SLEEPJ, 2021, 1–8

doi: 10.1093/sleep/zsaa151
Advance Access Publication Date: 18 August 2020
Original Article

ORIGINAL ARTICLE

Substantia nigra pars reticulata-mediated sleep and motor activity regulation

Yuan-Yang Lai[1,2,3,*], Tohru Kodama[1,4], Kung-Chiao Hsieh[2,5], Darian Nguyen[1] and Jerome M. Siegel[1,2]

[1]Department of Psychiatry & Biobehavioral Sciences, David Geffen School of Medicine, University of California, Los Angeles, CA, [2]VA Greater Los Angeles HealthCare System, North Hills, CA, [3]Greater Los Angeles Veterans Research and Education Foundation, Los Angeles, CA, [4]Physiological Psychology Lab, Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan and [5]Department of Medicine, David Geffen School of Medicine, University of California, Los Angeles, CA

*Corresponding author. Yuan-Yang Lai, VAGLAHS Sepulveda, 16111 Plummer Street, North Hills, CA 91343. Email: yylai@ucla.edu.

Abstract

Study Objectives: The substantia nigra pars reticulata (SNR) is a major output nucleus of the basal ganglia. Animal studies have shown that lesions of the SNR cause hyposomnia and motor hyperactivity, indicating that the SNR may play a role in the control of sleep and motor activity.

Methods: Eight 8- to 10-week-old adult male Sprague-Dawley rats were used. After 3 days of baseline polysomnographic recording, dialysates were collected from the lateral SNR across natural sleep–wake states. Muscimol and bicuculline were microinfused into the lateral SNR.

Results: We found that GABA release in the lateral SNR is negatively correlated with slow wave sleep (SWS; $R = -0.266$, $p < 0.01$, $n = 240$) and positively correlated with waking ($R = 0.265$, $p < 0.01$, $n = 240$) in rats. Microinfusion of muscimol into the lateral SNR decreased sleep time and sleep quality, as well as eliciting motor hyperactivity in wake and increased periodic leg movement in SWS, while bicuculline infused into the lateral SNR increased sleep and decreased motor activity in SWS in rats. Muscimol infusion skewed the distribution of inter-movement intervals, with most between 10 and 20 s, while a flat distribution of intervals between 10 and 90 s was seen in baseline conditions.

Conclusions: Activation of the lateral SNR is important for inducing sleep and inhibiting motor activity prior to and during sleep, and thus to the maintenance of sleep. Abnormal function of the lateral SNR may cause hyposomnia and motor hyperactivity in quiet wake and in sleep.

Statement of Significance

The basal ganglia are known to participate in the control of motor activity. However, the role of the substantia nigra pars reticulata (SNR) in the modulation of motor activity has received little attention. Here, we show that a GABAergic SNR mechanism not only participates in the regulation of motor activity, but may also be involved in sleep disruption. Inactivation of the SNR by the infusion of muscimol into the nucleus elicited motor hyperactivity in wake and in sleep and generated insomnia in the rat, symptoms resembling those seen in human restless legs syndrome patients. We conclude that the SNR plays a key role in inducing sleep and maintaining sleep via its inhibiting effect on the sensory-motor system and regulation of the activity of thalamic sleep nuclei.

Key words: restless legs syndrome; microdialysis; muscimol; bicuculline; infusion

Submitted: 14 April, 2020; **Revised:** 1 August, 2020

Introduction

It has been well established that dysfunction of the basal ganglia causes movement disorders, such as are seen in patients with Parkinson's disease, Huntington's disease, rapid eye movement (REM) sleep behavior disorder, and restless legs syndrome (RLS). The role of the striatum, globus pallidus, and dopaminergic system of the substantia nigra (SN) pars compacta (SNC) in the control of motor activity has been well documented. However, the neurophysiological function of SN pars reticulata (SNR) in the modulation of motor systems, as well as its pathological role in movement disorders has received less attention, despite the fact that SNR is one of the major output nuclei of the basal ganglia. Indeed, the basal ganglia's effects on motor activity are exerted via inhibition from the external globus pallidus and/or SNR of their target nuclei [1–4].

Anatomical and functional compartmentalization has been reported in the SNR, with the medial portion regulating emotion and the lateral portion regulating the sensory–motor system [5–7]. Our previous study found that neurotoxic N-methyl-D-aspartate lesions of the lateral SN, but not of the medial SN, induce hyposomnia and increase periodic leg movements (PLM) in sleep, which last over a 4-month recording, in the cat [8]. Insomnia and motor hyperactivity seen in the lateral SN-lesioned animals, symptoms resembling RLS, led us to hypothesize that inactivation and/or neuronal degeneration of the lateral SN may be involved in the generation of RLS. Our previous study also found that the number of midbrain dopamine neurons lost does not correlate with sleep duration changes [8]. Therefore, this study was designed to address the role of GABAergic system in the lateral SNR in the modulation of sleep and motor activity.

Methods

All procedures were approved by the Institutional Animal Care and Use Committee of the VA Greater Los Angeles Healthcare System.

Surgery for in vivo microdialysis and microinfusion experiments

Eight Sprague-Dawley male rats (Charles River) adult (8–10 weeks old), weighing 250–300 g, were used for the study. Surgery was performed to implant electrodes for electroencephalograph (EEG) and electromyograph (EMG) recordings, as described it in our previous study [9]. Briefly, under isoflurane (1.5%) anesthesia, three jewelers' screws were implanted over the cortex for cortical EEG recording. Three flexible multi-stranded stainless steel wires (7935, A-M Systems, Inc., Carlsborg, WA) were inserted into the nuchal and hindlimb musculature bilaterally for EMG recording. In the hindlimb, one wire was inserted into the gastrocnemius and two into the anterior tibialis muscle. Wires from all electrodes were soldered to a 15-pin Amphenol strip connector and encased in an acrylic head plug. Guide cannulae (CMA/11, CMA Microdialysis Inc.) were implanted unilaterally and terminated 1 mm above the target sites in the lateral SNR. The guide cannulae were sealed with stylets.

Sleep and motor activity recording and in vivo microdialysis experiments

Sleep and motor activity recordings were performed at least 7 days after surgery, by which time animals had recovered. Bipolar recordings were used to detect EEG and EMG. Animals were individually housed in a sound-attenuated chamber with a 12:12 light–dark cycle, and were allowed to adapt to the Raturn rodent bowls (MD-1404, BioAnalytical System, West Lafayette, IN). The Raturn rodent recording system rotates in the opposite direction of the animal's circular locomotion to prevent cable twisting. Food and water were provided for ad libitum consumption. The electrophysiological signals were collected and amplified through a polygraph (Model 78E or Model 15LT, Grass, MA), and then digitized and recorded via a Micro 1401 (Cambridge Electronics Design, Cambridge, UK). Sleep and phasic leg movements in slow wave sleep (SWS) and in wake were visually scored offline with a tailored script in Spike2 (Cambridge Electronics Design). Infrared cameras were used for video recordings. Video images were captured digitally through a four-channel surveillance video recorder card (Q-See QSPDVR04; RapidOS, New Taipei City, Taiwan). Video was recorded with timestamps matched to polysomnographic recordings.

After a 3-day baseline sleep and motor activity recordings, a CMA/11 probe with a membrane 1 mm in length (CMA Microdialysis) was inserted into the lateral SNR. The following day, artificial cerebrospinal fluid (aCSF) was microinfused through the probe into the lateral SNR at a flow rate of 2 μL/min with an infusion pump (ESP-64, Eicom, Japan). Infusions started at Zeitgeber (ZT) 2. About 10 μL of dialysate were collected consecutively 2 h after aCSF infusion in polypropylene sample tubes at 10°C and then stored at –80°C.

HPLC analysis experiment

The concentration of GABA in the perfusate was detected by HPLC (CTC-100, Eicom) with fluorescent detection (ECD-100, Eicom) and quantified with a PowerChrom (AD Instruments, Australia). The dialysate was first reacted with o-phthaldialdehyde, consisting of 500 μL of o-phthaldialdehyde in ethanol, 20 μL of mercaptoethanol, and 9.48 mL of sodium carbonate buffer (0.1 M, pH 9.5), by an autosampler (ESA Model 540) at 10°C. Following a 3-min reaction time, 15 μL of the derivatized sample mixture was injected into the HPLC system. The derivatives were then separated in a liquid chromatography column (MA-5ODS, Eicom) at 30°C with 30% methanol in 0.1 M phosphate buffer (pH 6), after being degassed by an on-line degasser (DG-100, Eicom).

The recovery rate of each probe was determined before and after each experiment. The probe was flushed for 20 min to be sure that there were no contaminants. Then the relative recovery rate of each probe was determined by placing it in aCSF containing known concentrations of GABA. Four solutions, covering the expected range of GABA concentrations were used: 0.05, 0.1, 0.5, and 1.0 fmol/mL.

Drug microinfusion experiment

The microinfusion experiment was performed the day after dialysate sampling. Sleep and motor activity recording obtained during the period of aCSF infusion/dialysate sampling served as control. On the day of the chemical infusion

experiment, aCSF was microinfused at ZT2 for 2 h, and then the infusion solution was switched to test drugs for 1 h, followed again by infusion of aCSF for another 1 h. The infusion rate was 2 μL/min. Sleep and motor activity recordings continued throughout. Test drugs used for the experiment included muscimol (Sigma), a $GABA_A$ receptor agonist, and bicuculline (Sigma), a $GABA_A$ receptor antagonist. The concentration of drug infusion was 50, 100, and 200 μM each for muscimol and bicuculline. Both muscimol and bicuculline were dissolved in aCSF.

At the end of the experiment, animals were deeply anesthetized with an overdose of sodium pentobarbital (100 mg/kg, ip), and then, intracardially perfused with 0.1 M phosphate buffer saline (PBS), pH 7.4, followed by 4% paraformaldehyde solution. Brains were removed and kept in 30% sucrose–PBS at 4°C.

The brains were cut into 40 micrometer sections, and then stained with neutral red. Brain sections were examined under the microscope. Infusion/dialysate collection sites were mapped using a NeuroLucida system.

Data analysis

Percent of 5-min intervals in wake, SWS, and REM sleep, corresponding to the 5-min period of dialysate collection, was obtained. Pearson's correlation analysis was then used to determine the relation between GABA concentration in the dialysate and sleep–wake states.

The CED 1401 Spike 2 program was used to analyze EEG power spectra, as well as to detect and score phasic muscle activity during quiet wake and sleep. A script (SUDSA Ver2.2) from CED was used to perform the Fast Fourier Transform with a resolution of 512 on each 10-s epochs of the EEG, which were sampled at 200 Hz. The spectral powers were calculated for each 2-Hz frequency bands between 0 and 50 Hz. The EEG was visually inspected for the occurrence of movement artifacts. Only artifact free epochs were included in the spectral power analysis. The sleep scores and powers in frequency bands along with their timestamps for each epoch were compiled in Microsoft Excel and statistically analyzed with IBM SPSS.

PLM in quiet wake and in SWS were analyzed by adapting the criteria outlined by the World Association of Sleep Medicine [10, 11] and described in our previous study [9]. In brief, phasic motor events in the leg satisfying the following criteria were counted as PLM: (1) the amplitude was twice of the tonic background activity; (2) the duration ranged between 0.2 and 5 s; (3) the interval between jerks was less than 90 s; and (4) at least four consecutive jerks fulfilled criteria described above. Phasic motor events, not meeting the criteria for PLM in SWS, were counted as isolated leg movements in sleep (ILMS). PLM epochs in SWS typically ended when the animal was awakened from sleep or the interval of leg movement was longer than 90 s. The index of PLM in quiet wake (PLMWI) and in SWS (PLMSI) was calculated as the total number of periodic motor movements divided by total time in quiet wake or in SWS per hour, respectively. Similarly, the index of ILMS (ILMSI) was calculated as the total number of ILM in SWS divided by total time in SWS per hour.

The total time in wake, SWS, and REM sleep, as well as PLMWI, PLMSI, and ILMSI were determined during the 1-h test chemical infusion, post hours 1 and 2, and post hours 3 and 4 after test chemical infusion into the lateral SNR. One-way ANOVA with replicated measures followed by a Bonferroni's post hoc, as well as paired *t*-test were used for statistical analysis.

Results

Dialysis and infusion sites

Figure 1, A shows an example of the tract of guide cannula and probe. All eight probes were located in the lateral SNR.

Microdialysis and HPLC analysis experiments

Thirty dialysates were collected from each animal. HPLC analysis showed that GABA levels in the lateral SNR are negatively correlated with SWS ($R = -0.266$, $p < 0.01$, $n = 240$, Pearson correlation test) and positively correlated with wake ($R = 0.265$, $p < 0.01$, $n = 240$, Pearson correlation test). Figure 1, B shows an example of normalized raw GABA level of the lateral SNR in dialysate and the percent time in wake and SWS of the corresponding period. Further analysis showed that GABA levels in the lateral SNR positively correlated with active wake ($R = 0.267$, $p < 0.01$, $n = 240$), but were not correlated with quiet wake ($R = -0.112$, $p > 0.05$, $n = 240$). GABA levels in the lateral SNR were not significantly correlated with REM sleep ($R = -0.06$, $p > 0.05$, $n = 240$, Pearson correlation test).

Microinfusion experiment

Sleep–wake pattern

aCSF infused into the lateral SNR had no effect on the sleep–wake pattern (Figure 2). Muscimol infused into the lateral SNR induced a dose-dependent decrease in SWS ($p < 0.001$, $df = 4$, ANOVA, Figure 2) and REM sleep ($p < 0.001$, $df = 4$, ANOVA, Figure 2) and an increase in wake ($p < 0.001$, $df = 4$, ANOVA, Figure 2). High dose (200 μM) muscimol infused into the lateral SNR elicited a decrease in both SWS and REM sleep and an increase in wake during the 1-h infusion and post 2 h post-infusion period (Figure 2). On the other hand, low and moderate doses of muscimol (50 and 100 μM) infused into the lateral SNR had no effect on sleep–wake pattern during the 1-h infusion. However, an increase in wake and a decrease in SWS were also observed during the post 2 h infusion period when the low and moderate doses muscimol were infused into the lateral SNR (Figure 2). The delayed effect of low to moderate doses muscimol infusion on sleep–wake pattern may have resulted from the low concentration of muscimol and slow diffusion of chemical into the lateral SNR. Muscimol infused into the lateral SNR not only decreased total sleep time but also affected sleep quality. EEG power spectral analysis showed a decrease in slow wave activity (Figure 1, C) and an increase in power at higher than 16 Hz (Figure 1, C) in SWS epochs, except for bands between 26 and 30 Hz which were not changed. A significant decrease in EEG power in 6–8 Hz bands and a significant increase in 2–4 Hz and 28–50 Hz (Figure 1, C) were also observed in REM sleep after muscimol infusion into the lateral SNR. Bicuculline infused into the lateral SNR increased REM sleep ($p < 0.05$, $df = 4$, ANOVA, Figure 2) and decreased wake time during the 1-h infusion ($p < 0.05$, $df = 4$, ANOVA, Figure 2). Bicuculline at high dose (200 μM) infused into the lateral SNR also increased SWS (Figure 2).

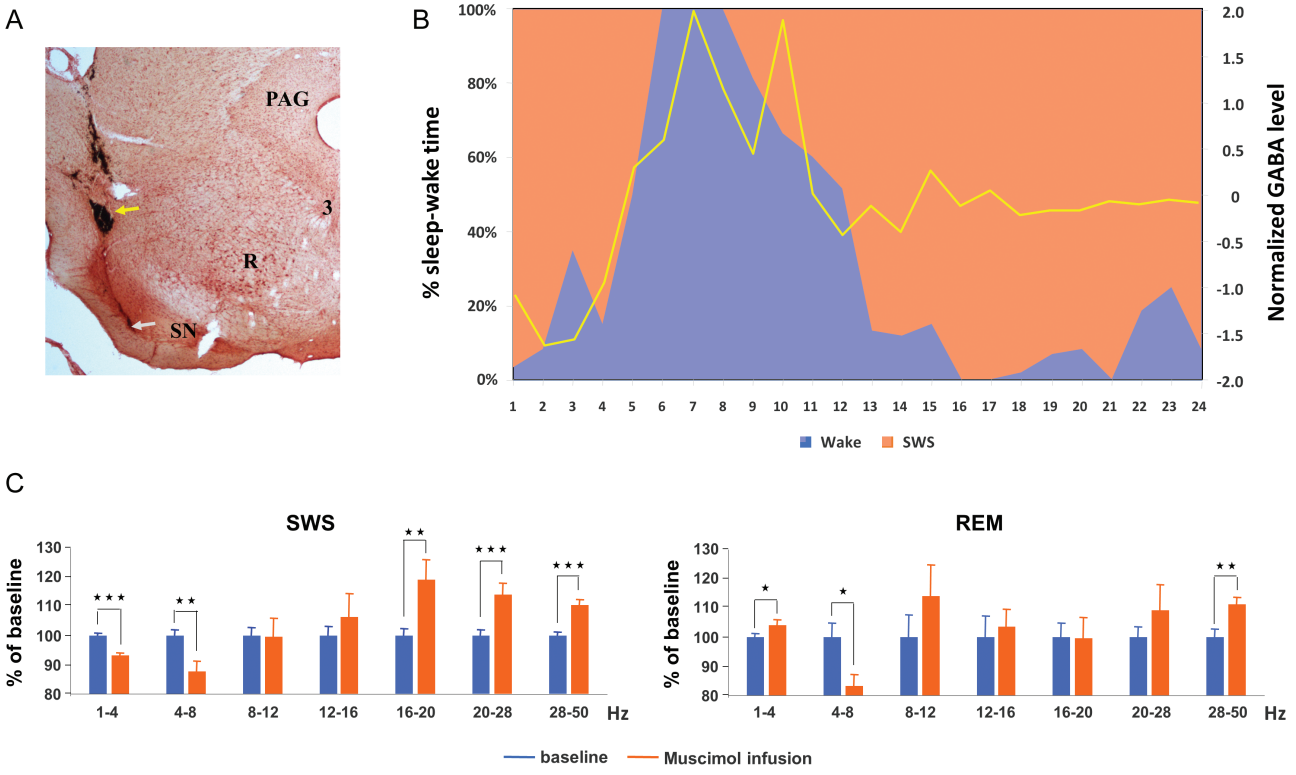

Figure 1. (A) Photomicrograph showing the dialysate collection and drug infusion sites in the SNR. (B) Normalized GABA level (yellow line) in the lateral SNR vs percent of wake (blue area) and SWS (orange area), respectively, in 24 consecutive dialysate collections. REM sleep did not occur during the period of dialysate collection. (C) EEG power spectral analysis showing changes in EEG frequency before (baseline) and after muscimol infusion. The yellow and white arrows, shown in A represent the tracts of guide cannulae and the tip of probe, respectively. PAG, periaqueductal gray; R, red nucleus; SN, substantia nigra; 3, oculomotor nucleus. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, ANOVA, n: SWS: 575, REM sleep: 47.

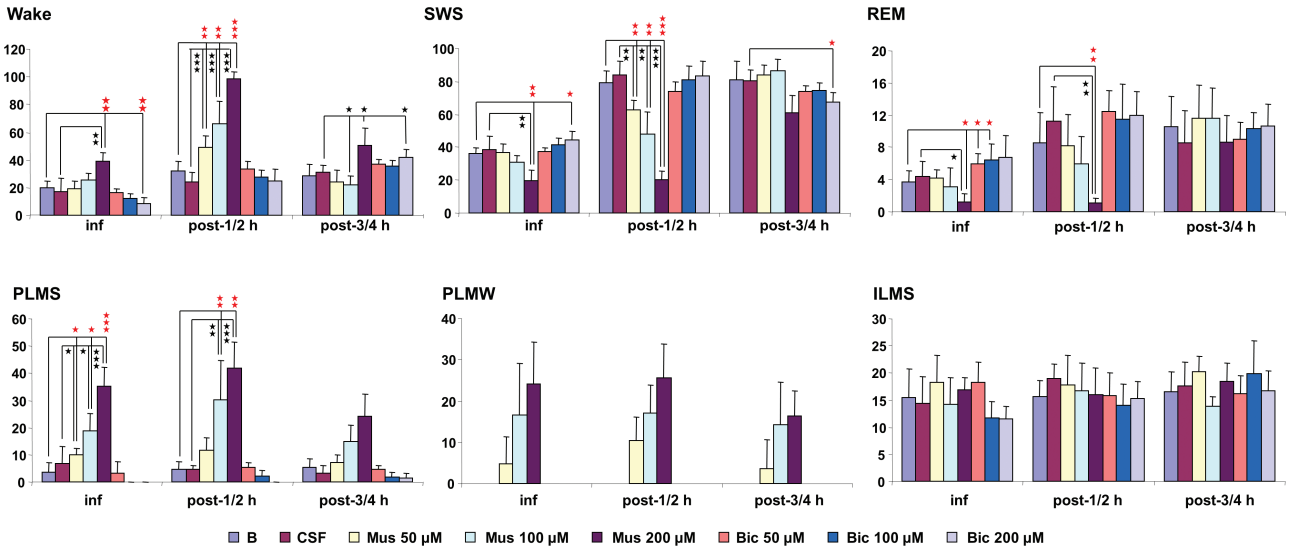

Figure 2. Effect of muscimol (Mus) and bicuculline (Bic) infused into the SNR on sleep–wake states and motor activity. Muscimol microinfused into the lateral SNR increased wake time and decreased SWS and REM sleep time. In contrast, bicuculline microinfused into the lateral SNR decreased wake time and increased SWS and REM sleep time. Infusion of muscimol into the lateral SNR also increased PLM in SWS (PLMS) and induced PLM in quiet wake (PLMW). In contrast, bicuculline infused into the lateral SNR suppressed PLMS. Isolated leg movements in SWS (ILMS) were not altered by either muscimol or bicuculline infused into the lateral SNR. The y-axis for wake, SWS, and REM is minutes. The y-axis for motor activity is the index of PLMS, PLMW, and ILMS. CSF, artificial cerebrospinal fluid; inf, infusion; post-1/2 h and post-3/4 h, post-infusion 1 and 2 h and post-infusion 3 and 4 h. The red and black stars represent significant differences between drug infusion and baseline and CSF infusion, respectively. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$, post hoc analysis, $n = 8$.

Motor activity

Microinfusion of muscimol into the lateral SNR increased motor activity (Figure 2). Motor hyperactivity, grooming, climbing, running, and circling during active wake were observed with high concentration (200 μM), but not with low to moderate concentration (50 and 100 μM) of muscimol infusion into the lateral SNR. PLM in quiet wake (Figure 3, E), which was never observed in the baseline recording or during aCSF infusion, was elicited by muscimol

infusion into the SNR. PLM in quiet wake appeared either immediately before falling sleep or during quiet sitting on the floor. Application of muscimol to the lateral SNR also increased PLM in SWS ($p < 0.001$, $df = 4$, ANOVA; Figure 2). PLM in quiet wake and in SWS occurred in the leg ipsilateral to the infusion site (Figure 3, A, E). Contralateral leg movements occasionally emerged simultaneous with ipsilateral leg twitching (Figure 3, D) in PLM in SWS. However, contralateral leg movements alone did not meet the criteria for PLM (Figure 3, D). Similar to our previous findings in iron-deficient (ID) rats [12] most of the leg movements intervals in SWS induced by muscimol infusion into the lateral SNR were between 10 and 20 s (Figure 3, B). In contrast, intervals of PLM in SWS during aCSF infusion or baseline recording were evenly distributed between 10 and 90 s (data not shown; Figure 3, C), as we previously reported in control rats [12]. In addition, PLM in SWS during aCSF infusion or baseline recording appeared bilaterally (Figure 3, C), while unilateral PLM were not observed. Isolated leg movements in SWS were not altered by muscimol infusion into the lateral SNR (Figure 2). Muscimol infusion into the lateral SNR had no effect on phasic or tonic muscle activity during REM sleep (data not shown).

In contrast to muscimol infusion, microinfusion of bicuculline into the lateral SNR dose-dependently suppressed PLM in SWS ($p < 0.001$, $df = 4$, ANOVA; Figure 2). Muscle tone in REM sleep was not altered by bicuculline infusion into the lateral SNR (data not shown).

Discussion

Technical limitations

Reverse microdialysis was used to infuse test drugs into the lateral SNR in this study. In contrast to conventional microinjection, in which the test drug is directly injected into the target area, the drug is slowly diffused into the target site with the microinfusion technique. The recovery rate of the microdialysis probe used for infusion was 10%. It takes a longer time to induce physiological changes with low and/or moderate concentrations of drug, and the effective dose of agonist and antagonist needed to induce a change in sleep and motor activity have to be higher when using a dialysis probe, as compared with conventional microinjection. Furthermore, one can assume that there is a gradient of concentration around the diffusion site. Crochet and Sakai [13] have shown that the perfusate diffuses into a 1 mm diameter region after 2 h of dialysis microinfusion. The diffusion area might be less than 1 mm diameter in our study because the animals received a 1-h drug infusion. A conclusion drawn from such a diffusion study is does not precisely locate the affected region. Further studies with unit recording could more precisely localize the neurons mediating the effects of microinfusion.

In the present study, we found that GABA release in the lateral SNR was positively correlated with wake and negatively correlated with SWS. It has been reported that brain temperature affects transmitter release. Nomura et al.[14] showed a significant and a non-significant decrease in GABA release in the cortex and hippocampus, respectively, after local brain temperature was cooled to 15°C for 30 min in epileptic patients. Thus, one may speculate that an increase in lateral SNR GABA level in wake may result from an increase in brain temperature during wake. However, it is unlikely that the increase in GABA release in the lateral SNR, seen in the present study, is due to an increase in brain temperature. The difference of brain temperature between wake and SWS has been reported to less than 0.5°C in the rat [15]. We also found that infusion of the $GABA_A$ receptor antagonist, bicuculline, which increases neuronal activity, into the

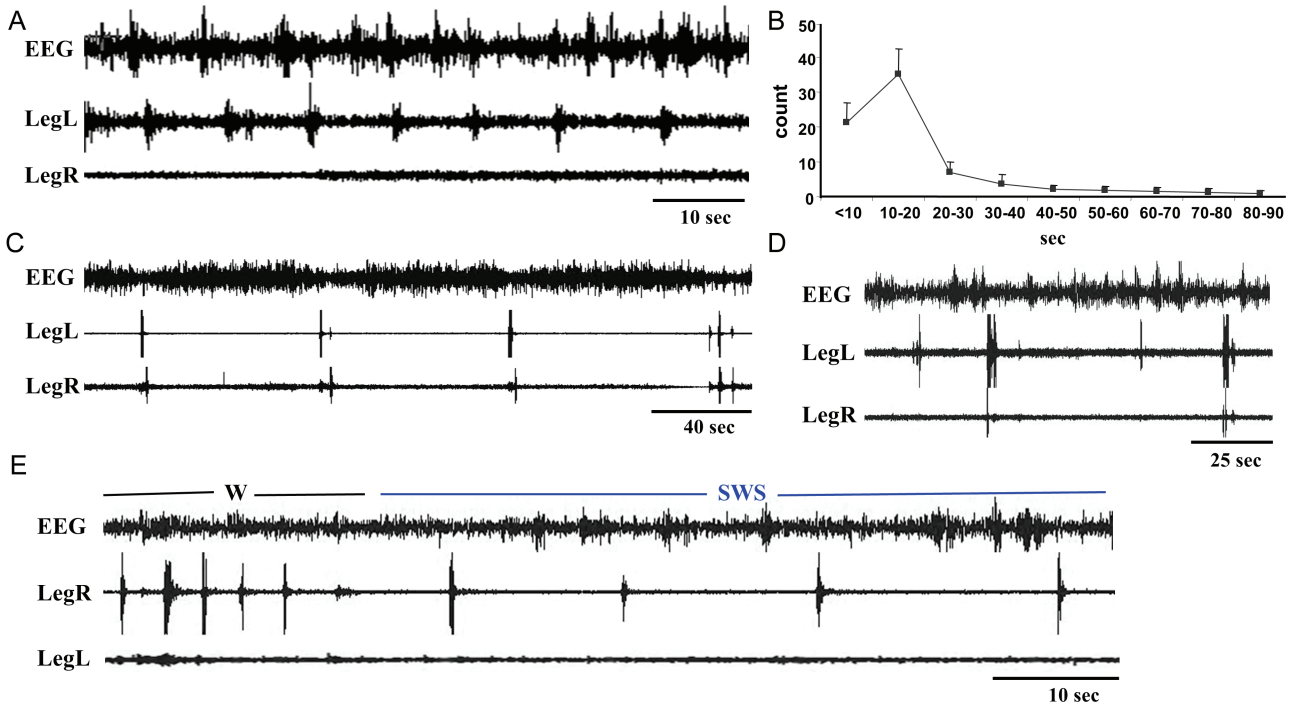

Figure 3. Example of muscimol infusion into the lateral SNR inducing PLM in SWS (A) and PLM in wake that extended into SWS (E). (B) The inter-leg movement interval of eight rats during muscimol infusion into the lateral SNR and during the 4 h post-infusion period. (C) Bilateral leg movements of PLM in SWS during artificial CSF infused into the lateral SNR. (D) Mixed ipsilateral and bilateral leg movements in SWS were observed after muscimol infused into the left lateral SNR, however, contralateral right leg movements alone did not meet the criteria for PLM. LegL and LegR, left and right leg, respectively.

lateral SNR produced an increase in sleep and a decrease in PLM in SWS. In contrast, infusion of $GABA_A$ receptor agonist, muscimol, which decreases neuronal activity, into the lateral SNR elicited insomnia and PLM in wake and increased PLM in SWS. Inactivation of the lateral SNR by muscimol infusion not only decreased sleep time but also worsened sleep quality. The distribution of leg movement recurrence intervals in SWS induced by muscimol-lateral SNR infusion was similar to that seen in ID rats [12] and in human RLS patients [16]. Clinical evidence revealed that RLS patients suffer insomnia, with more than 80% of patients also having PLM in quiet wake and/or in sleep [17, 18]. We found that lateral SNR muscimol-infusion generated insomnia and PLM in quiet wake and SWS in rats, resembling that seen in RLS patients.

Liu et al.[19] showed that the SNR plays a role in the modulation of sleep and motor activity. They found that GABAergic neurons in the medial SNR are responsible for sleep modulation, while the parvalbumin neurons in the lateral SNR contribute to the generation of motor activity. This result differs from our current study and other studies using the lesion technique. Gerashchenko et al.[20] showed that hypocretin-2-saporin lesions of the medial ventral midbrain including the medial portion of the SNC and SNR and the ventral tegmental area, have no effect on sleep or motor activity. In contrast, lesions of the lateral SNC and SNR caused insomnia and motor hyperactivity during waking in the rat [20], in agreement with our current findings. Similar findings also reported that lesions of the medial ventral midbrain do not alter sleep pattern, whereas, lesions of the lateral ventral midbrain generate insomnia and PLM in sleep in cats [8]. However, sleep-wake pattern changes may differ as a function of the techniques used. Yu et al.[21] showed that ventral tegmental area glutamatergic and GABAergic neurons are related to wake and sleep, respectively. Chemogenetic stimulation activate and/or inhibit specific neuronal phenotypes, whereas neurotoxic lesions damage all types of neuron in the area. Thus, both wake promoting and sleep promoting neurons in the ventral tegmental area are damaged resulting in no change in the sleep–wake pattern.

Striatal spiny medium GABAergic neurons project to the SNR via direct and indirect pathways. Striatal GABAergic neurons containing the dopamine D_1 receptor project to the SNR, whereas, striatal GABAergic neurons containing dopamine D_2 and adenosine A_{2A} receptors (D2R/A2R) project to the globus pallidus. GABAergic neurons in the globus pallidus project to the SNR. Yuan et al.[22] using optogenetic stimulation, found that activation of striatal GABAergic D2R/A2R neurons increases SWS in mice. The increased SWS induced by striatal activation was shown to be mediated by the inhibition of globus pallidal neuronal activity [22]. Yuan et al.[22] hypothesized that striato-pallidal modulation of sleep may be mediated by the pallidal projection to the thalamic reticular nucleus [23], whose activity has been reported to relate to spindles and slow waves [24–29]. However, a role of SNR projection through the striato-pallidal pathway on sleep regulation cannot be ruled out. Neurons of the SNR have been shown to send projections to the reticular thalamic nucleus [30, 31]. SNR neurons also innervate the dorsomedial thalamic nucleus [32]. The dorsomedial thalamic nucleus may also be involved in the control of sleep. Neurodegeneration of the dorsomedial thalamic nucleus is observed in patients with fatal familial insomnia [33, 34], whose total amount of sleep is dramatically decreased. We hypothesize that an increase in striatal GABAergic neuronal activity inhibits pallidal GABAergic neuronal activity, and consequently decreases GABA release onto the SNR, increases SNR neuronal activity, and increases sleep via the projections to the sleep-related thalamic nuclei (Figure 4).

The effect of SNR on motor activity may be mediated through the pedunculopontine nucleus. Takakusaki et al.[35, 36] reported that electrical or chemical (bicuculline injection) stimulation of the lateral SNR suppresses muscle atonia, a characteristic of REM sleep, induced by pedunculopontine activation in the decerebrate cat. However, activation of the lateral SNR itself has no effect on muscle tone [35, 36]. They also reported that activation of the medial but not the lateral SNR inhibits midbrain medial locomotor region-induced locomotion [35, 36]. Their results differ from ours and other studies. Motor hyperactivity was not reported in the rat with medial SN lesion [20]. On the other hand, lesions of the lateral SN elicited motor hyperactivity in the rat and cat [8, 20]. In the present study, we also showed that activation of the lateral SNR has no effect on REM sleep atonia. Thus, the effect of SNR on motor activity may not be mediated through the pedunculopontine nucleus.

It is possible that the suppression effect of SNR on motor activity may be mediated by the thalamus (Figure 4). SNR GABAergic neurons have been found to project to [37–42] and exert an inhibitory effect on ventrolateral and ventromedial thalamic neurons [43, 44]. Using intracellular recording technique, Ueki [44] demonstrated that electrical stimulation of the SNR elicits monosynaptic IPSPs in ventral thalamic neurons. Antal et al.[30] also reported that SN stimulation elicits an inhibitory effect on neurons of the thalamic ventropostero-lateral nucleus. Thus, activation of the SNR not only increases sleep but also inhibits motor activity via thalamic nuclei. In addition to the regulation of sleep and motor activity, SNR may also modulate the sensory–motor system. Injection of muscimol into the lateral SNR increases the auditory startle response [45, 46] and

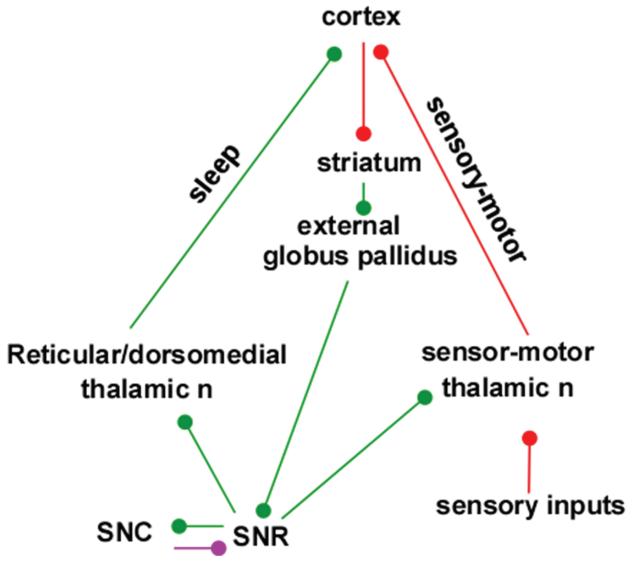

Figure 4. Hypothetical neural circuitry generating hyposomnia and PLM in sleep. The role of the SNR in the control of sleep and sensory–motor activity may be mediated by its thalamic projection. See text for details. Red, green, and pink lines represent glutamatergic, GABAergic, and dopaminergic projections. SNC, substantia nigra pars compacta.

decreases prepulse inhibition [46]. An increase in the auditory startle reaction has been reported in idiopathic RLS patients [47].

We hypothesize that inhibition from the lateral SNR plays an important role in keeping the motor system in a quiescent state before falling sleep and during sleep, and thus helps to induce and maintain sleep. As shown in Figure 4, sensory inputs ascend to the spinal cord, dorsal column nuclei, and trigeminal nucleus, at which point second order neurons project to the thalamus. Under normal circumstances, activation of the thalamus by sensory inputs during sleep may be inhibited by GABAergic projections from the SNR [30, 44]. Thus, inactivation and/or neuronal degeneration of the SNR may disinhibit neuronal activity of the thalamus resulting in sensory–motor hyperactivity. Indeed, a clinical study revealed that sensory leg discomfort and PLM in idiopathic RLS patients are correlated with thalamic activation [48]. Thalamic sensory–motor nuclei hyperactivity may thus activate sensorimotor cortex and release glutamate into the striatum resulting in RLS generation [49] (Figure 4). In addition to the suppression of thalamic sensory–motor neuronal activity, activity of SNR may be involved in the generation of spindles and slow wave by way of its projection to the reticular and dorsomedial thalamic nuclei [31, 32] (Figure 4). The GABAergic projection from the SNR also inhibits dopaminergic neuronal activity of the SNC [50, 51]. Thus, inactivation of GABAergic neuronal activity in the SNR disinhibits dopamine neuronal activity in the SNC, which in turn further suppresses SNR GABAergic neuronal activity [52] (Figure 4) and results in hyposomnia and motor hyperactivity. Abnormally high levels of tyrosine hydroxylase and phosphorylated tyrosine hydroxylase in the SNC have been reported in idiopathic RLS patients and in the ID rat [53], an animal model of RLS [9, 12].

In conclusion, we have shown, for the first time, that the lateral portion of the SNR plays a key role in maintaining sleep via its inhibitory effect on the sensory–motor system and regulating the activity of thalamic sleep nuclei. Dysfunction of the lateral SNR induces hyposomnia and generates motor hyperactivity in sleep and in quiet wake, that is, RLS-like activity.

Funding

This work was supported by the National Institute of Health grants, NS082242 (Y.Y.L.), DA034748 and HL148574 (J.M.S.), and the Department of Veterans Affairs.

Disclosure statements

Financial disclosures: All authors have nothing to declare.

Non-financial disclosures: All authors have nothing to declare.

References

1. Freeze BS, *et al*. Control of basal ganglia output by direct and indirect pathway projection neurons. *J Neurosci*. 2013;**33**(47):18531–18539.
2. Gittis AH, *et al*. New roles for the external globus pallidus in basal ganglia circuits and behavior. *J Neurosci*. 2014;**34**(46):15178–15183.
3. Mink JW. The basal ganglia: focused selection and inhibition of competing motor programs. *Prog Neurobiol*. 1996;**50**(4):381–425.
4. Redgrave P, *et al*. The basal ganglia: a vertebrate solution to the selection problem? *Neuroscience*. 1999;**89**(4):1009–1023.
5. Deniau JM, *et al*. The lamellar organization of the rat substantia nigra pars reticulata: distribution of projection neurons. *Neuroscience*. 1992;**46**(2):361–377.
6. Deniau JM, *et al*. The pars reticulata of the substantia nigra: a window to basal ganglia output. *Prog Brain Res*. 2007;**160**:151–172.
7. Ilinsky IA, *et al*. Organization of the nigrothalamocortical system in the rhesus monkey. *J Comp Neurol*. 1985;**236**(3):315–330.
8. Lai YY, *et al*. Neurotoxic lesions at the ventral mesopontine junction change sleep time and muscle activity during sleep: an animal model of motor disorders in sleep. *Neuroscience*. 2008;**154**(2):431–443.
9. Lai YY, *et al*. Motor hyperactivity of the iron deficient rat—an animal model of restless legs syndrome. *Move Disord*. 2017;**32**(12):1687–1693.
10. Allen RP, *et al*. Restless legs syndrome/Willis-Ekbom disease diagnostic criteria: updated International Restless Legs Syndrome Study Group (IRLSSG) consensus criteria–history, rationale, description, and significance. *Sleep Med*. 2014;**15**(8):860–873.
11. Zucconi M, *et al*. The official World Association of Sleep Medicine (WASM) standards for recording and scoring periodic leg movements in sleep (PLMS) and wakefulness (PLMW) developed in collaboration with a task force from the International Restless Legs Syndrome Study Group (IRLSSG). *Sleep Med*. 2006;**7**(2):175–183.
12. Lai YY, *et al*. Reply: the iron-deficient rat as a model of restless legs syndrome: was anything lost in translation? *Mov Disord*. 2018;**33**(1):182–183.
13. Crochet S, *et al*. Effects of microdialysis application of monoamines on the EEG and behavioural states in the cat mesopontine tegmentum. *Eur J Neurosci*. 1999;**11**(10):3738–3752.
14. Nomura S, *et al*. Effects of focal brain cooling on extracellular concentrations of neurotransmitters in patients with epilepsy. *Epilepsia*. 2017;**58**(4):627–634.
15. Obál F Jr, *et al*. Changes in the brain and core temperatures in relation to the various arousal states in rats in the light and dark periods of the day. *Pflugers Arch*. 1985;**404**(1):73–79.
16. Ferri R, *et al*. Different periodicity and time structure of leg movements during sleep in narcolepsy/cataplexy and restless legs syndrome. *Sleep*. 2006;**29**(12):1587–1594.
17. Montplaisir J, *et al*. Clinical, polysomnographic, and genetic characteristics of restless legs syndrome: a study of 133 patients diagnosed with new standard criteria. *Mov Disord*. 1997;**12**(1):61–65.
18. Montplaisir J, *et al*. Immobilization tests and periodic leg movements in sleep for the diagnosis of restless legs syndrome. *Move Disord*. 1998;**13**(2):324–329.
19. Liu D, *et al*. A common hub for sleep and motor control in the substantia nigra. *Science*. 2020;**367**(6476):440–445.
20. Gerashchenko D, *et al*. Insomnia following hypocretin 2-saporin lesions of the substantia nigra. *Neuroscience*. 2006;**137**(1):29–36.
21. Yu X, *et al*. GABA and glutamate neurons in the VTA regulate sleep and wakefulness. *Nat Neurosci*. 2019;**22**(1):106–119.
22. Yuan XS, *et al*. Striatal adenosine A_{2A} receptor neurons control active-period sleep via parvalbumin neurons in external globus pallidus. *eLife* 2017;**6**:e29055.
23. Mastro KJ, *et al*. Transgenic mouse lines subdivide external segment of the globus pallidus (GPe) neurons

and reveal distinct GPe output pathways. *J Neurosci*. 2014;**34**(6):2087–2099.
24. Fernandez LMJ, *et al*. Thalamic reticular control of local sleep in mouse sensory cortex. *eLife*. 2018;**7**:e39111.
25. Halassa MM, *et al*. State-dependent architecture of thalamic reticular subnetworks. *Cell*. 2014;**158**(4):808–821.
26. Marini G, *et al*. Changes in EEG spindle activity induced by ibotenic acid lesions of medialis dorsalis thalamic nuclei in the cat. *Brain Res*. 1989;**500**(1–2):395–399.
27. Steriade M, *et al*. Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticularis thalami. *J Neurophysiol*. 1985;**54**(6):1473–1497.
28. Steriade M, *et al*. Thalamocortical oscillations in the sleeping and aroused brain. *Science*. 1993;**262**(5134):679–685.
29. Vantomme G, *et al*. Regulation of local sleep by the thalamic reticular nucleus. *Front Neurosci*. 2019;**13**:576.
30. Antal M, *et al*. The substantia nigra conveys target-dependent excitatory and inhibitory outputs from the basal ganglia to the thalamus. *J Neurosci*. 2014;**34**(23):8032–8042.
31. Paré D, *et al*. Substantia nigra pars reticulata projects to the reticular thalamic nucleus of the cat: a morphological and electrophysiological study. *Brain Res*. 1990;**535**(1):139–146.
32. Russchen FT, *et al*. The afferent input to the magnocellular division of the mediodorsal thalamic nucleus in the monkey, Macaca fascicularis. *J Comp Neurol*. 1987;**256**(2):175–210.
33. Lugaresi E, *et al*. Fatal familial insomnia and dysautonomia with selective degeneration of thalamic nuclei. *N Engl J Med*. 1986;**315**(16):997–1003.
34. Tinuper P, *et al*. The thalamus participates in the regulation of the sleep-waking cycle. A clinico-pathological study in fatal familial thalamic degeneration. *Electroencephalogr Clin Neurophysiol*. 1989;**73**(2):117–123.
35. Takakusaki K, *et al*. Basal ganglia efferents to the brainstem centers controlling postural muscle tone and locomotion: a new concept for understanding motor disorders in basal ganglia dysfunction. *Neuroscience*. 2003;**119**(1):293–308.
36. Takakusaki K, *et al*. Evidence for a role of basal ganglia in the regulation of rapid eye movement sleep by electrical and chemical stimulation for the pedunculopontine tegmental nucleus and the substantia nigra pars reticulata in decerebrate cats. *Neuroscience*. 2004;**124**(1):207–220.
37. Beckstead RM. Long collateral branches of substantia nigra pars reticulata axons to thalamus, superior colliculus and reticular formation in monkey and cat. Multiple retrograde neuronal labeling with fluorescent dyes. *Neuroscience*. 1983;**10**(3):767–779.
38. Bodor AL, *et al*. Structural correlates of efficient GABAergic transmission in the basal ganglia-thalamus pathway. *J Neurosci*. 2008;**28**(12):3090–3102.
39. Kha HT, *et al*. Projections from the substantia nigra pars reticulata to the motor thalamus of the rat: single axon reconstructions and immunohistochemical study. *J Comp Neurol*. 2001;**440**(1):20–30.
40. Kuramoto E, *et al*. Complementary distribution of glutamatergic cerebellar and GABAergic basal ganglia afferents to the rat motor thalamic nuclei. *Eur J Neurosci*. 2011;**33**(1):95–109.
41. Rajakumar N, *et al*. Parvalbumin-containing GABAergic neurons in the basal ganglia output system of the rat. *J Comp Neurol*. 1994;**350**(2):324–336.
42. Sakai ST, *et al*. Nigrothalamic projections and nigrothalamocortical pathway to the medial agranular cortex in the rat: single- and double-labeling light and electron microscopic studies. *J Comp Neurol*. 1998;**391**(4):506–525.
43. Kase D, *et al*. Inhibitory synaptic transmission from the substantia nigra pars reticulata to the ventral medial thalamus in mice. *Neurosci Res*. 2015;**97**:26–35.
44. Ueki A. The mode of nigro-thalamic transmission investigated with intracellular recording in the cat. *Exp Brain Res*. 1983;**49**(1):116–124.
45. Aguilar BL, *et al*. Inhibition of the substantia nigra pars reticulata produces divergent effects on sensorimotor gating in rats and monkeys. *Sci Rep*. 2018;**8**(1):9369.
46. Rodrigues S, *et al*. Muscimol injection into the substantia nigra but not globus pallidus affects prepulse inhibition and startle reflex. *Neuropharmacology*. 2020;**162**:107796.
47. Frauscher B, *et al*. Auditory startle reaction is disinhibited in idiopathic restless legs syndrome. *Sleep*. 2007;**30**(4):489–493.
48. Bucher SF, *et al*. Cerebral generators involved in the pathogenesis of the restless legs syndrome. *Ann Neurol*. 1997;**41**(5):639–645.
49. Yepes G, *et al*. Targeting hypersensitive corticostriatal terminals in restless legs syndrome. *Ann Neurol*. 2017;**82**(6):951–960.
50. Saitoh K, *et al*. Nigral GABAergic inhibition upon mesencephalic dopaminergic cell groups in rats. *Eur J Neurosci*. 2004;**19**(9):2399–2409.
51. Tepper JM, *et al*. $GABA_A$ receptor-mediated inhibition of rat substantia nigra dopaminergic neurons by pars reticulata projection neurons. *J Neurosci*. 1995;**15**(4):3092–3103.
52. Windels F, *et al*. Dopamine action in the substantia nigra pars reticulata: iontophoretic studies in awake, unrestrained rats. *Eur J Neurosci*. 2006;**24**(5):1385–1394.
53. Connor JR, *et al*. Altered dopaminergic profile in the putamen and substantia nigra in restless leg syndrome. *Brain*. 2009;**132**(Pt 9):2403–2412.