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LOCUS COERULEUS NEURONAL ACTIVITY DURING THE SLEEP-WAKING CYCLE IN MICE

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Abstract—Using extracellular single-unit recordings in non-anesthetized, head-restrained mice, we examined spontaneous and evoked discharges of noradrenaline-containing locus coeruleus (NA-LC) neurons across the sleep-waking cycle. The neurons were all characterized by triphasic broad action potentials. They discharged as either slow (<6 Hz) tonic, single spikes or phasic clusters of spikes specific to wakefulness (W), the discharge rate being highest during active waking and significantly lower during quiet waking. They remained totally silent during both slow-wave sleep (SWS) and paradoxical (or rapid eye movement (REM)) sleep. The phasic unit activity was related to abrupt activation of electromyographic activity occurring either spontaneously or elicited by alerting sensory stimuli. At the transition from waking to sleep, they ceased firing before the onset of cortical synchronization (deactivation), the first sign of electroencephalographic sleep, a significant decrease in firing rate preceding the onset of unit activity of sleep-specific neurons in the basal forebrain (BFB)/preoptic (POA) hypothalamus, as described previously [Takahashi K, Lin JS, Sakai K (2009) Neuroscience 161:269–292]. At the transition from SWS to waking, they fired before the onset of both cortical activation and a significant decrease in activity of sleep-specific neurons. These findings support the previous view that the NA-LC system is involved in both tonic and phasic processes of arousal, and further support our previous proposals that initiation of sleep is caused by decreased activity of waking-promoting neurons (disfacilitation) and that NA-LC neurons play an important role in the sleep/waking switch, that is from waking to sleep and from sleep to waking [Takahashi K, Lin JS, Sakai K (2009) Neuroscience 161:269–292].

Key words: locus coeruleus, single unit recording, arousal, sleep-waking switch, disfacilitation.

The nucleus locus coeruleus (LC) in the mouse is a dense collection of noradrenaline (NA)-containing neurons in the dorsolateral pontine tegmentum (Ginovart et al., 1996), as first observed in the rat (Dahlström and Fuxe, 1964). In rats, cats, and monkeys, NA-LC neurons have been reported to display state-dependent neuronal activity, discharging at highest rates during wakefulness (W), at lower rates during slow-wave sleep (SWS), and exhibiting complete cessation of discharge during paradoxical sleep (PS), or rapid eye movement (REM) sleep (Foote et al., 1983; Jacobs, 1986; Steriade and McCarley, 1990). NA-LC neurons play an important role in selective attention, general arousal, stress reactions due to challenging environmental situations, and neuronal gene expression (Levine et al., 1990; Cirelli et al., 1996; Aston-Jones et al., 2000; Berridge and Waterhouse, 2003). Early brain section, lesion, and stimulation studies identified a critical role of the brainstem neural systems, including the NA-LC system, in the induction and maintenance of arousal (Moruzzi, 1972; Sakai and Crochet, 2003; Jones, 2005). On the other hand, both clinical and experimental evidence indicated a crucial role of the preoptic area (POA) of the anterior hypothalamus and adjacent basal forebrain (BFB) in the induction and maintenance of sleep, and suggested that a reciprocal inhibitory interaction between the sleep-promoting system in the POA/BFB and the waking-promoting system in the posterior hypothalamus (PH) accounts for the alternation between sleep and waking (von Economo, 1929; Nauta, 1946; Moruzzi, 1972). According to a current prevailing hypothesis, sleep-promoting, or hypnogenic, neurons, located in the POA/BFB, contain GABA and inhibit whole waking-promoting neurons in the brainstem neural systems at sleep onset and during sleep (McGinty and Szymusiak, 2001; Saper et al., 2001; Jones, 2005). According to this hypothesis, the sleep process starts and ends with the beginning or cessation, respectively, of firing of POA/BFB hypnogenic neurons. However, in a recent study in mice (Takahashi et al., 2009), we found both sleep-specific and waking-specific neurons in the POA/BFB and demonstrated that, during sleep-wake state transitions, activity of the sleep-specific neurons follows, and does not precede, that of waking-promoting neurons in the POA/BFB and of histamine (HA)-or orexin (also called hypocretin, Orx/Hcrt)-containing waking-promoting neurons in the PH. Because both POA/BFB sleep-promoting and waking-promoting neurons are under brainstem adrenergic and noradrenergic control (Osaka and Matsumura, 1994, 1995), we hypothesized that brainstem catecholaminergic neurons, in particular NA-LC neurons, may represent the critical waking-promoting neurons that control the sleep-waking

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Abbreviations: AW, active waking; BFB, basal forebrain; ChAT, choline acetyltransferase; CV, coefficient of variation; D, drowsy state; DV, dorso-ventral; ECG, electrocardiogram; EEG, electroencephalogram; EMG, electromyogram; HA, histamine; IMEANFR, instantaneous mean frequency; LC, nucleus locus coeruleus; NA, noradrenaline; Orx/Hcrt, orexin/hypocretin; PH, posterior hypothalamus; POA, preoptic area; PS, paradoxical sleep; QW, quiet waking; REM, rapid eye movement; SWS, slow-wave sleep; TH, tyrosine hydroxylase; TM, tuberomammillary nucleus; W, wakefulness.

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switch. Currently, no information is available about the activity of mouse NA-LC neurons in general and their unit activity profiles during state transitions in particular, despite the increasing use of the mouse in experimental models. We therefore recorded, for the first time, from mouse NA-LC neurons, determined their discharge properties during the complete sleep-waking cycle and their state transitions, and compared their activity profiles with those of the sleep-specific neurons in the POA/BFB and waking-specific neurons in the POA/BFB and PH in order to elucidate the role of NA-LC neurons in the sleep-waking switch.

EXPERIMENTAL PROCEDURES

Animals and surgery

All procedures were approved by the University of Lyon 1 Animal Care Committee, the standards of which meet those of the EEC Guidelines (86/609/EEC) and the Policy on Ethics approved by the Society for Neuroscience (1993). All efforts were made to minimize the number of animals used and their suffering.

Fifteen male adult C57BL/6 mice (Harlan, France; 25–34 g at the time of surgery) were used. The mice were anesthetized using a mixture of 0.8 mg/ml of ketamine (Imalgene 1000, Merial, Lyon, France) and 1 mg/ml of xylazine (Rompun 2%, Bayer Pharma, Puteaux, France) at an initial dose of 10 ml/kg, with 3 ml/kg boosters as required, given i.p. The mice were placed in a stereotaxic apparatus (SN-3, Narishige, Tokyo, Japan) with blunt ear bars and were implanted with electrodes to record the neocortical electroencephalogram (EEG), neck electromyogram (EMG), and electrocardiogram (ECG) as described previously (Takahashi et al., 2008). One cannula (30 gauge) was fixed on the skull (anteroposterior (AP) 2.0 mm from the bregma and 0.0 mm from the medio-lateral (ML)) and used as a stereotaxic reference during the experiment. In addition, a U-shaped plastic plate (18 mm wide, 16 mm long, 5 mm thick) was fixed stereotaxically to the skull using dental acrylic cement so that the cranium could be painlessly returned to the same stereotaxic position using a chronic head holder (SA-8, Narishige). A small hole was drilled in the skull above the LC region and covered with antibiotic cream for the subsequent insertion of microelectrodes.

Extracellular single unit and polygraphic recordings

After a recovery period of 1 week, the animals were progressively habituated to the head-restrained position (7–14 days) by placing them on a cotton sheet inside a plastic box, painlessly restraining the head with a head holder and preventing large body movements with a cotton-coated plastic cover. The head was covered to reduce visual stimuli. If defecation or urination occurred, the cotton sheet was slid along under the mouse to keep the animal clean and dry so as to minimize discomfort. Room temperature was maintained at 24±1 °C during the recording session. Under these conditions, the animals were able to move their bodies and limbs relatively freely and to sleep in a sphinx position. After habituation, they could be kept in this position for 3–6 consecutive hours without showing any signs of discomfort and displayed complete sleep-waking cycles, consisting of W, SWS, and PS. If any signs of discomfort were seen, the mouse was freed from the restrained position.

Single neuronal activity was recorded extracellularly using a glass pipette microelectrode filled with 0.5 M sodium acetate solution containing 2% Pontamine Sky Blue, as described previously (Takahashi et al., 2006). The mean (±SD) impedance of the electrodes (n=113) at the beginning of each recording session was 17.2±4.6 Mohm. The microelectrode was attached perpendicular to the electrode holder of a pulse motor microdrive manipulator (MO-81, Narishige). After determination of the AP and ML stereotaxic coordinates using the guide cannula as a reference, the electrode was placed over a target structure, the brain surface exposed and cleaned, and 1% xylocaine (Astra Zeneca, Rueil Malmaison, France) applied to the electrode insertion point. The electrode was then lowered until it touched the brain surface using an electrode carrier (SM-15, Narishige) equipped with a substage (SM-15M, Narishige), permitting a fine movement (10 μm) in the dorso-ventral (DV) or AP direction. The DV stereotaxic coordinate at the brain surface was noted. During unit recordings, the electrode was inserted into the brain using the microdrive manipulator in increments of 3 μm. The exposed brain surface was protected against drying by filling the hole in the skull with physiological saline and applying a gelatin sponge to the brain surface. When single unit activities were recorded, the driven distance of the electrode tip from the brain surface (displayed on the remote control unit of the microdrive manipulator) was noted and unit recordings were carried out during at least one complete sleep-waking cycle lasting 5–20 min. The neuronal activity was amplified and filtered (NeuroLog, Digitimer, Hertfordshire, UK) with a cut-off frequency of 100 Hz, then digitized at a sampling rate of 20.8 kHz using a CED 1410 data processor (Cambridge Electronic Design (CED), Cambridge, UK). The polygraphic signals were also digitized at a sampling rate of 508.1 Hz and stored on a personal computer.

Unit recordings were usually made bilaterally at intervals of 0.2–0.3 mm rostrocaudally and mediolaterally. At the end of each experiment, Pontamine Sky Blue was injected from the recording electrode by passing a negative current (5 μA for 5–6 min) so as to mark, in each electrode track, one or two recording sites, the locations of which were previously determined by the microdrive manipulator. During a series of laterally aligned unit recordings, the electrode was occasionally removed from the brain without marking any recording sites with dye, placed 0.2–0.3 mm medial or lateral to the previous electrode track, and lowered by the electrode carrier to the same DV coordinate as that determined in the first recording trial. At the end of the experiment, one or two recording sites were then marked with Pontamine Sky Blue in the last track as described above, allowing the location of the unit recording sites in the previous electrode track(s) to be determined histologically (see below). After each experimental session, the exposed brain surface was cleaned and covered with antibiotic cream after local application of xylocaine. Unit recordings were made during two experimental sessions per day and the experiment lasted 5–6 consecutive days. Animals were maintained on a 12-h light/dark schedule with lights on from 7 AM to 7 PM, and recordings were usually made between 10 and 12 AM and between 2 and 5 PM, periods in which mice normally sleep. When no neuronal activity was detected during sleep episodes, we checked for the presence of waking-active neurons by eliciting waking by hand clapping, touching the tail with a soft brush, or giving a puff of air through a plastic tube placed near (5–6 mm) the mouse's face. 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).

Histochemistry and determination of unit recording sites

Under deep anesthesia, the animals were perfused through the ascending aorta with 50 ml of Ringer's solution, followed by 120 ml of fixative consisting of 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer, pH 7.4 (PB). The brain was removed, postfixed for 24 h at 4 °C in the same fixative, then placed in PB containing 30% sucrose for 48 h at 4 °C. Twenty μm coronal sections were cut serially on a cryostat and collected in strict order in 0.1 M phosphate-buffered saline, pH 7.4, containing 0.3% Triton X-100 (PBST) in the wells of two 12-well tissue culture plates. They were then rinsed and stored at 4 °C in PBST.

Every third section was incubated for 4–5 days at 4 °C with (1) rabbit polyclonal anti-5-HT antibodies (a gift from Prof. Kimura,