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Are there Sleep-promoting Neurons in the Mouse Parafacial Zone?

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Abstract—Although recent studies have reported that gamma-aminobutyric acid (GABA) neurons in the parafacial zone (PZ) of the rostral medulla are needed for the induction of slow-wave sleep (SWS) and that the PZ is a medullary SWS-promoting center, it remains unknown whether the PZ contains SWS-active or sleep-promoting neurons. In the present study, a total of 125 neurons were recorded, for the first time, in non-anesthetized, head-restrained mice during the complete wake–sleep cycle throughout the PZ of the rostral medulla. The vast majority (87.2%) of the neurons displayed increased activity during both wakefulness (W) and paradoxical (or rapid eye movement) sleep (PS) compared to during SWS (W/PS-active neurons) and a few (8.0%) discharged phasically and selectively during PS (PS-active neurons), but none discharged maximally during SWS (SWS-active neurons) or displayed a higher rate of spontaneous discharge during both SWS and PS than during W (SWS/PS-active neurons). These findings do not support the view that the GABAergic PZ is a medullary SWS-promoting center.

Key words: medullary parafacial zone, parvicellular reticular nucleus alpha, GABAergic/glycinergic neurons, slow-wave sleep, sleep-promoting neurons.

INTRODUCTION

As first described by Bremer (1935), when the midbrain is completely transected just behind the third nerves, the cat exhibits ocular behavior and electroencephalographic (EEG) patterns similar to those of a sleeping cat, and the switch from sleep to waking is completely abolished throughout the survival period of an acute preparation. However, this switch is retained after acute spinal section at the C1 cervical segment, suggesting that certain brain structures responsible for the switch from sleep to waking lie between the midbrain and the lower medulla (for reviews, see Moruzzi, 1972). In contrast, transection at the mid-pontine level results in a wake-like state characterized by EEG activation and the presence of ocular tracking behavior (Batini et al., 1958; Moruzzi, 1972). When cats with midbrain transections are followed for a longer period of time, they begin to show periodically ocular and EEG patterns similar to those in a waking cat, but the isolated forebrain and hindbrain show different "sleep" and "waking" changes (Villablanca et al., 2001; Sakai and Crochet, 2003). These changes are also seen in chronically maintained cats with transection at the pontomedullary junction, although paradoxical, or rapid eye movement (REM), sleep (PS) is no longer evident (Webster et al., 1986; Vanni-Mercier et al., 1991).

A previous functional magnetic resonance imaging (fMRI) study in humans (Dang-Vu et al., 2008) demonstrated increased activity associated with SWS in the brainstem, particularly in a midbrain/pontine tegmental region near the locus coeruleus (LC). Interestingly, in a recent single-unit recording study in the mouse, I reported the existence of sleep-specific, possibly sleep-promoting, neurons near the LC in a region referred to as the sublaterodorsal tegmental nucleus (SubLDT) (Sakai, 2015). In addition, a recent study in transgenic mice demonstrated that pharmacogenetic excitation of glutamatergic neurons located in the rostral SubLDT promotes SWS (Hayashi et al., 2015). Although sleep-specific, possibly sleep-promoting, neurons have also been described in the dorsal raphe nucleus of the rostral pons in the cat (Sakai and Crochet, 2001a) and mouse (Sakai, 2011), little is known about whether sleep-promoting neurons are present in the medulla. Recently, Anaclet et al. (2012, 2014) reported that, in rodents, gamma-aminobutyric acid

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Abbreviations: AW, active wakefulness; ChAT, choline acetyltransferase; D, drowsy state; EEG, electroencephalogram; EMG, electromyogram; fMRI, functional magnetic resonance imaging; GABA, gamma-aminobutyric acid; IRt, intermediate reticular nucleus; LC, locus coeruleus; NTS, nucleus of the solitary tract; PCRtA, parvicellular reticular nucleus, pars alpha; PS, paradoxical sleep; PSt, transition period from slow-wave sleep to PS; PZ, parafacial zone; QW, quiet wakefulness; REM, rapid eye movement; SubLDT, sublaterodorsal tegmental nucleus; SWS, slow-wave sleep; W, wakefulness.

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(GABA)/glycine neurons located in the PZ play an important role in the induction of SWS and that the PZ is a medullary SWS-promoting center. However, it is not known whether the PZ contains sleep-active, sleep-promoting neurons in rodents. In the present study, therefore, extracellular single-unit recording with high-impedance glass pipette microelectrodes was used in non-anesthetized, head-restrained mice to record a large number of neurons throughout the PZ during the complete wake-sleep cycle in order to determine whether or not sleep-active, in particular SWS-active neurons that discharge maximally during SWS, are present in this discrete region of the rostral medulla. Here, I report that, in the mouse, no sleep-selective, possibly sleep-promoting, neurons are found in the PZ of the rostral medulla, which is inconsistent with the view that the PZ is a medullary SWS-promoting center.

EXPERIMENTAL PROCEDURES

Animals and surgery

The study was approved by the University of Lyon 1 Animal Care Committee, the standards of which meet those of the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering.

Six male adult C57BL/6 mice weighing 28–32 g at the time of surgery were used. As described in detail previously (Sakai, 2015), electrodes were implanted to record the cortical EEG, neck electromyogram (EMG), and electrocardiogram, a 30-gauge stainless steel tube was fixed on the skull as a stereotaxic reference and a U-shaped plastic plate was fixed to the skull so that the cranium could be painlessly returned to the same stereotaxic position.

Extracellular single-unit and polygraphic recordings

After recovery, all animals were progressively habituated to the head-restrained position for 7–14 days. Single neuronal activity was then recorded extracellularly using a glass pipette microelectrode filled with 0.5 M sodium acetate solution containing 2% Direct Blue 15 (Sigma, St Louis, USA). Neuronal activity was recorded after amplification and filtering (0.1–50.0 kHz) using a NeuroLog system (Digitimer, Hertfordshire, UK). Neuronal activity and the polygraphic signals were digitized at a sampling rate of, respectively, 20.8 kHz and 508.1 Hz using a CED 1410 data processor (Cambridge Electronic Design [CED], Cambridge, UK) and stored on a personal computer.

Unit recordings were made either unilaterally or bilaterally at intervals of 0.2 mm rostrocaudally and 0.1–0.2 mm mediolaterally. In order to mark the recording site, Direct Blue 15 was injected from the recording electrode at the end of each experiment. Unit recordings were carried out during two experimental sessions per day for 5–10 consecutive days. During the experiment, behavior was monitored using a video camera placed in front of the mouse and Logitech QuickCam software (Logitech France SA, Paris, France), as described previously (Sakai, 2015).

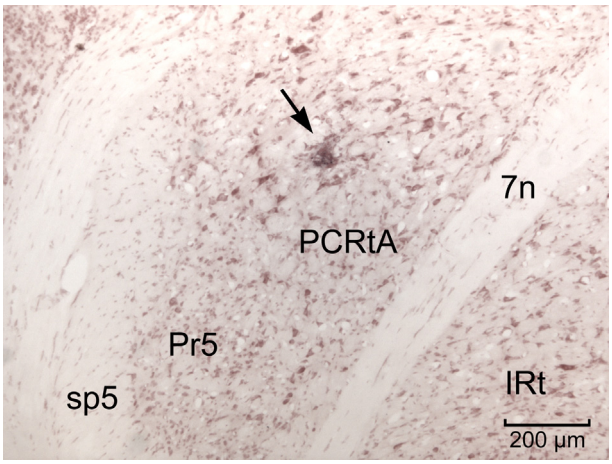

Fig. 1. Photomicrograph showing a unit recording site marked with Direct Blue 15 (arrow) in the mouse parvicellular reticular nucleus, pars alpha (PCRtA). The section was counterstained with Neutral red. 7n, facial nerve; IRt, intermediate reticular nucleus; Pr5, principal sensory trigeminal nucleus; sp5, spinal trigeminal tract. Nomenclature according to the mouse atlas of Paxinos and Franklin (2001).

Histochemistry and determination of unit recording sites

Under deep pentobarbital anesthesia, all animals were perfused transcardially with Ringer's solution, followed by fixative consisting of 4% paraformaldehyde, 0.05% glutaraldehyde, and 0.2% picric acid in 0.1 M phosphate buffer, pH 7.4. The brain was then removed, postfixed for 24 h at 4 °C in glutaraldehyde-free fixative, and placed in 0.1 M phosphate buffer, pH 7.4, containing 30% sucrose. Twenty-micrometer coronal sections were then cut serially on a cryostat and the localization of the unit recording sites determined histologically, as previously described (Sakai, 2015). Choline acetyltransferase (ChAT) immunostaining was performed as described in a previous paper (Sakai, 2012).

Data analysis

Wake–sleep stages. Wake–sleep stages were scored using 3-s bins. Mean discharge rates were calculated from all of the recordings for each unit using 1- to 10-s bins for each of the following 7 states: (1) active or attentive W (AW; 1- to 10-s bins); (2) quiet W (QW; 2- to 10-s bins); (3) drowsy state (D; 3-s bins); this state corresponded to the first 3-s period from the onset of EEG synchronization during the transition from W to SWS; (4) light SWS or S1 (10-s bins); (5) deep SWS or S2 (10-s bins); (6) PSt, the transition period from SWS to PS (10-s bins); and (7) PS (10-s bins), as described in detail in a previous paper (Sakai, 2015).

Spike shape and duration. Spike shape and duration were determined for each unit from averaged action potentials using a low frequency cutoff at 100 Hz. The