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DISCHARGE PROPERTIES OF PRESUMED CHOLINERGIC AND NONCHOLINERGIC LATERODORSAL TEGMENTAL NEURONS RELATED TO CORTICAL ACTIVATION IN NON-ANESTHETIZED MICE

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Abstract—We have recorded, for the first time, in non-anesthetized, head-restrained mice, a total of 339 single units in and around the laterodorsal (LDT) and sublaterodorsal (SubLDT) tegmental nuclei, which are located, respectively, in, or beneath, the periaqueductal gray and contain cholinergic neurons. The recordings were made during the complete wake–sleep cycle including wakefulness (W), slow-wave sleep (SWS), and paradoxical (or rapid eye movement) sleep (PS). The tegmental neurons displayed either a biphasic narrow or triphasic broad action potential. Seventy-six LDT or SubLDT neurons characterized by their triphasic long-duration action potentials were judged to be cholinergic and this was verified in anesthetized mice using neurobiotin juxtacellular labeling combined with choline acetyltransferase immunohistochemistry of the recorded cell. The 76 presumed cholinergic neurons discharged tonically at the highest rate during W and PS (W/PS-active neurons) as either single isolated spikes or clusters of two to five spikes, and 26 of them discharged selectively during W and PS, these W/PS-selective neurons being found mainly in the SubLDT. The clustering discharge was particularly prominent during PS, when it was associated with an obvious phasic change in the cortical electroencephalogram (EEG), and during waking periods, when it was accompanied by abrupt body movements. During the transition from sleep to waking, the cholinergic W/PS-selective neurons and the LDT or SubLDT noncholinergic W-selective neurons showed firing before the onset of W, while, at the transition from waking to sleep, they ceased firing before sleep onset. At the transition from SWS to PS, all the cholinergic neurons exhibited a significant increase in discharge rate before the onset of PS. The present study in mice supports the view that cholinergic and noncholinergic LDT and SubLDT neurons play an important role in tonic and phasic processes of arousal and cortical EEG activation occurring during W or PS, as well as in the sleep/waking switch.

Key words: laterodorsal and sublaterodorsal nuclei, acetylcholine, single-unit recording, neurobiotin, cortical activation, sleep/waking switch.

INTRODUCTION

The dorsolateral mesopontine tegmentum contains several key structures responsible for the initiation and maintenance of wakefulness (W) and/or paradoxical sleep (PS), also known as rapid eye movement (REM) sleep. Of these, cholinergic neurons in the laterodorsal (LDT), sublaterodorsal (SubLDT), and adjacent pedunculopontine (PPN) tegmental nuclei provide the major cholinergic inputs to the thalamus or hypothalamus (for reviews, see Wainer and Mesulam, 1990; Jones, 1993). It is well established that, in the cat, the activation of cholinergic thalamic afferents produces activation (desynchronization) of the cortical electroencephalogram (EEG) by suppressing slow cortical waves (0.3–1.0 Hz), delta waves (1–4 Hz), and spindle wave oscillations (11–14 Hz) (Currò Dossi et al., 1991; Steriade et al., 1991, 1993a,b). Cholinergic LDT neurons can drive dopamine (DA) release in the nucleus accumbens by exciting ventral tegmental area DA neurons, thereby activating the limbic system and enhancing motivational components of arousal (Forster and Blaha, 2000). Previous single-unit recording studies in non-anesthetized cats and rats reported that activity of the mesopontine cholinergic neurons is linked to arousal, as well as to cortical activation occurring during W and PS (El Mansari et al., 1989; Steriade et al., 1990a; Kayama et al., 1992; Koyama and Sakai, 2000; Datta and Siwek, 2002).

Evidence is accumulating for the presence of multiple waking-promoting structures in various regions of the brain, such as the locus coeruleus (LC) containing noradrenaline (NA) neurons, the tuberomammillary (TM)

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Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); Ach, acetylcholine; AW, active or attentive waking; BFB, basal forebrain; ChAT, choline acetyltransferase; D, drowsy state; DA, dopamine; DAB, 3,3′-diaminobenzidine tetrahydrochloride; DMT, dorsomedial tegmental area; DR, dorsal raphe nucleus; EEG, electroencephalogram; EMG, electromyogram; GAD, glutamate decarboxylase; HA, histamine; IMF, instantaneous mean frequency; ir, immunoreactive; LC, locus coeruleus; LDT, laterodorsal tegmental nucleus; LGNd, dorsal lateral geniculate nucleus; LTS, low-threshold calcium spike; MS, medial septum; NA, noradrenaline; Nb, neurobiotin; Orx/Hcrt, orexin/hypocretin; PBST, phosphate-buffered saline containing Triton X-100; PAG, periaqueductal gray; PGO, pontogeniculo-occipital; PH, posterior hypothalamus; POA, preoptic area; PPT, pedunculopontine tegmental nucleus; PPTm, diffuse medial part of the PPT; PS, paradoxical sleep; QW, quiet waking; REM, rapid eye movement; S1 and S2, light and deep slow-wave sleep respectively; SD, standard deviation; SubLDT, sublaterodorsal tegmental nucleus; SWS, slow-wave sleep; TH, tyrosine hydroxylase; TM, tuberomammillary nucleus; W, wakefulness.

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nuclei containing histamine (HA) neurons, the posterior hypothalamus (PH) containing orexin (also called hypocretin, Orx/Hcrt) neurons, the dorsal raphe nucleus (DR) containing serotonin (5-hydroxytryptamine, 5-HT) neurons, and the mesopontine tegmentum containing acetylcholine (Ach) neurons (Sakai and Crochet, 2003; Jones, 2005). In order to understand the sleep/waking switch, it is necessary to identify critical waking-promoting neurons and determine their activity profiles during state transitions from waking to sleep and from sleep to waking in the same species. In our recent studies in mice, we demonstrated waking-specific discharge of NA-LC neurons (Takahashi et al., 2010) and HA-TM neurons (Takahashi et al., 2006; Sakai et al., 2010) and waking-selective, but not waking-specific, discharge of Orx/Hcrt-PH neurons (Takahashi et al., 2008) and 5-HT-DR neurons (Sakai, 2011a). At the transition from sleep to waking, NA, Orx/Hcrt, and 5-HT neurons discharge before the onset of EEG activation (Takahashi et al., 2008, 2010; Sakai, 2011a), whereas HA neurons fire after onset, with a pronounced delay (Takahashi et al., 2006; Sakai et al., 2010). At the transition from waking to sleep, all the waking-promoting neurons exhibit a significant decrease in discharge rate before the onset of EEG synchronization, the decrease occurring in the order NA-LC = HA-TM > Orx/Hcrt $\geqslant$ 5-HT-DR, suggesting that these waking-promoting neurons play different roles in the sleep/waking switch and control of arousal, though they act in a coordinated manner to stimulate cortical activity. Currently, no information is available about the single-unit activity of mouse LDT and SubLDT neurons during the complete sleep/waking cycle and their unit-activity profiles during the state transitions, knowledge which is a prerequisite for an understanding of their roles in the generation and/or maintenance of behavioral states and the sleep/waking switch. We have therefore recorded, for the first time in non-anesthetized, head-restrained mice, a large number of neurons in, and immediately adjacent to, the LDT and SubLDT, which are characterized by their clusters of cholinergic neurons, to (i) determine their electrophysiological properties and discharge profiles during the complete sleep–waking cycle and at state transitions and (ii) elucidate their roles in behavioral state control and the sleep/waking switch. In addition, using neurobiotin (Nb) juxtacellular labeling combined with choline acetyltransferase (ChAT) immunohistochemistry of the recorded cell in anesthetized mice, we examined the validity of distinguishing cholinergic and non-cholinergic tegmental neurons on the basis of their triphasic or biphasic spike form.

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.

Twenty-six male adult C57BL/6 mice (Janvier S.A.S., St Berthevin, France; 29–35 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), given intraperitoneally, then were placed in a stereotaxic apparatus (SN-3, Narishige, Tokyo, Japan) and implanted with electrodes to record the neocortical EEG, neck electromyogram (EMG), and electrocardiogram. One cannula (30 gauge) was fixed on the skull [anteroposterior (AP) 1.0 or 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, and 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 LDT region and covered with antibiotic cream for the subsequent insertion of microelectrodes, as described previously (Sakai, 2011a).

Another three mice did not undergo electrode implantation, but were treated with colchicine (Sigma–Aldrich, St. Louis, 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 2.0 μl of Ringer's solution) was 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, after 30–43 h, 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 26 animals with implanted electrodes were progressively habituated to the head-restrained position for 7–14 days. 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, slow-wave (or non-REM) sleep (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 (GC150F-15, Harvard Apparatus, Kent, UK; 1–2 μm tip diameter) filled with 0.5 M sodium acetate solution containing 2% Direct Blue 15 (Sigma, St Louis, USA). The mean (±standard deviation (SD)) impedance of the electrodes measured *in vivo* at the beginning of each recording session was 18.2 ± 3.7 Mohms (n = 191). The microelectrode was attached at an angle of 90° to the electrode holder of a pulse motor microdrive manipulator (MO-81, Narishige). After determination of the AP and medio-lateral (ML) stereotaxic coordinates using the guide cannula as a reference, the electrode was placed over a target structure and the brain surface exposed and cleaned under local application of 1% xylocaine (Astra Zeneca, Rueil Malmaison, France). The electrode was then lowered until it touched the brain surface and the dorso-ventral (DV) stereotaxic coordinate at the brain surface 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 from 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 was noted and unit recordings carried out during at least one complete sleep–waking cycle lasting 5–30 min. The neuronal activity was recorded after amplification and filtering (at 500 Hz low pass and 50 kHz high pass) using a NeuroLog system (Digitimer,