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ORIGINAL PAPER

# Inactivation of the Ventral Pallidum by $GABA_A$ Receptor Agonist Promotes Non-rapid Eye Movement Sleep in Rats

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## Abstract

GABA, the most abundant inhibitory neurotransmitter in the brain, is closely linked with sleep and wakefulness. As the largest area input to the ventral pallidum (VP), the nucleus accumbens (NAc) has been confirmed to play a pivotal role in promoting non-rapid eye movement (NREM) sleep through inhibitory projections from NAc adenosine $A_{2A}$ receptor-expressing neurons to VP GABAergic neurons which mostly express $GABA_A$ receptors. Although these studies demonstrate the possible role of VP GABAergic neurons in sleep–wake regulation, whether and how its modulate sleep–wake cycle is not completely clear. In our study, pharmacological manipulations were implemented in freely moving rats and then the EEG and the EMG were recorded to monitor the sleep–wake states. We found that microinjection of muscimol, a $GABA_A$ receptor agonist, into the VP increased NREM sleep in both light and dark period. Microinjection of bicuculline, a $GABA_A$ receptor antagonist, into the VP increased wakefulness in the light period. Collectively, our data identify the important role of VP $GABA_A$ receptor-expressing neurons in NREM sleep of rats which may help improve the understanding of the pathological sleep disorders.

**Keywords** Ventral pallidum · GABA · Muscimol · Sleep–wake states

## Introduction

The ventral pallidum (VP) is a heterogeneous region of the basal forebrain (BF) that strongly regulates cortical activation, sleep homeostasis, and attention [1, 2]. Besides, VP is also widely considered to be an output structure of the basal ganglia [3]. It has long been studied that the VP mediates a variety of neurobiological behaviors, such as reward [1, 4–6] and motivation [1, 4, 7], as well as drugs of abuse [1, 8, 9] and behaviors related to cognition [1, 10].

The largest input to VP is from the nucleus accumbens (NAc), a major component of the ventral striatum [11, 12], which mainly contains two subtypes of GABA (gamma-aminobutyric acid)-ergic projection neurons: one expresses dopamine $D_1$ receptor/adenosine $A_1$ receptor ($D_1R/A_1R$), and the other one expresses dopamine $D_2$ receptor/adenosine $A_{2A}$ receptor ($D_2R/A_{2A}R$) [13, 14]. In addition, the abundant glutamic acid decarboxylase (Gad)-immunoreactive neurons of VP, mostly expressing ionotropic $GABA_A$ receptors, are heavily innervated by GABAergic fibers from the NAc [1]. The VP together with the NAc belonging to the ventral striatopallidal system receives dense inputs from limbic structures, leading to the hypothesis that this system is a critical interface between reward-related process and motor output [5, 15–17].

The BF is a richly heterogeneous structure, containing a number of genetically distinct cell populations, including cholinergic, glutamatergic and GABAergic neurons [18, 19]. The cholinergic neurons are classic wake-promoting neurons in the forebrain. However, the number of GABAergic neurons in the basal forebrain is more than twice that of cholinergic neurons. Emerging evidence indicates that activation of GABAergic neurons in the forebrain also produces wakefulness and inhibition increases sleep [18, 19]. In addition, lesion degeneration study [20] suggests that the basal ganglia, which include the VP, are important neural circuitry regulating sleep–wake states and cortical activation. Recently, Oishi et al. found that the activation of the adenosine $A_{2A}R$ neurons in the core subcompartment (NAc core) strongly induces non-rapid eye movement (NREM) sleep where this effect is mediated by inhibitory projections from $A_{2A}R$ neurons to VP GABAergic neurons. They further

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demonstrated that activation of VP GABAergic neurons in mice suppresses NREM sleep [21, 22]. Soon after, a study revealed that NAc $D_1R$ neuron circuits play a fundamental role in the production and maintenance of wakefulness [14]. Taken together, we hypothesize that VP GABAergic neurons may be important for sleep–wake regulation. However, whether and how the VP GABAergic neurons mediate the sleep–wake cycle is not completely clear.

The present study was aimed to explore the function of the VP $GABA_A$ receptor-expressing neurons in sleep–wake regulation with pharmacology and electroencephalogram/electromyogram (EEG/EMG) recording methods.

## Materials and Methods

### Animals

Adult male Sprague–Dawley rats (270–320 g) were purchased from Beijing Huafukang Bioscience Co. Ltd (Beijing, PR China). They were individually housed in an appropriate temperature (22.0 ± 1.0 °C) and humidity (50%) under a 12 h light/dark cycle (lights on at 7:00) with food and water available ad libitum. The protocol for animal study was approved by the Ethics Committee of Wuhan University (IACUC permit number: 2019124).

### Drugs and Chemicals

Muscimol and (-)-bicuculline methiodide were purchased from Abcam (Cambridge, MA, USA) which were dissolved in saline. These drugs were administrated at 0.25 µl per side.

### Surgery

Rats were anesthetized with 1% pentobarbital sodium (40 mg/kg, i.p.) for surgical operations and mounted in a stereotactic instrument (RWD, Shenzhen, PR China). Two guide cannulas (internal diameter: 0.34 mm) were implanted bilaterally 0.5 mm above the VP (anteroposterior (AP) + 0.24 mm, mediolateral (ML) ± 2.0 mm, dorsoventral (DV) − 7.1 mm) depending on the rat brain atlases of Paxinos and Watson [23] for drug application (Fig. 5a). Rats were also chronically implanted with EEG and EMG electrodes. Briefly, the implants comprised two pairs of stainless-steel screws (1.0 mm diameter) serving as EEG electrodes, which were implanted into the dura located in the frontal (2.5 mm anterior and 2.0 mm lateral to the bregma) and parietal (1.0 mm anterior and 2.0 mm lateral to the lambda) cortices respectively. Two multi-stranded PFA-coated stainless steel wires serving as EMG electrodes were placed bilaterally into the dorsal cervical neck muscles. EEG and EMG electrodes were soldered into a headmount, and the whole assembly was attached to the skull with dental cement. The stainless-steel stylets (outside diameter: 0.30 mm) were inserted through each guide cannula to prevent its occlusion.

### EEG/EMG Recordings and Pharmacological Treatments

For habituation prior to data collection, rats were singly transferred to recording chambers and connected to the EEG/EMG headstages for 3 days beginning 1 week after surgery. The recording cable was connected to a slip-ring unit in order to make the movement of the rats unrestricted.

On the day of recording, two injector cannulas (outside diameter: 0.30 mm) fitting with collar were protruded 0.5 mm beyond the guide cannulas, so that the injector cannulas were lowered bilaterally into the VP (DV − 7.6 mm). These injectors were connected through snugly fitting polyethylene tubings (PE 20, RWD) to a pair of 1.0 µl microsyringes mounted on a controlled dual-syringe infusion/withdrawal pump (Legato 180, KD Scientific Inc., USA) at the rate of 100 nl/min. The rats were randomly separated into different experimental groups. The following groups were investigated: (1) Baseline control recordings of the normal sleep–wake cycle lasted for 24 h started at 7:00 without any treatment. The baseline control animals were the rats with EEG/EMG electrodes but no cannula implantation. (2) Muscimol, a $GABA_A$ receptor agonist, at 0.25 or 0.50 µg/side or saline was dosed at 21:00 (i.e. 2 h after light-off). (3) Bicuculline methiodide, a $GABA_A$ receptor antagonist, at 0.25 µg/side or saline was dosed at 9:00 (i.e. 2 h after light-on). (4) Muscimol at 0.50 µg/side or saline was dosed at 9:00. The rats we used were all naive animals and they were used only once and were not reused.

### Histology

After EEG/EMG recordings, rats were deeply anesthetized with chloral hydrated (400 mg/kg), and perfused through the left ventricle of the heart with 200 ml of saline, followed by 300 ml of 4% paraformaldehyde (PFA). Brains were removed, postfixed for 12 h in 4% PFA, and then immersed in 30% sucrose phosphate buffer (PB) at 4 °C until they sank. Next, the brains were frozen in liquid nitrogen for a moment and then coronally sectioned at 30 µm on a freezing microtome (CM1900, Leica Micro-systems, Germany) at − 20 °C. Finally, the sections were mounted on glass slides, dried, dehydrated, and coverslipped. Images were captured by a fluorescent microscopy (BX53, Olympus, Japan).

### Data Analysis

EEG/EMG signals were amplified and filtered (EEG: 0.5–30 Hz, EMG: 40–200 Hz), then digitized at a sampling rate of 128 Hz and recorded with the Apollo II™16 data acquisition system (Bio-signal Inc., PR China). Sleep–wake states were scored offline using Sirenia sleep pro software (Pinnacle Technology Inc., USA). All scoring was semi-automatic based on EEG and EMG waveforms and behaviors monitored by an infrared camera in 10 s epochs. The wakefulness state was characterized by a high-frequency, low-amplitude, desynchronized EEG and highly variable muscle tone on EMG. NREM sleep was identified by a predominant high-amplitude, low-frequency (0.5–4 Hz) synchronized EEG and relative less motor activity. Rapid eye movement (REM) sleep was defined by pronounced theta like (4–9 Hz) EEG activity and the absence of EMG tone according to previously described standard criteria validated for rats [24, 25].

### Statistical Analysis

Data are presented as the mean ± SEM. Statistical comparisons among each group were performed by one-way analysis of variance (ANOVA) and post hoc Fisher's least significant difference (LSD) test. Prisma 8.0 was used to create the graphs, and then photoshop 7.0 was used to combine the pictures and graphs together. In all statistical comparisons, *P*-values less than 0.05 were considered significant.

## Results

### Microinjection of Muscimol into the VP Increased NREM Sleep in the Dark Period

First, we examined the sleep–wake profile after bilateral microinjection of muscimol, a $GABA_A$ receptor agonist, into the VP in freely moving rats at 21:00, a time when rodents exhibit maximal wakefulness and active behaviors. Typical examples of EEG, EMG, and the corresponding hypnograms for 10 h from rats respectively given saline or muscimol at a dose of 0.50 μg/side are represented in Fig. 1a. As shown, muscimol at 0.50 μg/side increased NREM sleep marked by slow and high-voltage EEG and low EMG activity accompanied with decreases in wakefulness and REM sleep, as compared with saline injection.

There was not significant effect of saline microinjected into the VP on sleep–wake states compared to baseline control. The hourly analysis of sleep–wake amounts for 12 h revealed that muscimol at 0.25 and 0.50 μg/side significantly increased NREM sleep lasted for 5 h (23:00–4:00) and 8 h (21:00–5:00), respectively, as compared with saline control. The enhancement of NREM sleep was concomitant with decreases in wakefulness and REM sleep. Besides, the effect was greater at a dose of 0.50 μg/side than 0.25 μg/side on the NREM sleep promotion and wakefulness suppression (Fig. 1b). There was a main effect of muscimol on wakefulness ($F_{3,20} = 90.885$, $P < 0.001$), NREM sleep ($F_{3,20} = 114.170$, $P < 0.001$) and REM sleep ($F_{3,20} = 7.147$, $P = 0.002$) during the 8 h post-injection time period. For the amount of time spent in each stage, muscimol at 0.25 and 0.50 μg/side increased NREM sleep by 50.49% (261.61 ± 19.18 min vs. 173.84 ± 8.94 min, $P < 0.001$) and 150.77% (435.94 ± 10.16 min vs. 173.84 ± 8.94 min, $P < 0.001$), respectively, as compared with saline control. Correspondingly, muscimol at 0.25 and 0.50 μg/side decreased wakefulness by 24.87% and 84.59%, respectively, and REM sleep by 82.11% and 100%, respectively, relative to the saline control. Compared to the muscimol at 0.25 μg/side, muscimol at 0.50 μg/side increased NREM sleep by 66.64%, and decreased wakefulness by 79.48%, while no significant difference in REM sleep (Fig. 1c). Thus, the effects of muscimol on the VP appeared to be dose-related.

To better understand the sleep–wake profile alterations of muscimol in different doses, we calculated the distribution of bouts in wakefulness, NREM and REM sleep for 8 h after microinjection. Muscimol at 0.25 μg/side increased the number of wakefulness and NREM sleep bouts in < 120 s, decreased the number of REM sleep bouts in < 120 s and 120–240 s, and decreased the number of wakefulness bouts in > 240 s, as compared with saline control. Muscimol at 0.50 μg/side decreased the number of NREM and REM sleep bouts both in < 120 s and 120–240 s, and decreased wakefulness and NREM sleep bouts in > 240 s, relative to the saline control (Fig. 2a). Further, muscimol at 0.25 μg/side increased the episode number of wakefulness and NREM sleep, decreased the episode number of REM sleep, and decreased the mean duration of wakefulness. Muscimol at 0.50 μg/side significantly decreased the episode number of each stage. However, the mean time spent in NREM sleep was robustly increased in accompany with decreases in wakefulness and REM sleep (Fig. 2b, c). An analysis of stage transition number for 8 h after muscimol administrated with 0.25 μg/side showed that the transition from wakefulness to NREM sleep and NREM sleep to wakefulness were increased, and NREM sleep to REM sleep and REM sleep to wakefulness were decreased, while muscimol at 0.50 μg/side showed that the transition between each stage was decreased, as compared with saline control (Fig. 2d).

### Microinjection of Bicuculline into the VP Increased Wakefulness in the Light Period

Next, we microinjected bicuculline, a $GABA_A$ receptor antagonist, at a dose of 0.25 μg/side into the bilateral VP

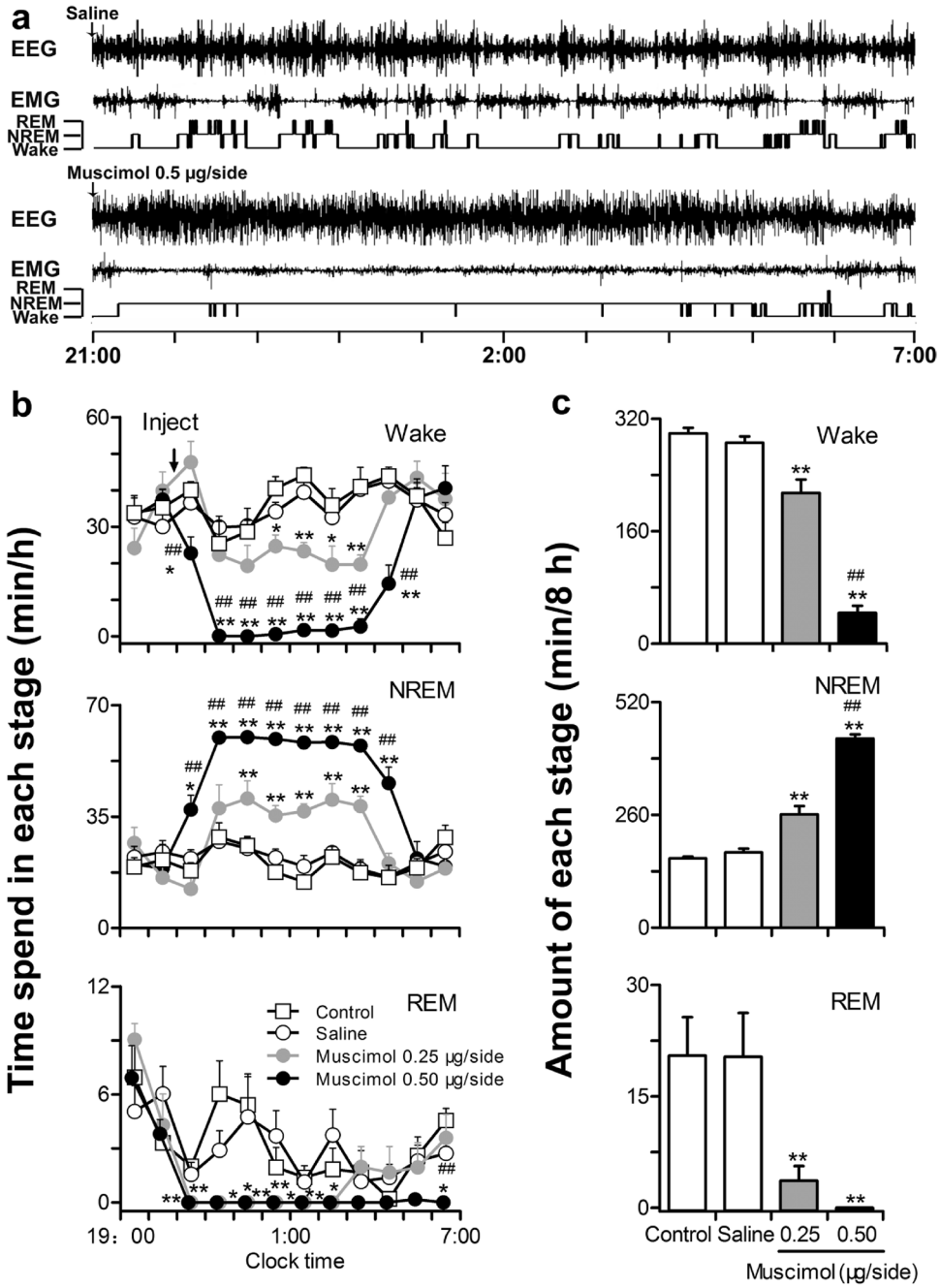


**Fig. 1** Microinjection of muscimol into the VP increased NREM sleep in the dark period. **a** Typical examples of the EEG/EMG recordings and the corresponding hypnograms after administration of saline or muscimol at 0.50 μg/side for 10 h (from 21:00 to 7:00). **b** 12 h time-course changes in wakefulness, NREM, and REM sleep in rats treated with muscimol at 0.25 or 0.50 μg/side. **c** Amount of time spent in wakefulness, NREM, and REM sleep for 8 h after the microinjection of muscimol at 0.25 or 0.50 μg/side. Note: There was not significant effect of saline microinjected into the VP on sleep–wake states compared to control. The *black arrow* indicates the time of injection. Values are the means ± SEM (n = 6). $^{*}P < 0.05$ and $^{**}P < 0.01$ versus saline, $\#P < 0.05$ and $\#\#P < 0.01$ 0.50 μg/side versus 0.25 μg/side. Data were analyzed by one-way ANOVA and followed by Fisher's LSD test

in freely moving rats at 09:00, a time when rodents usually show a high level of spontaneous sleep. Typical examples of EEG, EMG, and the corresponding hypnograms for 10 h from rats respectively given saline or bicuculline at 0.25 μg/side are represented in Fig. 3a. As shown, bicuculline at 0.25 μg/side increased wakefulness and decreased NREM and REM sleep, as compared with saline control. There was not significant effect of saline microinjected into the VP on sleep–wake states compared to baseline control. The hourly analysis of sleep–wake amounts for 12 h revealed that the wakefulness was increased by 143.27% and 99.78% during the first-hour and second-hour post-injection, respectively, as compared with saline control. The NREM sleep was decreased during the first-hour post-injection and the REM sleep was decreased during the first-hour, third-hour, sixth-hour and tenth-hour post-injection, relative to the saline control (Fig. 3b). There was a main effect of bicuculline on wakefulness ($F_{2,15} = 25.487$, $P < 0.001$), NREM sleep ($F_{2,15} = 16.845$, $P < 0.001$) and REM sleep ($F_{2,15} = 10.030$, $P = 0.002$) during the 2 h post-injection time period. For the amount of time spent in each stage, bicuculline at 0.25 μg/side increased wakefulness by 126.76% (77.80 ± 8.23 min

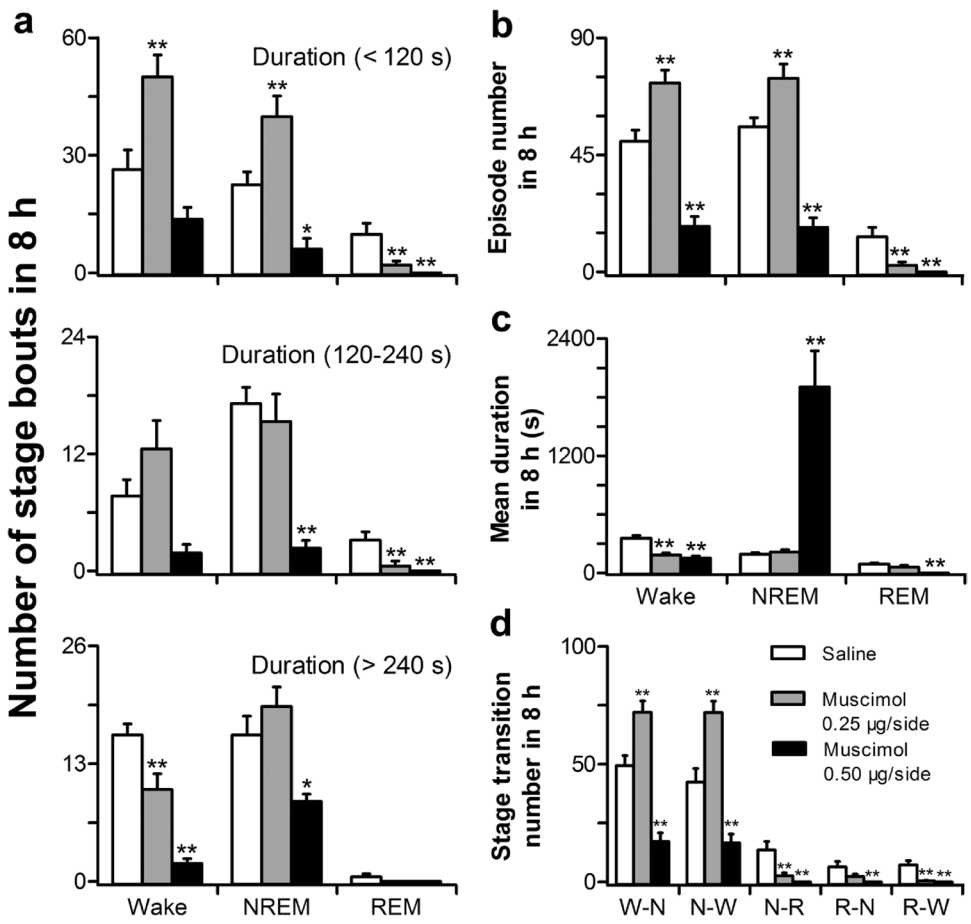


**Fig. 2** Characteristics of sleep–wake bouts, episode number, mean duration and stage transition number in 8 h after the administration of muscimol into the VP. The distribution of the number of each stage bouts in < 120 s, 120–240 s and > 240 s duration (**a**), episode number (**b**), mean duration (**c**) and stage transition number (W, N and R represent wakefulness, NREM sleep and REM sleep, respectively) (**d**) in 8 h. Values are the means ± SEM (n = 6 per group). *$P < 0.05$ and **$P < 0.01$ versus saline. Data were analyzed by one-way ANOVA and followed by Fisher's LSD test

vs. 34.31 ± 4.35 min, $P < 0.001$), and decreased NREM and REM sleep by 47.07% (42.06 ± 8.27 min vs. 79.47 ± 4.08 min, $P < 0.001$) and 97.75% (0.14 ± 0.14 min vs. 6.22 ± 1.32 min, $P = 0.014$), respectively, as compared with saline control (Fig. 3c).

## Microinjection of Muscimol into the VP Increased NREM Sleep in the Light Period

We have confirmed that inactivation of the VP GABAergic neurons increased NREM sleep prominently after microinjection of muscimol in the dark period. To further examine whether the muscimol will affect sleep–wake rhythm in the light period, muscimol at 0.50 μg/side was administrated bilaterally into the VP in freely moving rats at 9:00. Typical examples of EEG, EMG, and the corresponding hypnograms for 12 h from rats respectively given saline or muscimol at 0.50 μg/side are represented in Fig. 4a. As shown, muscimol at 0.50 μg/side increased NREM sleep and decreased wakefulness and REM sleep, as compared with saline control. There was not significant effect of saline microinjected into the VP on sleep–wake states compared to baseline control. The hourly analysis of sleep–wake amounts for 14 h revealed that the NREM sleep was increased lasted for 10 h (10:00–20:00), while the wakefulness and the REM sleep were decreased lasted for 5 h (10:00–15:00) and 10 h (9:00–19:00), respectively, as compared with saline control (Fig. 4b). There was a main effect of muscimol on wakefulness ($F_{2,15} = 35.339$, $P < 0.001$), NREM sleep ($F_{2,15} = 87.413$, $P < 0.001$) and REM sleep ($F_{2,15} = 23.304$, $P < 0.001$) during the 11 h post-injection time period. For the amount of time spent in each stage, muscimol at 0.50 μg/side increased NREM sleep by 32.75% (577.20 ± 10.91 min vs. 434.80 ± 5.18 min, $P < 0.001$), and decreased wakefulness and REM sleep by 54.11% (72.22 ± 8.57 min vs. 157.37 ± 7.86 min, $P < 0.001$) and 84.40% (10.58 ± 3.63 min vs. 67.84 ± 4.65 min, $P < 0.001$), respectively, relative to the saline control (Fig. 4c).

Schematic representation of the implantation sites of the guide cannulas and injector cannulas is shown in the Fig. 5a. In our study, the bilateral microinjection sites of rats located only in the VP were used for this study (Fig. 5b-e).

## Discussion

Our data here demonstrated that the microinjection of muscimol into the bilateral VP in freely moving rats during the dark period induced a dose-dependent increases in NREM

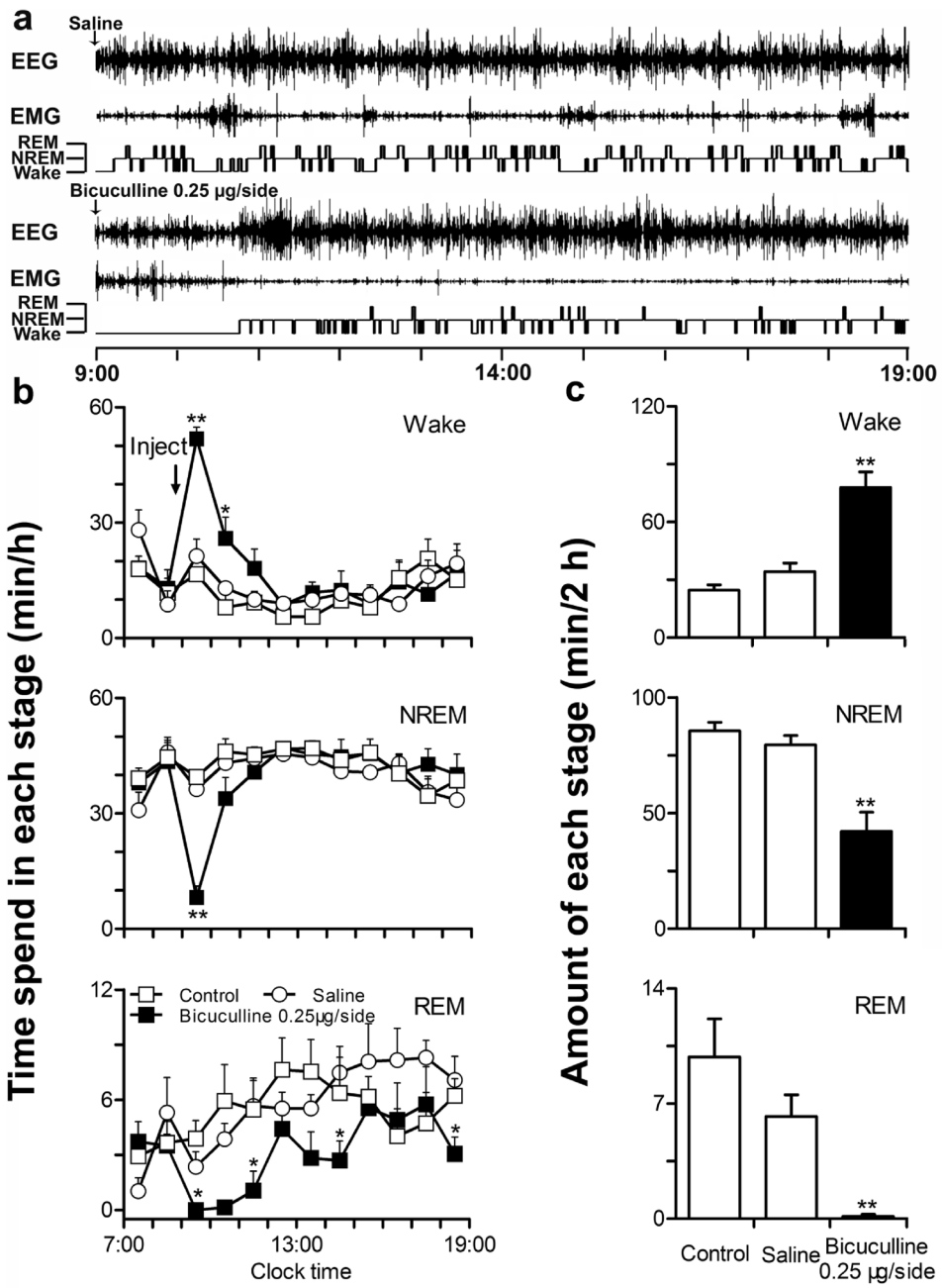


**Fig. 3** Microinjection of bicuculline into the VP increased wakefulness in the light period. **a** Typical examples of the EEG/EMG recordings and the corresponding hypnograms after administration of saline or bicuculline at 0.25 μg/side for 10 h (from 9:00 to 19:00). **b** 12 h time-course changes in wakefulness, NREM, and REM sleep in rats treated with bicuculline at 0.25 μg/side. **c** Amount of time spent in wakefulness, NREM, and REM sleep for 2 h after the microinjection of bicuculline at 0.25 μg/side. Note: There was not significant effect of saline microinjected into the VP on sleep–wake states compared to control. The *black arrow* indicates the time of injection. Values are the means ± SEM (n = 6). $^{*}P < 0.05$ and $^{**}P < 0.01$ versus saline. Data were analyzed by one-way ANOVA and followed by Fisher's LSD test

sleep, and decreases in wakefulness and REM sleep. In a further analysis of the characteristics of NREM sleep bouts, we documented that muscimol at 0.25 μg/side promoted NREM sleep by increasing the episode number, whereas muscimol at 0.50 μg/side promoted NREM sleep mainly through extending the sleep duration time even if the episode number decreased, as compared with saline control. Moreover, the further study was performed by bicuculline or muscimol bilaterally administrated into the VP during the light period. The results indicated that bicuculline at 0.25 μg/side increased wakefulness accompanied with decreases in NREM and REM sleep and the effect of muscimol at 0.50 μg/side in the light period was similar to the dark period but lasted longer.

In current study, only male rats were used, given that unstable hormone level of female rats during proestrus and estrous phases since the high hormone of proestrus phase decreases the amount of NREM sleep and REM sleep and increases the amount of wakefulness compared with estrous phase in female rats [26]. Secondly, the illustrations of bilateral injection sites are shown in Fig. 5c-e for each treatment animal. The modest volume of drugs and saline (0.25 μl) used in our study were slightly less [27] or more [28] than used in other studies where the drugs diffusion

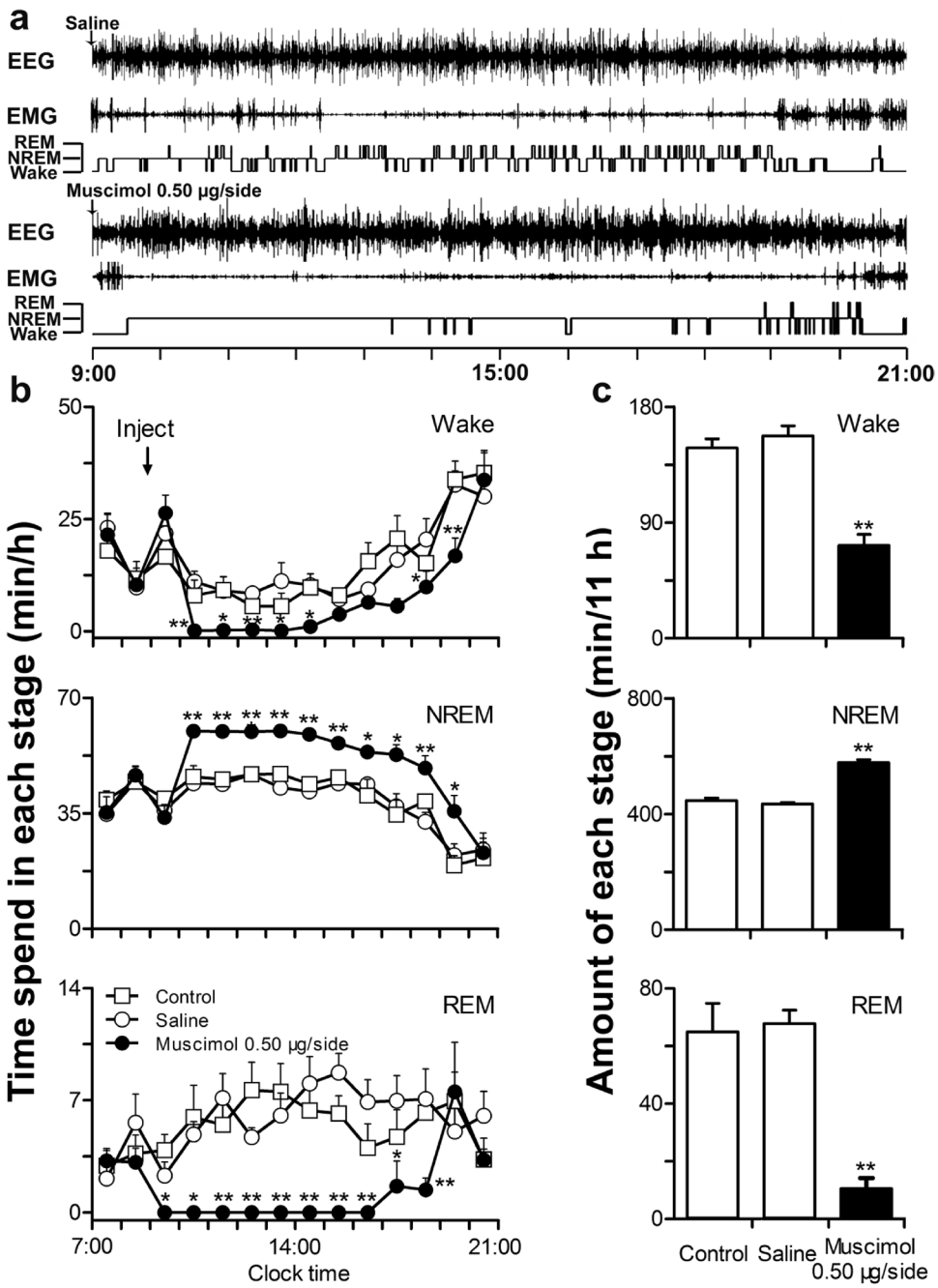


**Fig. 4** Microinjection of muscimol into the VP increased NREM sleep in the light period. **a** Typical examples of the EEG/EMG recordings and the corresponding hypnograms after administration of saline or muscimol at 0.50 μg/side for 12 h (from 9:00 to 21:00). **b** 14 h time-course changes in wakefulness, NREM, and REM sleep in rats treated with muscimol at 0.50 μg/side. **c** Amount of time spent in wakefulness, NREM, and REM sleep for 11 h after the microinjection of muscimol at 0.50 μg/side. Note: There was not significant effect of saline microinjected into the VP on sleep–wake states compared to control. The *black arrow* indicates the time of injection. Values are the means ± SEM (n = 6). $^{*}P < 0.05$ and $^{**}P < 0.01$ versus saline. Data were analyzed by one-way ANOVA and followed by Fisher's LSD test

areas occupied approximately spherical volumes between 0.5 and 1.0 mm in diameter. Thus, it proves that the compound infused was almost restricted to the VP area. Given the pharmacological microinfusion of VP cells without being able to prove restriction to the VP area, the immunoreactivity of c-fos, a marker of neuronal activation, shall be performed to help delineate the spreading of their injections in the future study.

GABA, the most abundant inhibitory neurotransmitter in the brain, interacts with two different receptor subtypes, namely $GABA_A$ and $GABA_B$. However, GABA mediates inhibition mainly via $GABA_A$ receptors which are the ligand-gated chloride ion channels [29]. Many sedatives and anaesthetics target has been identified as the $GABA_A$ receptor. These include intravenous anesthetics, such as propofol, and benzodiazepines, such as diazepam [30]. More importantly, recent findings suggest that BF GABAergic neurons play a major role in the sleep–wake regulation [18, 19]. Using chemogenetic method, Anaclet et al. [18] proved that activation of BF GABAergic neurons, including the VP neurons, produces sustained wakefulness, whereas inhibition increases sleep. Our results further support the prominent

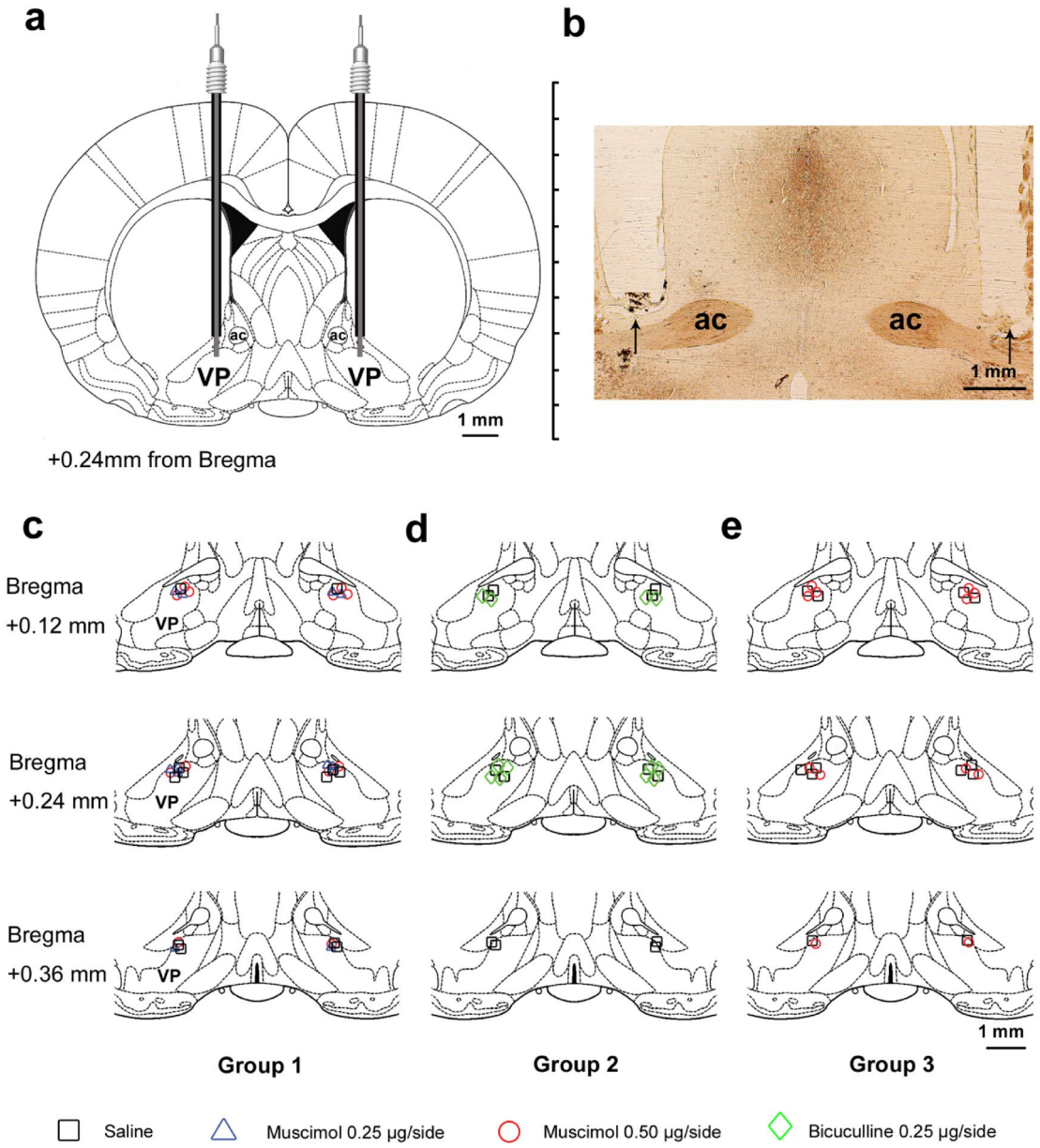


**Fig. 5** The illustrations of bilateral microinjection into the VP in rats. **a** Schematic representation of the implantation sites of the guide cannulas (*black*) 0.5 mm above the VP. The injector cannulas (*grey*) are within the VP (AP: + 0.24 mm from bregma). **b** Photomicrograph of a coronal brain section showing the tracks of the guide cannulas 0.5 mm above the VP (×4). The *black arrows* show the tip of the guide cannulas. **c-e** Schematic representation of the injection sites in the VP in group 1 (microinjection of muscimol into the VP in the dark period) (**c**), group 2 (microinjection of bicuculline into the VP in the light period) (**d**), group 3 (microinjection of muscimol into the VP in the light period) (**e**). Sections (**a, c-e**) were according to Paxinos and Watson (2007). Scale bar: 1 mm. *VP* ventral pallidum, *ac* anterior commissure

role of VP GABAergic neurons in the control of sleep–wake states by pharmacological manipulations. Moreover, based on the neural tracing studies established that BF GABAergic neurons directly innervate inhibitory cortical interneurons, which may be the circuitry that regulates sleep–wake states [31, 32]. The mechanisms that regulate sleep and wakefulness by VP GABAergic neurons need to be uncovered in the future study.

The VP is a central convergent point for input from several structures related to reward, and serves as one of the most important sites for motivation and reward in the brain [4]. These behaviors ensure survival and reproduction in nature, which depend on heightened arousal. For example, injection of the $GABA_A$ receptor antagonist within VP results in sniffing, gnawing, tongue protrusion, and chewing behaviors in rats [33], whereas infusion of $GABA_A$ receptor

agonist bilaterally into VP suppresses locomotion and rearing [34]. In our study, after infusion of bicuculline into VP bilaterally, the rats presented abnormal pivoting movements and compulsive gnawing, which is consistent with previous work [33–35]. It is still unknown the underlying mechanisms of such abnormalities, but their relationship with generalized locomotor activation are relatively minimal. In view of this, one may be inclined to speculate that descending projections of the VP are related to the intended movement, or alternative to the intentional actions [33].

According to the differences between neurotensin- and calbindin d28k-immunoreactivities (Enk-IR or CalB-IR, respectively), the VP is divided into the ventromedial VP (VPm), dorsolateral VP (VPl) and rostral VP (VPr). Earlier studies identified the VPm and VPl receiving projections from the NAc shell and core, respectively, and with distinct projection targets [11, 12, 36, 37]. Recent findings revealed that the NAc shell and core have different function for sleep–wake regulation [21, 38]. In the present study, the microinjection of muscimol into VP mimics the effect of NAc GABA neurotransmitter released into VP. Although the injector cannulas we inserted through were mainly targeted the VPl, the infusion would penetrate into other VP region. Therefore, the subregions of VP in sleep–wake regulation still needs further exploration.

The ventral mesencephalon (VM) and the mediodorsal thalamus (MDT) are the main downstream areas of the VP [12, 39]. They received both NAc $D_1$- and $D_2$- medium spiny neurons (MSNs) through output neurons of the VP [39]. Previous neuroanatomical studies have reported that VPl and VPm efferent projections are largely segregated in dopaminergic mesencephalon. VPm mostly targets the ventral tegmental area (VTA) while the VPl preferentially innervates the substantia nigra pars reticulata (SNr) and compacta (SNc) [1, 12]. In recent years, growing evidence suggests that VTA dopaminergic neurons are necessary for arousal and their inhibition suppresses wakefulness [40, 41]. Besides, in Parkinson's disease, where dopamine neurons in the substantia nigra degenerate, waking is interrupted by sleep episodes [42]. Moreover, combining neural activity recordings with EEG recordings revealed a strong vigilance state dependent modulation of neuronal activity with increased activity during wakefulness and REM sleep relative to NREM sleep in both SN and VTA [43]. Thus, the indirect pathways of NAc core-VPl-VTA and NAc shell-VPm-SN may play a role in the sleep–wake regulation.

In conclusion, we found that muscimol inactivated the VP GABAergic system and enhanced NREM sleep in rats. VP $GABA_A$ receptor-expressing neurons played an important role in the sleep–wake cycle.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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