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MORPHOLOGICAL AND ELECTROPHYSIOLOGICAL CHARACTERISTICS OF NEURONS WITHIN IDENTIFIED SUBNUCLEI OF THE LATERAL HABENULA IN RAT BRAIN SLICES

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Abstract—Based on the specificity of its inputs and targets, the lateral habenular complex (LHb) constitutes a pivotal motor-limbic interface implicated in various cerebral functions particularly in regulating monoamine transmission. Despite its functional significance, cellular characteristics underlying LHb functionality have not been examined systematically. The present study aimed to correlate morphological and electrophysiological properties of neurons within the different subnuclei of the LHb using whole-cell recording and neurobiotin labeling in rat slice preparations. Morphological analysis revealed a heterogeneous population of projection neurons randomly distributed throughout the LHb. According to somatodendritic characteristics four main categories were classified including spherical, fusiform, polymorphic and vertical cells. Electrophysiological characterization of neurons within the different categories demonstrated homologous profiles and no significant differences between groups. Typically, LHb neurons possessed high input resistances and long membrane time constants. They also displayed time-dependent inward rectification and distinct afterhyperpolarization. A salient electrophysiological feature of LHb neurons was their ability to generate rebound bursts of action potentials in response to membrane hyperpolarization. Based on the pattern of spontaneous activity, neurons were classified as silent, tonic or bursting. The occurrence of distinctive firing modes was not related to topographic allocation. The patterns of spontaneous firing and evoked discharge were highly sensitive to alterations in membrane potential and merged upon de- and hyperpolarizing current injection and synaptic stimulation. Besides projection neurons, recordings revealed the existence of a subpopulation of cells possessing morphological and physiological properties of neocortical neurogliaform cells. They were considered to be interneurons. Our data suggest that neurons within the different LHb subnuclei behave electrophysiologically more similar than expected, considering their morphological heterogeneity. We conclude that the formation of functional neuronal entities within the LHb may be achieved through defined synaptic inputs to particular neurons, rather than by individual neuronal morphologies and intrinsic membrane properties.

Key words: basal ganglia, habenular subnuclei, reward, whole-cell recording, neurobiotin, neurogliaform cell.

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Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, afterhyperpolarization; BST, bursting cell; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; EPSP, excitatory postsynaptic potential; FC, fusiform cell; HCN, hyperpolarization-activated cyclic nucleotide-gated non-selective cation channel; I_h, HCN-mediated membrane current; LHb, lateral habenular complex; LHbL, lateral division of the lateral habenular complex; LHbLB, basal part of the lateral division of the lateral habenular complex; LHbLMc, magnocellular part of the lateral division of the lateral habenular complex; LHbLMg, marginal part of the lateral division of the lateral habenular complex; LHbLO, oval part of the lateral division of the lateral habenular complex; LHbLPc, parvocellular part of the lateral division of the lateral habenular complex; LHbM, medial division of the lateral part of the habenular complex; LHbMA, anterior part of the medial division of the lateral part of the habenular complex; LHbMC, central part of the medial division of the lateral part of the habenular complex; LHbMMg, marginal part of the medial division of the lateral part of the habenular complex; LHbMPc, parvocellular part of the medial division of the lateral part of the habenular complex; LHbMS, superior part of the medial division of the lateral part of the habenular complex; MHb, medial habenular complex; NGC, neurogliaform cell; PBS, phosphate-buffered solution; PC, polymorphic cell; PTX, picrotoxin; R_m, membrane input resistance; SC, spherical cell; SIL, silent cell; tau, membrane time constant; TIR, tonic irregular-spiking cell; TR, tonic regular-spiking cell; VC, vertical cell; V_m, membrane resting potential.

The lateral habenular complex (LHb), a distinct constituent of the epithalamic habenula, represents a highly conserved structure in the mammalian diencephalon (Bianco and Wilson, 2009). It consists of two core parts: a medial (LHbM) and a lateral (LHbL) division (Herkenham and Nauta, 1977, 1979). The LHbM receives afferents primarily from limbic forebrain areas (Herkenham and Nauta, 1977; Groenewegen et al., 1993; Kowski et al., 2008). Its efferents predominantly descend to dopaminergic and serotonergic midbrain nuclei (Herkenham and Nauta, 1979; Araki et al., 1988; Behzadi et al., 1990; Omelchenko et al., 2009). On the other hand, the LHbL is mainly innervated by the basal ganglia, in particular the globus pallidus internus (Herkenham and Nauta, 1977; Parent et al., 1981; Rajakumar et al., 1993). Its efferents preferentially target cholinergic nuclei of the mesopontine tegmentum (Herkenham and Nauta, 1979; Semba and Fibiger, 1992). Based on its prominent relations with the monoaminergic systems, the LHb is particularly implicated in the regulation of important motor and cognitive behaviors (Klemm, 2004; Geisler and Trimble, 2008; Bianco and Wilson, 2009).

Quite recently, it has been discovered that the LHb processes reward information that controls reward-related activity of midbrain dopaminergic neurons, and hence may be substantially involved in reinforcement learning (Ullsperger and von Cramon, 2003; Matsumoto and Hikosaka, 2007; Hikosaka et al., 2008; Hong and Hikosaka, 2008; Salas et al., 2010). In addition, increasing experimental evidence suggests that dysfunction of the LHb plays a crucial role in the pathophysiology of major psychiatric

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diseases, such as schizophrenia and depression (Ellison, 1994; Shumake et al., 2003; Lecourtier et al., 2004; Shepard et al., 2006; Ranft et al., 2010; Sartorius et al., 2010).

The variety of the biological functions and behaviors in which the LHb is implicated, is morphologically reflected by its complex subnuclear organization. Detailed anatomical analysis of the rodent LHb revealed a medial and a lateral division comprising of at least 10 different subnuclei (Andres et al., 1999; Geisler et al., 2003). The functional relevance of some of the discovered anatomical entities has been indicated by hodological and electrophysiological data, which show subnucleus-related afferent and efferent connectivity as well as targeted subnucleus-specific dopaminergic modulation (Gruber et al., 2007; Kowski et al., 2008, 2009; Brinschwitz et al., 2010). Given the absence of direct physiological evidence, however, it remains unclear whether the morphologically defined subnuclei represent separate functional entities that serve individual tasks within the overall function of the LHb.

An important requirement for understanding the physiological role of any nuclear group is a thorough analysis of the structural and functional properties of its constituents, such as axonal and dendritic arborization patterns and intrinsic membrane characteristics. Although the morphology of neurons within the LHb has been investigated in various mammalian species including dog and rabbit (Ramon y Cajal, 1911), cat (Iwahori, 1977), mouse (Meyer and Ferres-Torres, 1981) and rat (Kim and Chang, 2005), much less is known about electrophysiological characteristics of LHb neurons. So far, there are only two reports providing information about intrinsic membrane properties and electroresponsiveness of LHb neurons (Wilcox et al., 1988; Chang and Kim, 2004). The available data suggest that the LHb is composed of different neuronal groups. However, the relationship between cellular morphologies and electrophysiological properties of individual LHb neurons and their topographic allocation within the habenular nucleus has not yet been studied.

In the present investigation, we used whole-cell recording and neurobiotin labeling in rat brain slice preparations to examine membrane properties and morphological characteristics of topographically identified neurons within the different subnuclei of the LHb. Two principal questions were addressed: First, are there differences in the somatodendritic and axonal morphologies between neurons of individual subnuclei? And second, do neurons located in separate subnuclei display particular electrophysiological characteristics? The present study is the first comprehensive approach to correlate structural and functional attributes of individual LHb neurons with their subnucleus-specific topography.

EXPERIMENTAL PROCEDURES

Slice preparation

Brain slices were prepared from 10 to 21-d old Wistar rats, which were obtained from an institutional breeder (Forschungseinrichtungen für Experimentelle Medizin, Charité-Universitätsmedizin Berlin, Krahmerstraβe 6–10, D-12207 Berlin, Germany). All experiments were approved by the Regional Berlin Animals Ethics Committee (T0127/02) and performed in strict accordance with the European Communities Council directive regarding care and use of animals for experimental procedures. All efforts were made to minimize the number of specimens and animal suffering. Animals were deeply anesthetized with diethylether and decapitated. Brains were quickly dissected and placed into ice-cold sucrose-artificial cerebrospinal fluid (ACSF; dissection solution, see below). Coronal slices (250 to 500 μm) containing the habenular complex were cut using a vibrating microtome (VT 1000 S; Leica Instruments, Nussloch, Germany). Slices were placed in a holding vial and incubated in oxygenated ACSF (recording solution, see below) at 32–35 °C. After 60–90 min recovery, individual slices were transferred into a recording chamber, maintained submerged at room temperature and continuously perfused with recording solution at a rate of 2.5–5.0 ml/min.

Electrophysiological recordings and data acquisition

Patch-clamp recordings were made from individual neurons visualized by differential interference contrast video microscopy using a Newvicon camera (C2400; Hamamatsu Photonics, Hamamatsu City, Japan) mounted to an upright microscope (Axioskop FS 1; Zeiss, Oberkochen, Germany) equipped with Nomarsky optics, a 630.9 NA water immersion objective (Zeiss), and an infrared filter (RG-9; Schott, Melsungen, Germany). Patch pipettes were made from borosilicate glass tubing (outer diameter, 1.5 mm; wall thickness, 0.64 mm; Science Products, Hofheim, Germany) using a Sutter micropipette puller (P-97; Sutter Instruments, Novato, CA, USA). When filled with internal solution pipettes had resistances of 3–5 MΩ. Whole cell recordings were performed employing an EPC-9 patch-clamp amplifier (Heka Elektronik, Lambrecht, Germany). Data acquisition was controlled with Pulse software (Pulse 8.77, Heka Elektronik). Signals were digitized at 5–10 kHz and filtered with low-pass Bessel filter characteristic of 2 kHz cut-off frequency.

Extracellular recordings were made with glass microelectrodes filled with ACSF (see below). Electrode resistance amounted to 5–10 MΩ. Single-unit activity was recorded using an EXT-01C amplifier (NPI Instruments, Tamm, Germany). Extracellular spikes were acquired with a CED 1401 plus interface (Cambridge Electronic Design Limited, Cambridge, UK) controlled by Spike2 software (CED Limited). Signals were digitized at 10 kHz and bandpass filtered at 0.3 and 3 kHz cutoff frequencies. Synaptic activation of LHb neurons was achieved by electrical stimulation of afferent fibers in the white matter of the stria medullaris with glass-insulated bipolar platinum wire electrodes (tip diameter, 50 μm; tip separation, 100–200 μm). Orthodromic stimuli consisted of 0.05 ms square pulses with intensities ranging between 0.5 and 5 V. Single pulses or short repetitive trains of stimuli (10–25 pulses) were used. Repetitive stimulation was delivered with a frequency of 100 Hz and an interval of 30 s.

Solutions and drugs

The recording solution (ACSF) contained (in mM): 125 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgCl_2$, and 25 D-glucose, pH 7.4 maintained by saturation with carbogen (95% O_2/5% CO_2). Fast excitatory and inhibitory transmission were inhibited employing the glutamate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM; Biotrend, Köln, Germany) and the GABA antagonist picrotoxin (PTX, 25 μM; Biotrend). For dissection and during slicing a sucrose-ACSF was used in which NaCl was partially substituted with 50 mM sucrose (final NaCl, 75 mM). Additionally, the solution contained (in mM): 0.1 $CaCl_2$, 6 $MgCl_2$, and 3 kynurenic acid (Sigma-Aldrich, Taufkirchen, Germany). Pipettes were filled with internal solution containing (in mM): 128 K-gluconate, 20 KCl, 10 HEPES, 0.1 EGTA, 2 $MgCl_2$, and 2 Na_2ATP (~300 mOsm), pH adjusted to 7.2 with NaOH. For intracellular labeling of recorded neurons, 2 mg/ml neurobiotin