Sleep Medicine 14 (2013) 1076–1084

Contents lists available at ScienceDirect

Sleep Medicine

journal homepage: www.elsevier.com/locate/sleep

Original Article

# Electrical stimulation of the rostral ventrolateral medulla promotes wakefulness in rats

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ARTICLE INFO

*Article history:*
Available online 21 August 2013

*Keywords:*
Rostral ventrolateral medulla
Electrical stimulation
Sleep
Heart rate variability
Sympathetic nervous system
Arousal

ABSTRACT

*Objectives:* Previous studies suggest that central sympathetic activity might carry information on wakefulness, so we tested the hypothesis that direct activation of the rostral ventrolateral medulla (RVLM), a well-studied sympathetic vasomotor center, promotes wakefulness.

*Methods:* A bipolar stimulating electrode was implanted in the right RVLM of Wistar-Kyoto rats or in a brainstem control site. Bioelectrical signals were recorded using a telemetry system. The experiment comprised a baseline session and a 6-h electrical stimulation session (50 μA, 50 Hz for 3 min every 20 min). Sleep–wake stages were defined by the electroencephalogram (EEG) and electromyogram (EMG) as active waking (AW), nonrapid eye movement (NREM) sleep, and rapid eye movement (REM) sleep. Autonomic function was assessed using cardiovascular variability analysis.

*Results:* During the RVLM stimulation session, AW time increased from 38.48 ± 5.82 to 99.91 ± 8.23 min compared with baseline ($P < .001$), while REM sleep was decreased from 110.10 ± 4.91 to 50.74 ± 13.01 min ($P = .004$). Analysis of the RVLM stimulation bouts delivered during NREM sleep showed a significantly higher probability of awakening; it also showed that the latency to arousal was significantly shorter than the latency for 10% blood pressure (BP) increase (1.50 ± 0.30 vs 7.42 ± 1.83 s; $P = .009$).

*Conclusions:* Our findings show that direct stimulation of the RVLM promotes wakefulness, suggesting that sleep disturbance may result from central sympathetic activation.



## 1. Introduction

Autonomic functioning closely fluctuates with the sleep–wake cycle. There is a decrease in sympathetic activity from wakefulness to nonrapid eye movement (NREM) sleep, and a reversal of this change may occur during rapid eye movement (REM) sleep [1]. Inadequate sleep disrupts the sympathovagal balance, and thus increases the risk for developing hypertension [2,3]. However, less is known about the impact of sympathetic overactivity on sleep. Indeed poor sleep quality is common among hypertensive individuals [4–6], and the degree of sleep disturbance has been shown to be associated with the severity of hypertension [6,7]. Thus central sympathetic overactivity may not only play a critical role in the development and maintenance of hypertension [8,9], but it also may account for sleep problems in hypertensive individuals. To shed light on this speculation, we explored an essential question related to this; namely, does activation of the rostral ventrolateral medulla (RVLM), a sympathetic center, disturb normal sleep–wake cycles?

The RVLM consists of a neurochemically heterogeneous collection of neurons. Mounting evidence has solidly established the RVLM as a major vasomotor center that harbors sympathetic premotor neurons involved in maintaining blood pressure (BP) [9–11]. Beyond this neuroanatomic and electrophysiologic studies have revealed that substantial inputs to the locus coeruleus, a well-studied wake-promoting center, emanate from the RVLM [12–18]. In addition, afferents to the sleep center, the ventrolateral and median preoptic area, have been shown to originate in part from the RVLM [12,19]. Moreover, clonidine injected to the RVLM has been shown to be both hypotensive and sedative [20]. Based on these findings, Samuels and Szabadi [17,21] pointed out the potential role of the RVLM as an arousal promoting area and in the modulation of waking and sleep behavior. The most promising evidence to date comes from a recent work by Abbott et al. [22],

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1389-9457/$ - see front matter 
http://dx.doi.org/10.1016/j.sleep.2013.06.011

who found an arousal effect of the activation of C1 neurons during sleep. The hypothetic scheme may be that the RVLM neurons send excitatory ascending axonal projections to the locus coeruleus, which then ascends to the cortex to desynchronize its electrical activity.

A functional role of the central sympathetic network in the arousal mechanism has been suggested from a number of studies. In human subjects, analysis of beat-to-beat intervals or muscle sympathetic nerve activity in proximity to cortical arousal showed that heart rate accelerated and sympathetic nerves fired before the onset of arousal [23–25]. With the aid of cardiovascular variability analysis, we observed that sympathetic activation preceded the transition to wakefulness in rats during NREM sleep [26]. Moreover, indirect stimulation of the sympathetic activity by acute hypotension has been shown to arouse sleeping lambs [27]. However, these findings are insufficient to demonstrate the causal role of central sympathetic activation in promoting wakefulness. Therefore, our study aimed to test the hypothesis that electrical stimulation of the RVLM will promote wakefulness in rats.

## 2. Methods

### 2.1. Animals and surgery

Male Wistar-Kyoto rats (200–250 g) were used in our experiment (eight for the control group and eight for the experimental group). These animals were an inbred strain and were obtained from National Laboratory Animal Center in Taiwan based on the principles listed in the Position of the American Heart Association on Research Animal Use. The animals were housed under a 12-h light–dark cycle (8:00 am–8:00 pm lights on) with food and water provided ad libitum. The experiments were performed during the light period. All surgical and testing procedures were approved by the Institutional Animal Care and Use Committee of National Yang Ming University. Under anesthesia with 50 mg/kg pentobarbital, the skull surface was exposed and five screws were fixed into it, one of which served as the right parietal electrode (2 mm lateral and 2 mm posterior to bregma), one of which served as the occipital electrode (2 mm lateral and 2 mm anterior to lamda), and another in the occipital bone acted as the reference electrode.

The electromyogram (EMG) was recorded from two electrodes inserted into the dorsal neck muscles, and the electrocardiogram (ECG) was recorded from two electrodes placed dorsally under skin. A telemetry transmitter (TA11PA-C40, Data Sciences, St. Paul, MN, USA) was implanted in the abdomen to collect BP signals of the abdominal aorta. A concentric bipolar stainless-steel stimulating electrode (SNEX-100, David Kopf, Tujunga, CA, USA) was implanted in the RVLM (4.2–4.5 mm posterior to lamda, 1.6–1.8 mm lateral to midline, and 8–9 mm from dura) [28]. The final position of the electrode was verified using online monitoring of BP and was determined as the site at which a train of electrical stimulation (50 μA, 50 Hz, 10 s) provoked at least a 50-mmHg increase in BP. For the control group, all of the above mentioned procedures were done in the same way, except that the stimulating electrode was implanted above the RVLM approximately 5 mm from dura where electrical stimulation induced no response in BP (Fig. 1). However, some of the control electrodes fell outside the brainstem, and this approach was considered a limitation of our study. These electrodes were joined to a connector fixed to the skull by dental acrylic. After 7 days of recovery from surgery, the rats began the experimental procedures.

### 2.2. Experimental protocols

On day 1, the experimental or control rat was placed in the recording cage for habituation. On day 2, a telemetry sensor and a sham stimulator were worn on the head of the rat, and the rat was recorded for 24 h (from lights on) to collect a baseline dataset. On day 3, a telemetry electrical stimulator incorporated with a telemetry recording sensor was mounted on the head of the rat, and the rat received a 10-h recording (from lights on), in which a 6-h electrical stimulation session started from 2 h after light on. The electrical stimulation was delivered at 50 μA at 50 Hz (pulse duration, 1 ms; interpulse interval, 20 ms) for 3 min with an interstimulation interval of 17 min, thus a total of 18 stimulations over 6 h. The electrical stimuli were wirelessly commanded from a nearby computer where the stimulation pattern was defined. When the electrical stimulation was not able to cause a visible increase in BP, the recording was terminated and the animal was discarded ($n = 2$).

### 2.3. Signal recording

The electrophysiologic signals were recorded by a wireless sensor (KY4C, K&Y Lab, Taiwan) (size, 25 × 21 × 13 mm; weight, 8.3 g). The electroencephalogram (EEG), EMG, and ECG signals were amplified 1000-, 1000-, and 500-fold, respectively, and filtered at 0.16–48 Hz, 34–103 Hz, and 0.72–103 Hz, respectively

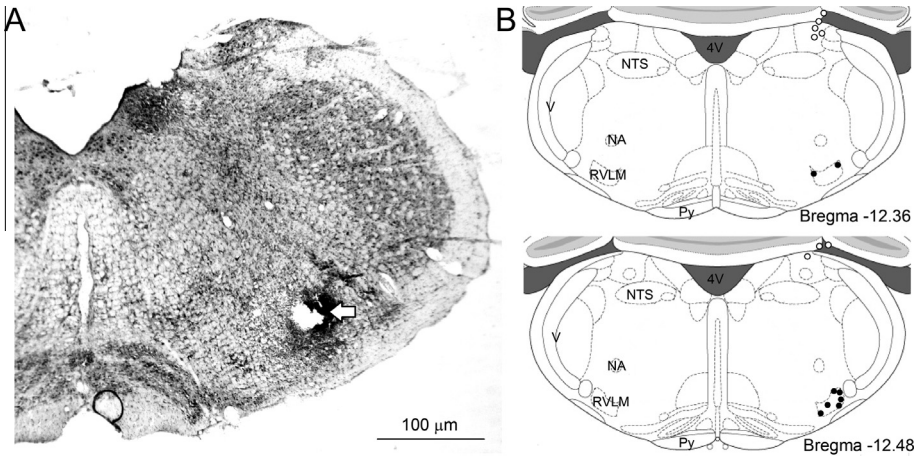


**Fig. 1.** Anatomic location of the stimulating electrode. Photomicrograph of a coronal section taken through the rostral medulla (A). Diagrammatic representation of two levels of the rostral medulla showing electrode locations of the experimental (closed circles) and control (open circles) groups (B). *Abbreviations:* NTS, nucleus tractus solitarius; NA, nucleus ambiguus; RVLM, rostral ventrolateral medulla. Arrow indicates the location of the electrode tip.