Neuroscience 182 (2011) 144–161

SLEEP-WAKING DISCHARGE PROFILES OF MEDIAN PREOPTIC AND SURROUNDING NEURONS IN MICE

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Abstract—The median preoptic nucleus (MnPO), part of the anteroventral third ventricular region, plays a key role in body fluid homeostasis and cardiovascular regulation. Recently, a cluster of neurons showing sleep-related c-fos immunoreactivity was found in the rat MnPO, and a subsequent electrophysiological study found that nearly 76% of rat MnPO neurons exhibit increased discharge during sleep. In a recent single unit recording study in mice, we found that sleep-active neurons are not localized in any specific region of the preoptic/basal forebrain (POA/BFB). However, the discharge profiles of mouse MnPO neurons across wake-sleep states remained to be determined. In this study, we therefore examined whether the mouse MnPO contains a high proportion of sleep-active neurons and constitutes a distinct cluster of sleep-promoting neurons in the median preoptic region. We recorded a total of 234 single units in the MnPO, the laterally adjacent peri-MnPO, the dorsally adjacent medial septum (MS), and the ventrally adjacent periventricular (Pe)/medial preoptic (MPO) area (Pe/MPO). We found that the MnPO contained similar proportions of sleep-active (31.9%) and waking (W)-active (33.0%) neurons, together with many waking/paradoxical sleep (W/PS)-active neurons (23.4%), whereas the Pe/MPO and MS contained a high proportion of sleep-active neurons (66.0 and 62.9%, respectively), while the peri-MnPO contained a high proportion of W-active neurons (57.1%). In the MnPO, both W-active and W/PS-active neurons were distributed throughout the nucleus, whereas sleep-active neurons were mostly located on its border. Only slowly discharging (<5 Hz) slow-wave sleep (SWS)/PS-selective neurons were found in the MnPO. During the transition from W to SWS, all of these SWS/PS-selective neurons fired not before, but after, sleep onset, with a gradual increase in discharge rate. In addition to its well-known homeostatic and cardiovascular functions, the MnPO might modulate the sleep-waking cycle by playing different roles in sleep/wake state regulation.

Key words: median preoptic nucleus, periventricular nucleus, medial septum, single unit recording, sleep-waking switch, cardiovascular regulation.

The nucleus preopticus medianus, or median preoptic nucleus (MnPO), a structure composed of densely packed small cells attached to the rostral wall of the third ventricle, is a part of the anteroventral third ventricular region (AV3V) in the anterior hypothalamus and plays a key role in body fluid homeostasis and cardiovascular regulation (for reviews, see Andersson, 1978; Fitzsimons, 1998; Lind and Johnson, 1982; Nicolaïdis and Fitzsimons, 1984). MnPO neurons are sensitive to blood pressure changes (Gambino and Felix, 1986; Knuepfer et al., 1985; Nicolaïdis, 1970; Stocker and Toney, 2005; Tanaka et al., 1993). Lesions of the MnPO in rats cause attenuated pressor responses to centrally acting agents, failure to develop experimental hypertension, and dysfunction of fluid intake and fluid output controls, leading to severe dehydration and hypernatremia (Gardiner and Stricker, 1985; Lind and Johnson, 1982; Mangiapane et al., 1983; Manning et al., 1985). Anatomical studies have shown that the MnPO receives a unique set of inputs from chemosensitive and barosensitive systems, such as the subfornical organ (SFO), organum vasculosum lamina terminalis (OVLT), paraventricular nucleus of the hypothalamus (PVN), lateral parabrachial nucleus (PbL), nucleus of the solitary tract (NTS), and ventrolateral medulla (Kawano and Masuko, 1993; Saper and Levisohn, 1983; Saper et al., 1983). MnPO neurons, in turn, send axons to the SFO, OVLT, PVN, medial preoptic area (MPO), hypothalamic supraoptic nucleus (SON), PbL, dorsal raphe nuecleus, and locus coeruleus (Camacho and Phillips, 1981; Chiba and Murata, 1985; Lind et al., 1982; Saper and Levisohn, 1983; Uschakov et al., 2007; Zardetto-Smith and Johnson, 1995).

Recently, using the c-fos immunostaining method, two clusters of neurons that exhibit Fos protein expression after sustained sleep, but not after waking, were reported in the rat, first in the ventrolateral preoptic area (VLPO) (Sherin et al., 1996), then in the MnPO (Gong et al., 2000), and have been proposed to be the principal sleep-promoting cell groups within the preoptic area (POA) and basal forebrain (BFB). However, a more recent c-fos expression study in the rat reported that, when stress is minimized prior to, and during, the experimental period, fewer neu-

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Abbreviations: AP, arterial blood pressure; AV3V, anteroventral third ventricular region; AW, active waking; BFB, basal forebrain; ChAT, choline acetyltransferase; D, drowsy state; EEG, electroencephalogram; EMG, electromyogram; HA, histamine; IMEANFR, instantaneous mean frequency; LPO, lateral preoptic area; MnPO, median preoptic nucleus; MPO, medial preoptic area; MS, medial septal nucleus; NA, noradrenaline; NTS, nucleus of the solitary tract; Orx/Hcrt, orexin/hypocretin; OVLT, organum vasculosum lamina terminalis; PbL, lateral parabrachial nucleus; Pe, periventricular nucleus; PF/LH, perifornical-lateral hypothalamic area; PH, posterior hypothalamus; POA, preoptic area; PS, paradoxical sleep; PVN, paraventricular nucleus of the hypothalamus; QW, quiet waking; SFO, subfornical organ; SON, supraoptic nucleus; SWS, slow-wave sleep; TH, tyrosine hydroxylase; TM, tuberomammillary nucleus; VLPO, ventrolateral preoptic area; W, wakefulness.

0306-4522/11 $ - see front matter
doi:10.1016/j.neuroscience.2011.03.010

rons are active during sleep than during waking in these structures (Modirrousta et al., 2004). Only one electrophysiological study has reported that the rat MnPO contains a high proportion (nearly 76%) of neurons showing increased discharge during sleep (Suntsova et al., 2002). In this study, the authors employed a microwire bundle method using 10 Formvar-insulated stainless-steel microwires (20 μm) with a low impedance (500–700 kΩ). Because MnPO neurons are small (<15 μm; see Kawano et al., 1989) and densely packed, single unit recording would be very difficult using the microwire bundle method (see Discussion). In addition, the authors did not describe the firing properties of adjacent median preoptic neurons. Single unit recordings from the adjacent median preoptic areas are of interest, as these structures form part of the AV3V and there is a possibility that the functional MnPO may extend beyond its Nissl-defined borders into these adjacent areas. A previous c-fos expression study showed, for example, that Fos-positive, presumed sleep-active neurons extend beyond the MnPO boundary (Gong et al., 2000). Recently, we demonstrated that, in the mouse, sleep-promoting neurons are found throughout the POA and adjacent BFB (Takahashi et al., 2009), rather than in specific sites within the POA/BFB (Sherin et al., 1996; Szymusiak et al., 1998). We also found that significant changes in the activity of POA/BFB sleep-promoting neurons during state transitions follow, and do not precede, those in POA/BFB and posterior hypothalamic waking-promoting neurons, contrary to the current hypothesis that the sleep process starts and ends with the beginning or cessation, respectively, of firing of POA/BFB hypnogenic neurons (for reviews, see Jones, 2005; McGinty and Szymusiak, 2001; Saper et al., 2001). In our previous study, we did not record MnPO neurons and this prompted us to investigate the discharge characteristics of MnPO and adjacent median preoptic neurons in the mouse during the sleep-waking cycle. For this purpose, we used a high-impedance (>10 MΩ) glass pipette microelectrode (2–4 μm outer tip diameter) and examined the firing profiles of median preoptic neurons across the sleep-waking cycle and the temporal relationship between their unit activities and behavioral state transitions.

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 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.

Twelve male adult C57BL/6 mice (Harlan, France; 30–34 g at the time of surgery) 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., then were placed in a stereotaxic apparatus (SN-3, Narishige, Tokyo, Japan) with blunt ear bars.

Ten were implanted with electrodes to record the neocortical electroencephalogram (EEG), neck electromyogram (EMG), and electrocardiogram as described previously (Takahashi et al., 2006). One cannula (30 gauge) was fixed on the skull (AP 2.0 mm from the bregma and 0.0 mm from the midline) 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 MnPO and covered with antibiotic cream for the subsequent insertion of microelectrodes.

The other two mice did not undergo electrode implantation and were treated with colchicine (Sigma-Aldrich, St. Louis, MO, USA). A glass micropipette (tip diameter 35–40 μm) was coupled to the needle of a 5 μl Hamilton syringe (Hamilton Bonaduz AG, Switzerland) using hot melted wax, then the syringe was attached to a microinjector (IMS-3, Narishige) and colchicine (20 μg in 2 μl of Ringer's solution) injected into the lateral ventricle (AP 0.3 mm from the bregma, 1.1 mm from the midline, and 2.0 mm under the brain surface) with the aid of a stereotaxic carrier (SM-15, Narishige), then 30 h later, the mice were deeply anesthetized and perfused as described in the Histochemistry section below.

Extracellular single unit and polygraphic recordings

After the recovery period, the 10 animals with implanted electrodes 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 three to six consecutive hours without showing any signs of discomfort and displayed complete sleep-waking cycles, consisting of wakefulness (W), slow-wave (or non-rapid eye movement) sleep (SWS), and paradoxical (or rapid eye movement) sleep (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 (2–4 μm tip diameter) filled with 0.5 M sodium acetate solution containing 2% Direct Blue 15 (Sigma, St Louis, USA). The mean (±SD) impedance of the electrodes (n=69) measured *in vivo* at the beginning of each recording session was 18.1±4.4 MΩ. The microelectrode was attached to the electrode holder of either a pulse motor microdrive manipulator (MO-81, Narishige) or a stereotaxic micromanipulator (SM-25A, Narishige). The pulse motor microdrive manipulator was attached to an electrode carrier (SM-15, Narishige) equipped with a substage (SM-15M, Narishige), permitting fine movement (10 μm) in the dorso-ventral (DV) or antero-posterior (AP) direction. The microelectrode was fixed either at an angle of 90° or at an angle of 88 or 86° to the sagittal plane to gain access to the MnPO located in the midline. After determination of the AP and mediolateral 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 seen just lateral to the midsagittal sinus, and the DV stereotaxic coordinate at the brain surface was noted. During unit recordings, the electrode was inserted into the brain in 3 μm steps using the microdrive manipulator. 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 distance that the electrode tip was driven from the brain surface (displayed on the remote