cable twisting. The electrophysiological signals were amplified (Model 15LT, Grass Technologies, West Warwick, RI, USA), digitised (Micro 1401 mkII, Cambridge Electronics Design, Cambridge, UK) and recorded in Spike2 (Cambridge Electronics Design, Cambridge, UK). Infrared cameras capable of recording animal behaviours in total darkness were used for video recordings. The video images were captured digitally through a four-channel surveillance video recorder card (Q-See QSPDVR04; RapidOS, New Taipei City, Taiwan) in the same computer for polygraphic recordings. The video was recorded continuously with time stamps at a frame rate between 7 and 30 fps. The time stamps allow the polygraphic and video recordings to be matched.

### 2.4. Experimental protocol

The drugs were delivered to the target site by reverse microdialysis with an infusion pump (BioAnalytical System, West Lafayette, IN, USA). In the evening before the day of the experiment, a microdialysis probe (1 mm membrane, CMA11, CMA Microdialysis AB, Kista, Sweden) was inserted through a guide cannula into the ICX. On the day of the experiment, artificial cerebrospinal fluid (aCSF; Harvard Apparatus, Holliston, MA, USA) was infused at 2 μl $min^{-1}$ continuously from ZT2 to ZT7. The animals were undisturbed during simultaneous polygraphic and video recordings. A $GABA_B$ receptor agonist, baclofen (200 and 500 μM), or a $GABA_B$ receptor antagonist, saclofen (200 and 500 μM), both drugs dissolved in aCSF, were used for the 1-h drug infusion between AT4 and ZT5. Polygraphic and video recordings were continuously collected for another 24 h post-infusion. Drug infusions were spaced at least 48 h apart when multiple infusions were conducted in the same animal.

### 2.5. Histology

The site of drug infusion in each animal was verified by locating the tract of the microdialysis probe in the brain with a standard histology technique. In brief, animals were deeply anaesthetised by sodium pentobarbital (100 mg $kg^{-1}$, i.p.) and perfused transcardially with saline followed by 4% paraformaldehyde (PFA). The brains were retrieved and submerged in 4% PFA for 2-h, and then transferred to 30% sucrose solution in phosphate saline buffer. The brains were then cryosectioned into 50-μm slices and mounted on gelatin-coated slides. The brain sections were stained in neutral red (0.5%), dehydrated with alcohol, cleared with xylene and coverslipped.

### 2.6. Data analysis

Sleep and motor activity in the neck and leg were visually scored offline with a tailored script in Spike2 (Sleepscore v2.02 by Dr. Geoff Horseman, Cambridge Electronics Design, Cambridge, UK). Sleep–wake states were scored as waking, SWS or REM sleep. Motor events were scored as periodic movements (PMs) and isolated movements (IMs) in SWS and as twitches in REM sleep. PMs in SWS were scored based on the criteria adapted from those for scoring periodic leg movements (PLMs) in humans [32]. In brief, phasic motor activities in the neck and leg EMG channels satisfying the following criteria were counted as PM: (1) four or more repetitive movements that appear at a 5–90 s interval from each other, (2) the duration of heightened phasic EMG activity between 0.5 and 5 s and (3) the amplitude of the phasic motor event at least twice the baseline activity. The baseline EMG activity changed throughout the 24-h period and in different sleep–wake states. Therefore, the baseline EMG activity was defined as the average activity of 5 s before and 5 s after the phasic motor event. Phasic motor activities that satisfied the 2nd and 3rd criteria, but not the first, were counted as IM. The PM index (PMI) and IM index (IMI) were the number of periodic and isolated motor activities per h of total SWS time. Twitches in REM sleep were different from the phasic motor activity in SWS, with a shorter duration, from 0.2 to 2 s. The amplitude was at least three times the baseline level. If the twitches occurred in a cluster at 0.5 s intervals, they were counted as a single twitch. If the elevation in EMG lasted longer than 2 s, it was counted as an episode of REM without atonia. Muscle twitch index for REM sleep (TWI-REM) was the number of muscle twitches per hour of REM sleep. Percent of REM sleep without atonia (RWA) was calculated as the total duration of REM sleep without atonia divided by the total REM sleep time. An RBD-like episode was noted when variable motor activity recorded in the video was accompanied by either EEG or EMG features of REM sleep. A spreadsheet of visual scores of individual motor events was generated by the Spike2 script, which allowed us to calculate all motor variables and to tally them in hourly sums. The duration of each sleep–wake state and quantified motor activity in SWS and REM sleep were pooled in 2-h intervals in baseline and post-infusion periods, and 1-h intervals for the drug infusion period. Analysis of variance (ANOVA) and *post hoc t*-tests with Bonferroni correction were performed among different time periods for each dose and drug conditions in IBM SPSS Statistics v.20 (IBM, Armonk, NY, USA).

## 3. Results

### 3.1. Infusion sites

A total of seven sites received baclofen and saclofen infusions. Among them, five were in the ICX, one was in the area ventral to

the ICX and the last one was in the pedunculopontine tegmental nucleus (PPT; Fig. 1).

### 3.2. Effect of baclofen and saclofen ICX infusion on sleep

The sleep pattern was not significantly changed by the $GABA_B$ receptor agonist, baclofen, or antagonist, saclofen, infused into the ICX during the infusion period (Table 1). However, there was a significant increase in REM sleep in the first 2-h post-infusion period, compared to pre-infusion levels, by baclofen at both concentrations (200 μM, $n = 5$, $F_{(3,34)} = 3.66$, *post hoc* $p < 0.05$; 500 μM, $n = 4$, $F_{(3,27)} = 3.60$, *post hoc* $p < 0.05$). No significant changes were found in any sleep states in the post-infusion periods with saclofen infusion.

### 3.3. Effect of baclofen and saclofen ICX infusion on motor activity in wake and SWS

No change was observed in motor activity in waking with either baclofen or saclofen infused into the ICX. The baseline levels of the PLM index in SWS varied between animals, ranging from 0 to 13.6. However, a high dose (500 μM) of saclofen infused into the ICX completely suppressed PLM (Fig. 2). On the other hand, neither 200 nor 500 μM of saclofen significantly altered PMs in the neck (ANOVA; 200 μM: $n = 4$, $F_{(3,27)} = 0.85$, $p = 0.48$; 500 μM: $n = 5$, $F_{(3,34)} = 0.91$, $p = 0.45$) or hindlimb (200 μM: $n = 4$, $F_{(3,27)} = 0.84$, $p = 0.49$; 500 μM: $n = 5$, $F_{(3,34)} = 0.82$, $p = 0.49$) in SWS (Fig. 3). In contrast, baclofen infusion into the ICX increased PMs of the leg in SWS in a dose-dependent fashion, but did not affect motor activity in the neck. However, this increase barely missed the $p = 0.05$ level in the *post hoc* test (500 μM, one-way ANOVA, $n = 4$, $F_{(3,27)} = 3.02$, $p = 0.049$; *post hoc* $p = 0.059$ for a difference between pre-infusion and late post-infusion levels of leg PMI; 200 μM, $n = 5$, $F_{(3,34)} = 1.10$, $p = 0.36$; Fig. 3). However, the comparison among different drugs and doses reveals a highly significant difference among these four conditions at all time points for the infusion, early post-infusion and late post-infusion periods ($F_{(3,14)} = 7.18$, $F_{(3,35)} = 5.78$, $F_{(3,35)} = 5.15$, respectively, all $p < 0.01$ in one-way ANOVA tests) while there was no difference among drug-dose conditions for the pre-infusion periods. A bell shape of distribution of inter-leg movement intervals during PLM, with the majority of intervals between 2 and 20 s (Fig. 4), was found with ICX-baclofen infusion-induced PLM. Isolated movements, which may normally occur as postural changes in SWS, were not changed by either baclofen or saclofen infusions in the ICX (Fig. 5).

### 3.4. Effect of baclofen and saclofen ICX infusion on motor activity in REM sleep

Phasic motor activity, which was the total counts of periodic and non-periodic twitches in REM sleep, was significantly increased after a low dose (200 μM) of baclofen infused into the ICX (Figs. 6 and 7; one-way ANOVA, $n = 5$, $F_{(3,34)} = 6.18$, *post hoc* $p < 0.01$), but not a higher dose (500 μM, $n = 4$, $F_{(3,27)} = 2.03$, *post hoc* $p = 0.14$) in the leg muscle. Baclofen infused into the ICX also produced RWA (Fig. 8). The percentage of time in REM sleep showing RWA in the neck muscle was significantly increased by baclofen infused into the ICX at higher concentrations (500 μM: $n = 4$, $F_{(3,27)} = 4.58$, $p < 0.05$, Fig. 9) but not at lower concentrations (200 μM: $n = 5$, $F_{(3,34)} = 1.06$, $p = 0.38$, Fig. 9). The *post hoc* tests showed that the increase in % RWA was significant during the infusion period and the first 2-h post-infusion period with high dose infusion (Bonferroni correction, $p < 0.05$; Fig. 9). Percent of RWA in the hindlimb was not increased by baclofen infusion (200 μM: $n = 5$, $F_{(3,34)} = 0.89$, $p = 0.46$; 500 μM: $n = 4$, $F_{(3,27)} = 0.45$, $p = 0.72$).

RBD-like episodes were not found in the pre-infusion periods. However, RBD-like activities, which appeared in the video as body jerking and head and tail movements during REM sleep (Supple-

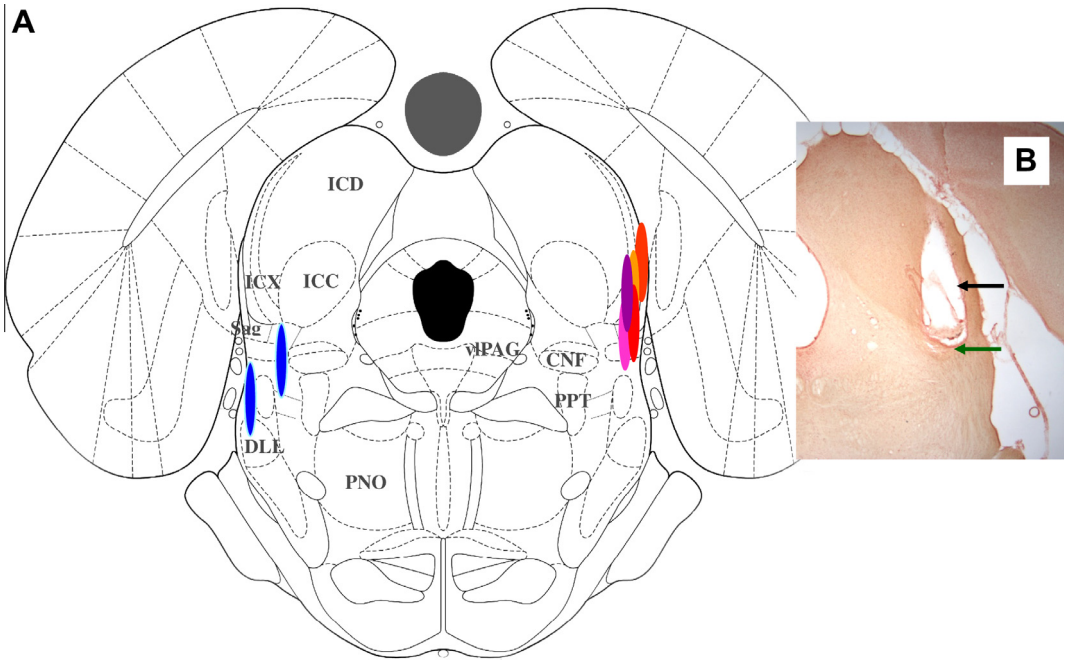


**Fig. 1.** Reconstruction of effective and ineffective sites for baclofen induction of motor hyperactivity during sleep (A) and photomicrograph showing the infusion site (B). A: The effective and ineffective sites are shown at the right and left, respectively. The effective sites were exclusively located in the ICX. Baclofen infused into the pedunculopontine nucleus (PPT, blue, right side) or area ventral to the ICX failed to elicit similar motor hyperactivity in sleep. B. The black and green arrows indicate the tract of the guide cannula and the site of infusion. CNF: cuneiform nucleus, DLL: dorsal nucleus of the lateral lemniscus, ICC: central nucleus of the inferior colliculus, ICD: dorsal nucleus of the inferior colliculus, ICX: external cortex of the inferior colliculus, PNO: nucleus pontis oralis, Sag: sagulum, vlPAG: ventrolateral periaqueductal grey. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 1**
Effect of baclofen and saclofen infused into the ICX on sleep–wake states.

| | | Pre (%) | Inf (%) | Post1 (%) | Post2 (%) |
|---|---|---|---|---|---|
| Baclofen 200 μM | Wake | 30.3 | 31.2 | 20.1 | 33.8 |
| | SWS | 60.2 | 59.3 | 62.9 | 54.7 |
| | REM | 9.5 | 9.5 | 17.0* | 11.5 |
| Baclofen 500 μM | Wake | 31.2 | 13.8 | 17.7 | 26.1 |
| | SWS | 61.2 | 73.3 | 66.9 | 59.3 |
| | REM | 7.6 | 12.9 | 15.4* | 14.6 |
| Saclofen 200 μM | Wake | 40.6 | 25.1 | 28.1 | 30.6 |
| | SWS | 52.1 | 62.2 | 59.4 | 57.8 |
| | REM | 7.3 | 12.7 | 12.5 | 11.6 |
| Saclofen 500 μM | Wake | 38.1 | 28.1 | 25.7 | 31.8 |
| | SWS | 54.0 | 57.5 | 63.0 | 56.1 |
| | REM | 7.9 | 14.4 | 11.3 | 12.0 |

Pre: aCSF pre-drug infusion; Inf: drug infusion; Post1: the first 2-h post-drug infusion; Post2: the second 2-h post-drug infusion.
* Significant different from Pre-infusion levels in *post hoc* tests, $p < 0.05$.

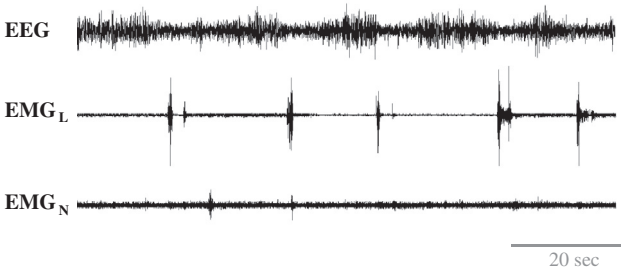


**Fig. 2.** Periodic leg movements (PLM) in sleep induced by baclofen (200 μM) infused into the ICX. Leg movements were accompanied by EEG arousal. Two isolated twitches can be seen in the neck EMG. $EMG_L$ and $EMG_N$: electromyographic leg and neck muscles, respectively.

mental videos), were observed during baclofen infusion and post-infusion periods. A dose-dependent pattern (Fig. 10) of baclofen-ICX infusion-induced RBD-like activity was found. At a high concentration of baclofen, RBD-like activities were observed in all of the infusion periods ($n = 4$) and in most of the post-infusion periods. The differences in inducing RBD-like activity was highly significant (ANOVA, $F_{(3,27)} = 10.51$, $p < 0.001$). The *post hoc* tests showed that infusion ($p < 0.001$), early post-infusion ($p < 0.001$) and late infusion periods ($p < 0.01$), all have significantly higher levels of RBD-activities compared to pre-infusion levels. The lower concentration of baclofen infusion also induced RBD-like activity; however, the frequency was lower than the high dose infusion (among five infusions of baclofen 200 μM, the numbers of infusions showing at least one RBD-like episode during infusion, early

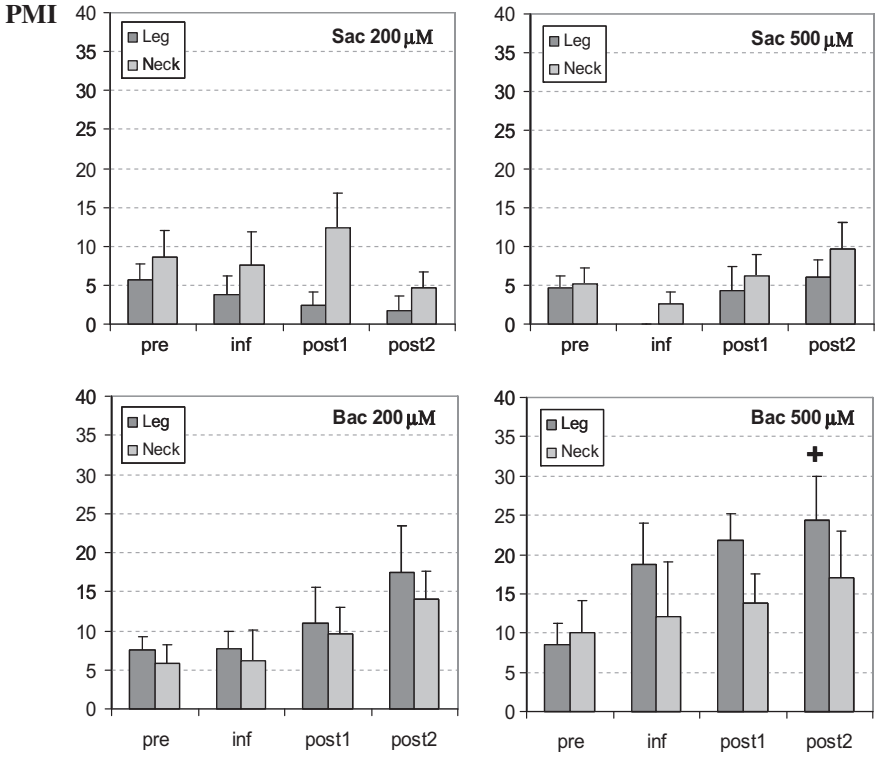


**Fig. 3.** Changes in periodic movement index (PMI) in the neck and leg in SWS after baclofen and saclofen infusions. Low doses (200 μM) of baclofen infused into the ICX failed to change motor activity in SWS, whereas high doses (500 μM) of baclofen infusion increased periodic movements in the leg ($p < 0.05$, ANOVA; *post hoc* $p = 0.059$ for post2 vs. pre) but not the neck during the post-infusion period. Saclofen infused into the ICX did not change periodic movements in either neck or leg muscles. The *y*-axis shows the index of periodic movements. Error bars = S.E.M. $^{+}p < 0.06$ compared to pre-drug infusion. Pre: aCSF pre-drug infusion, inf: drug-infusion, post1: the first 2-h post-drug infusion, post2: the second 2-h post-drug infusion.

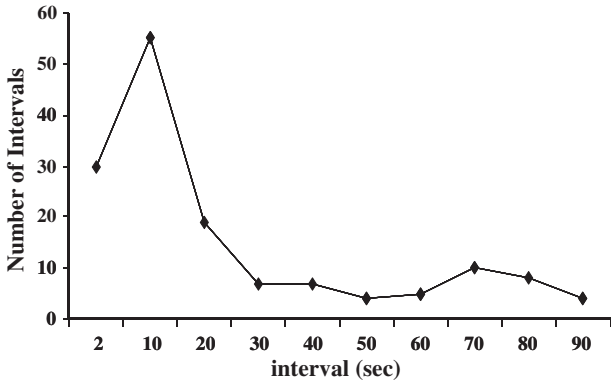


**Fig. 4.** Distribution of intervals between leg movement during PLM. The *y*-axis represents the total number of intervals collected from all animals that received baclofen infusion in the ICX. The majority of intervals fell between 2 and 20 s.

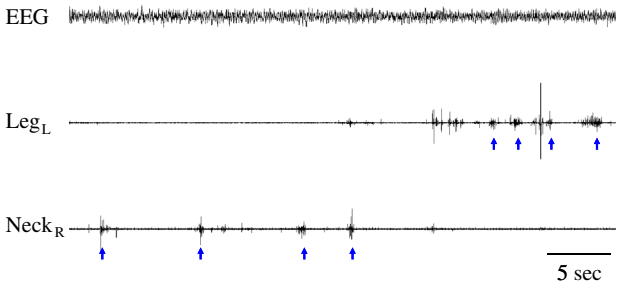


**Fig. 6.** Periodic movements (blue arrows) in REM sleep induced by baclofen infusion into the ICX. Neck muscle activities were temporally dissociated from leg activities. The dose of baclofen used in this experiment was 200 μM. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

post-infusion and late post-infusion were 1, 3 and 1, respectively). One RBD-like episode was seen in the second 2-h post-infusion period with the high concentration of saclofen infusion. However, the frequency of RBD-like activity induced by a high dose of saclofen infusion during the post-infusion period was not significantly different from zero ($F_{(3,19)} = 0.75$ among time periods after saclofen 500 μM, *post hoc* test $p = 1.0$). Saclofen infusions in ICX even at high concentration did not significantly change phasic (TWI-REM, leg: $F_{(3,33)} = 0.98$, $p = 0.42$; neck: $F_{(3,33)} = 0.24$, $p = 0.87$) or tonic (% RWA, leg: $F_{(3,33)} = 0.80$, $p = 0.50$; neck: $F_{(3,33)} = 0.50$, $p = 0.68$) motor activities in REM sleep, though both showed a trend of increase in the post-infusion periods, which echoed the rare appearance of RBD-like activity after infusion of saclofen 500 μM.

Motor hyperactivity in SWS and REM sleep or RBD-like activity was not found in ICX-aCSF infusions (i.e., pre-infusion period, $n = 18$). One located in the area ventral to the ICX and the other located in the PPT also received baclofen and saclofen infusions. Changes in motor activity in REM sleep and RBD-like activity were not found in either baclofen or saclofen infusion into these two sites.

## 4. Discussion

We demonstrated in the present study that the $GABA_B$ receptor agonist, baclofen, infused into the ICX elicits motor hyperactivity in SWS and REM sleep. Baclofen infusion also induced RBD-like activities, movements of head, leg and tail and body jerks. In contrast, the $GABA_B$ receptor antagonist, saclofen, infused into the ICX did not significantly alter motor activity in either SWS or REM sleep. Baclofen infused into the ICX increased REM sleep in the post-infusion period, while saclofen infused into the same site did not alter the sleep pattern. The increase in motor activity in SWS and REM

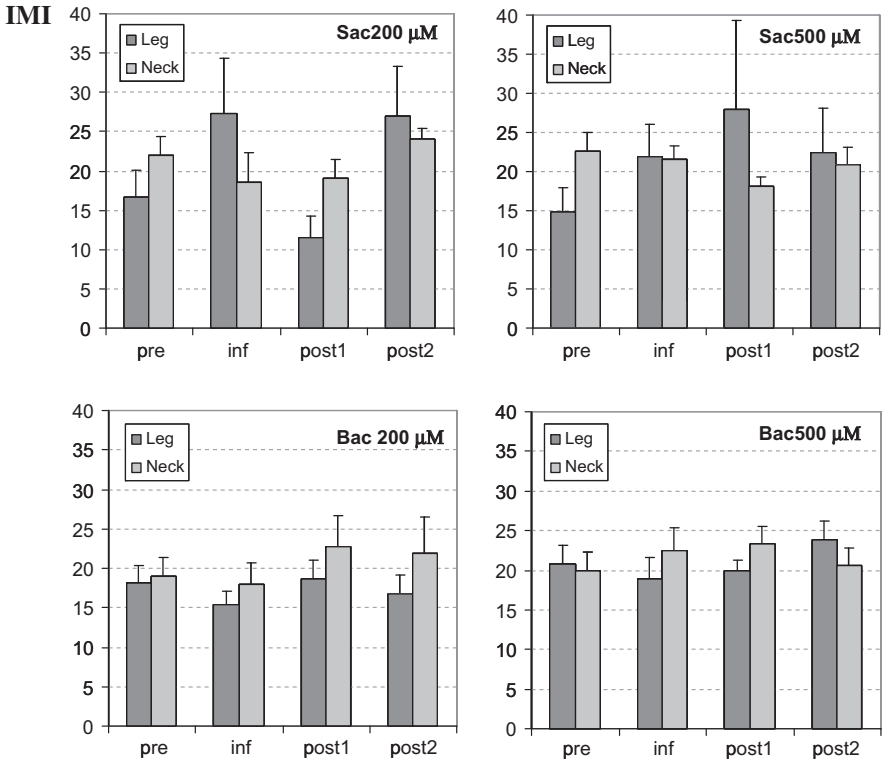


**Fig. 5.** The isolated movement index (IMI) was not affected by either baclofen or saclofen infusions. Both baclofen and saclofen at either high or low doses infused into the ICX failed to change isolated movements in the neck and leg muscles. The *y*-axis shows isolated movements index.

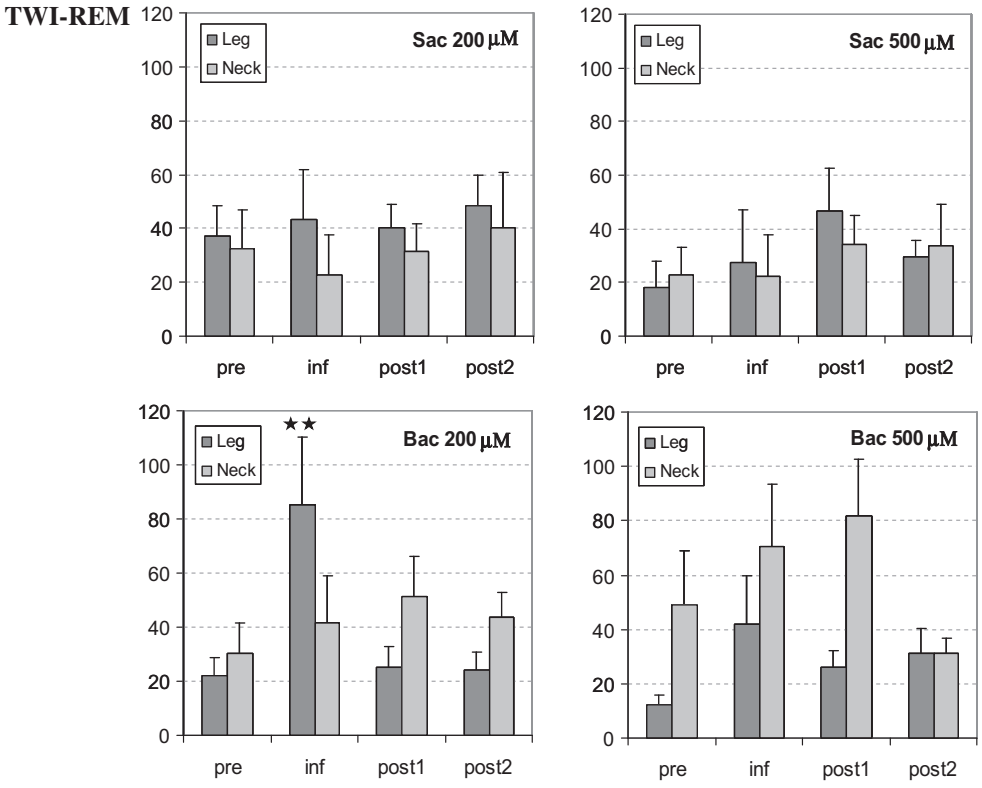


**Fig. 7.** Effect of baclofen infused into the ICX on muscle twitches during REM. The index of muscle twitches (TWI-REM) including periodic and non-periodic movements in REM sleep was calculated as the number of twitches per hour of REM sleep. Error bars = S.E.M. TWI-REM was significantly increased in the leg EMG during infusion periods of low concentrations of baclofen. $^{**}p < 0.01$ compared to pre-infusion.

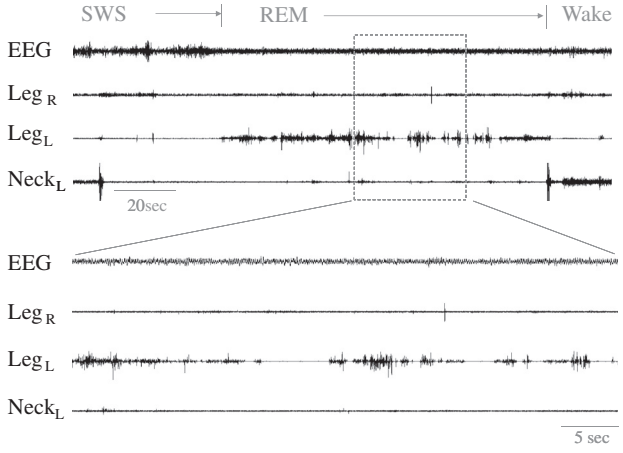


**Fig. 8.** REM sleep without atonia induced by baclofen infusion into the ICX. Baclofen (500 μM) infused into the right side of ICX elicited REM sleep without atonia in the leg contralateral to the infusion site. The neck and ipsilateral side of the leg muscles were atonic. Muscle activity was low in SWS. Tonic muscle activity appeared at the onset of REM sleep, and then had superimposed phasic activities. The high speed polygraphic recording showing EEG theta waves shown in the lower panel was taken from the rectangular area shown in the upper panel.

sleep with an increase in REM sleep induced by ICX baclofen infusion resembles features of human RBD [33].

Several factors should be considered in interpreting the results of this study. First, we observed that much of the drug effects started in the second half of the infusion period and continued into the post-infusion period. The method of drug delivery, reverse microdialysis, used in this study may explain the delay and prolonged effect of baclofen infusion on motor activity. In contrast to pressure injection, in which the total amount of drug is administered to the tissue in a bolus, in reverse dialysis the drug is slowly diffused through the dialysis membrane to the surrounding tissue. Thus, it may require a certain period of time for the concentration of the drug to reach to the threshold level for its motor effects. Second, the drug delivered to the tissue, using the microdialysis infusion technique, may diffuse to the structures surrounding the ICX and cause behaviour changes. Using the same infusion technique, Hoistad et al. [34] demonstrated that the radioactive compounds diffuse to the surrounding tissue less than 1 mm from the membrane after a 5-h infusion into the striatum. We showed in the present study that the effective and ineffective areas, identified by microscopic examination, in inducing RBD-like activity are located 0.5 mm apart (Fig. 1). Thus, it is unlikely that changes in motor activity in sleep induced by baclofen infusion result from a disinhibition of the brain structures surrounding ICX. Third, unlike electrical stimulation, which activates both passing fibres and cell bodies, chemical infusion activates and/or inactivates neurons containing the corresponding receptor. Thus, the effect of baclofen infused into ICX on motor activity in sleep results from activation of ICX neuronal activity but not activation of the passing fibres. Fourth, most of the animals used in this study received multiple infusions with different drugs and doses at the same infusion site. The infusions were delivered into the same site 48 h apart to minimise drug interactions. Fifth, the mechanical lesions and/or inflammation, due to probe insertion at the target site, may affect the results of infusion. Therefore, we used data collected from the 2-h aCSF infusion (ZT2–ZT4), that is, pre-drug infusion, on the day of the experiment as the baseline. Finally, circadian variations play

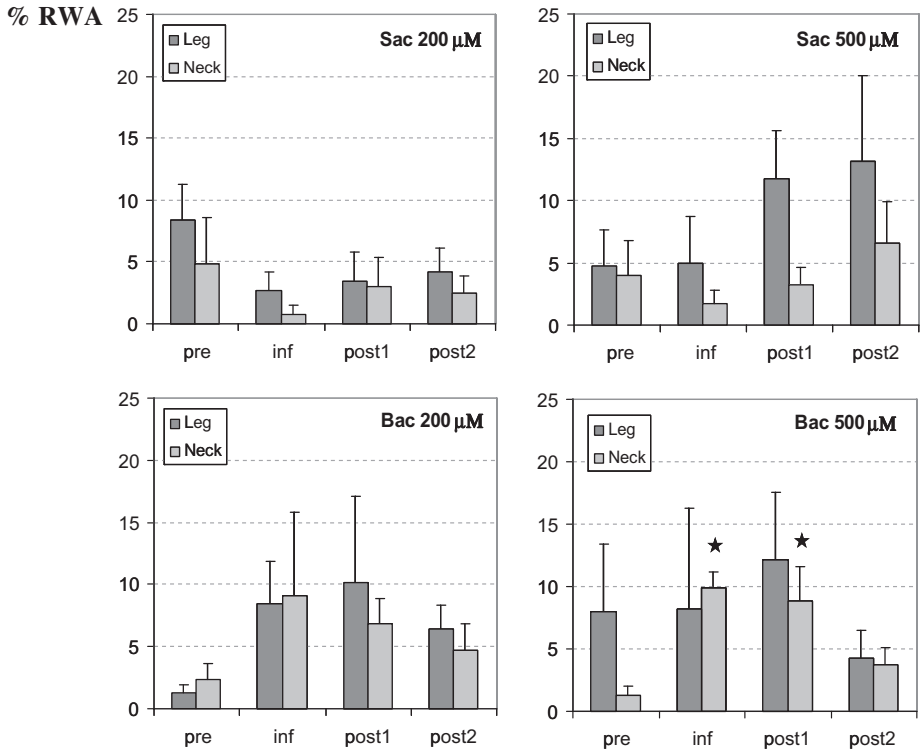


**Fig. 9.** Effect of baclofen infused into the ICX on tonic muscle activity in REM sleep. Time in REM sleep without atonia, as percentage of REM sleep time, was increased by high concentration baclofen infusion in the neck during the infusion period and during the first post-infusion period. Although a low concentration (200 μM) of baclofen and saclofen infused into the ICX tended to increase and decrease leg muscle tone respectively, the change of muscle tone induced by both drugs were not different from the baseline levels. The *y*-axis represents percent of REM sleep without atonia in REM sleep. Error bars = S.E.M.

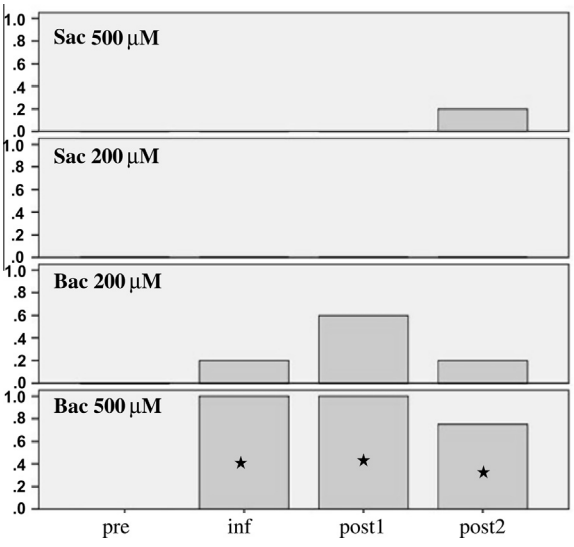


**Fig. 10.** Probability of at least one occurrence of RBD-like activity during the baclofen- and saclofen-ICX pre-infusion, infusion, and post-infusion periods. RBD-like activity occurred during the infusion of low and high doses of baclofen. The probability of the occurrence of RBD-like activity was also high during the post-infusion periods of high concentration of baclofen. $^{*}p < 0.05$ compared to pre-infusion.

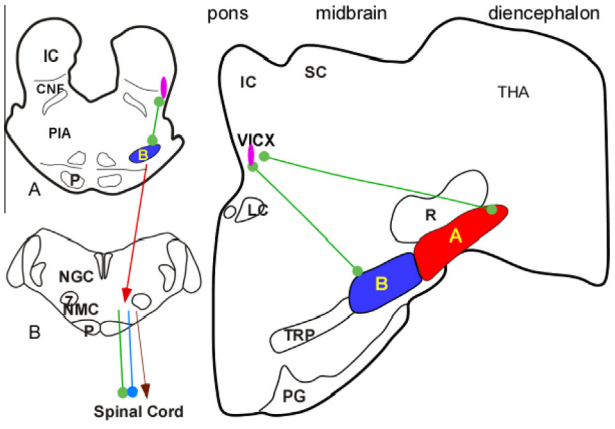


**Fig. 11.** Hypothetical neural circuitry involve in the generation of motor hyperactivity. The left and right panels represent coronal and sagittal sections, respectively. Green, blue, red, and brown lines represent GABAergic, glycinergic, glutamatergic, and monoaminergic projections. The pink areas shown in the coronal and sagittal sections represent the baclofen infusion site. Reciprocal GABAergic innervations between the ICX and rostrolateral substantia nigra, as well as the ICX and the caudal ventral mesopontine junction (VMPJ) have been reported (right panel). Lesions of the rostral VMPJ (red area, A) including substantia nigra pars reticulata elicit PLM in sleep, whereas, lesions of the caudal VMPJ (blue area in the coronal and sagittal sections, B) induce PLM and RBD-like activity. Neurodegeneration and/or inactivation of the rostral and/or caudal VMPJ result in hyperactivity of the ICX. Hyperactivity of the ICX causes hypoactivity of the caudal VMPJ via disinhibition of GABAergic mechanism (left panel), which subsequently decreases activity of the nucleus magnocellularis via disfacilitation of glutamatergic mechanism, and decreases glycine and GABA release and increases monoamine release to the motoneuron nuclei. Thus, hyperactivity of the ICX induced by baclofen infusion generates abnormal motor activity in sleep and abnormal auditory evoked potential, as seen in RBD patients. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

a major role in the control of sleep and motor activity in rodents. A circadian rhythm of PLM activity with movements occurring predominantly during the first half of the night has been reported in patients diagnosed with PLM disorder and in the elderly [35,36]. Although there are no prior published data on the circadian rhythm of PLM in the rat, our unpublished observations on PLM

in the iron-deficient rat suggest that more PLMs occur in the first half of the light phase, and the finding of PLM increasing during baclofen post-infusion periods in the second half of the light phase compared to control infusions, cannot be attributed to simple circadian variation in the PLM.

Our results lead us to suggest that abnormal activity of the ICX can cause PMs in sleep. The frequency of PLMs was high in ICX-baclofen infusion-induced animal. Unlike the RBD patients, who show a bimodal distribution of inter-leg movement intervals [37], a bell-shaped distribution of intervals between leg movements was found in the present study. On the other hand, isolated movements, which may result from postural changes in normal sleep, were not changed by either baclofen or saclofen infusion into ICX. Although we found that a delayed RBD-like activity was seen in one high-dose saclofen infusion, this activity may have resulted from an up-regulation of $GABA_B$ receptors induced by antagonist infusion [38].

The PPT, located about 1 mm ventral and medial to the ICX, is known to contain several classes of cholinergic REM-On neurons [39,40]. $GABA_B$ mechanisms in the PPT have been reported to be involved in the control of REM sleep in the rat [41,42]. However, changes in motor activity in sleep were not reported after injections of $GABA_B$ receptor-related substances in the PPT [41,42], and baclofen microinjection in the PPT suppressed REM sleep [42], which is the opposite of the REM-promoting effect observed in this study with the same drug in ICX. We also found in the present study that drug infusions into the area, 2–3 mm ventral to the ICX, failed to elicit motor hyperactivity. Thus, the effects of promoting REM sleep or motor activity in sleep during the post-infusion period were not due to drug diffusion into the PPT, and our findings of baclofen infusion-induced motor hyperactivity were likely specific to ICX. The ICX has been hypothesised to serve as a subcortical integration centre for multisensory inputs and for acousto-motor behaviours [27]. In contrast to the central and dorsal nuclei of the IC, which process acoustic signals and receive inputs primarily from the auditory structures, the ICX receives inputs not only from acoustic structures [43] but also from sensory–motor systems including the globus pallidus, substantia nigra pars lateralis (SNL), VMPJ, dorsal column nuclei, spinal trigeminal nucleus and spinal dorsal horn [44–47]. Neurons from the ICX project to the extrapyramidal system that includes the superior colliculus, pontine nucleus, posterior thalamus, cerebellum and SNL [48–54] and VMPJ [24]. Anatomical connections between the ICX and peri-locus coeruleus alpha or nucleus subcoeruleus, areas involved in the generation of RBD [55,56] have not been reported. Electrical stimulation of the ICX increases muscle tone and tonic and stretch reflexes [57], as well as eliciting vocalisation [58]. It has been reported that RBD patients show increased muscle tone in REM sleep, increased phasic motor activity in SWS and REM sleep and vocalisation during REM sleep [33,59,60].

The substantia nigra and C-VMPJ are both implicated in the generation of RBD [21,61–63]. Progressive dopaminergic degeneration and abnormal axonal integrity in the substantia nigra [62,63] and an increase in cerebral blood flow in the C-VMPJ [61] have been reported in idiopathic RBD patients. Both substantia nigra and C-VMPJ send GABAergic projections to the ICX [48,64,65]. The ICX contains GABAergic and glycinergic neurons [66]. $GABA_B$ receptors are densely distributed in the ICX [67]. It has been demonstrated that GABAergic inhibition in IC neurons is reduced by application of the $GABA_B$ receptor agonist, baclofen, through a pre-synaptic mechanism [68]. The systemic injection of baclofen has been reported to decrease the amplitude and prolong the latency of auditory stimulation-induced evoked potential recorded from the ICX [69]. A prolongation of latency of the auditory evoked potential wave V has been reported in idiopathic RBD patients [29].

A recent study showed that $GABA_A$, $GABA_B$ and glycine receptor agonisms at the level of motor nucleus are required in the generation of REM sleep paralysis [70]. In contrast, we found that $GABA_B$ receptor agonism in the ICX is sufficient for changes in motor activity in SWS and REM sleep. We have hypothesised that activation of the inhibitory system and disfacilitation of the monoamine system in the motor nucleus are required to produce muscle atonia [4]. However, $GABA_B$ agonism in the ICX inducing RBD-like activity may be mediated through the inactivation of the C-VMPJ and the ventromedial medulla of the nucleus magnocellularis. As shown in our model in Fig. 11, reciprocal GABAergic innervations between ICX and C-VMPJ have been reported [24,65]. Disinhibition of GABAergic and glycinergic neuronal activity induced by baclofen infusion in the ICX suppresses C-VMPJ neuronal activity. The decrease in C-VMPJ activity results in a decrease in nucleus magnocellularis activity via the excitatory glutamatergic projections [71]. Activation of the nucleus magnocellularis increases GABA and glycine release and decreases monoamine release into motor nuclei and simultaneously produces muscle atonia [4]. We hypothesise that activation of GABAergic neurons in the rostrolateral substantia nigra including the lateral substantia nigra pars reticulata (SNR) and SNL and C-VMPJ is required to inhibit ICX activity and suppress motor hyperactivity in normal sleep (Fig. 11). We conclude that inactivation and/or neuronal degeneration in the rostrolateral SNR/SNL and/or in the C-VMPJ facilitate ICX neuronal activity, which in turn prolongs auditory evoked potentials and elicits motor hyperactivity via inactivation of the C-VMPJ and ventromedial medulla in SWS and REM sleep.

## Conflict of interest

The ICMJE Uniform Disclosure Form for Potential Conflicts of Interest associated with this article can be viewed by clicking on the following link: http://dx.doi.org/10.1016/j.sleep.2012.08.008.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.sleep.2012.08.008.

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