Neuroscience 145 (2007) 851–865

INTRINSIC MEMBRANE PROPERTIES AND SYNAPTIC RESPONSE CHARACTERISTICS OF NEURONS IN THE RAT'S EXTERNAL CORTEX OF THE INFERIOR COLLICULUS

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Abstract—The inferior colliculus (IC) can be divided into three anatomical subdivisions: the central nucleus (ICc), the dorsal cortex (ICd) and the external cortex (ICx). ICx receives its primary auditory inputs from ICc and auditory cerebral cortical areas, and non-auditory inputs from regions of motor and other sensory systems. This wide array of projections makes the ICx a distinct structure within the auditory brainstem. The purpose of the current study was to comprehensively characterize the neuronal population of ICx, by intrinsic and synaptic response properties. Visual whole-cell patch clamp recordings were taken from ICx neurons (N=129) from rats between postnatal days 8 to 12. Neurons displayed various types of firing patterns in response to current injection, including *regular*, *adapting*, *pauser* and *bursting*. The regular cells constitute the majority (66%), followed by adapting (18%), pauser (13%) and bursting cells (2%). In response to hyperpolarizing current injection, many neurons illustrated a pronounced sag in the membrane potential, representing a hyperpolarization-activated current (I_h). Some neurons (25%) displayed a Ca^{2+}-dependent rebound depolarization following negative current injection. In response to depolarizing current injection, 70% of ICx neurons displayed a Ca^{2+}-mediated potential expressed as Ca^{2+} spikes/humps, uncovered when Na^+ and K^+ currents were removed. Also, spikes displayed an undershoot which was in part mediated by Ca^{2+}. Stimulation of the ICc elicited graded synaptic responses, which displayed a combination of excitatory and/or inhibitory potentials, with excitation being predominant across firing patterns. Neurons displayed temporal summation in response to repetitive stimulation at 20 Hz and higher.

The results indicate a relatively modest diversity in firing pattern and in intrinsic membrane properties, making this subnucleus distinct from its counterparts within the IC. The data show that ICx receives major excitatory input from ICc, supporting its role in integrating signals from brainstem and directing information to higher brain centers.

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Abbreviations: ACSF, artificial cerebrospinal fluid; AHP, after-hyperpolarization potential; AP, action potential; CoIC, commissure of the inferior colliculus; EPSP, excitatory postsynaptic potential; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid; IC, inferior colliculus; ICc, central nucleus of the inferior colliculus; ICd, dorsal cortex of the inferior colliculus; ICx, external cortex of the inferior colliculus; I_h, hyperpolarization-activated current; $I_{K(L)}$, low threshold K^+ current; IPSP, inhibitory postsynaptic potential; IV, current–voltage; MGB, medial geniculate body; R_{input}, input resistance; RLF, rate-level function; RMP, resting membrane potential; TEA, tetraethylammonium; TTX, tetrodotoxin; τ, time constant.

Key words: action potential, auditory brainstem, ICx, firing pattern, postsynaptic potential, whole-cell patch-clamp.

The inferior colliculus (IC) is a principal component of the auditory midbrain serving as a major integrative center within the central auditory system, critical for processing auditory information. The IC can be categorized into three main subdivisions; central nucleus of the inferior colliculus (ICc), dorsal cortex of the inferior colliculus (ICd) and the external cortex of the inferior colliculus (ICx). The ICx is found around the central nucleus laterally and ventrally, which has also been called the lateral cortex (Malmierca and Merchán, 2004; Oliver, 2005). Neurons in ICx are organized in three layers, showing a cortical-like neuronal architecture.

In contrast to ICc, the ICx receives very limited ascending lower auditory brainstem projections directly through the major ascending pathway, the lateral lemniscus. The ICx receives its most significant lower auditory brainstem projection through a local connection from the ipsilateral ICc and some minor input from the contralateral ICc and ICd through the commissure of the inferior colliculus (CoIC) (Moore and Goldberg, 1963; Willard and Ryugo, 1979; Adams, 1980; Saldaña and Merchán, 2005). However, ICx receives substantial inputs from the auditory cortex, including the primary and non-primary auditory cortex (Faye-Lund, 1985; Saldaña et al., 1996; Druga et al., 1997).

The ICx is distinct from the ICc and ICd since it possesses multisensory connections, and for its role in acoustico-motor responses. Anatomical studies have illustrated that ICx receives input from somatosensory structures including the spinal cuneate and gracile nuclei, and the trigeminal nucleus (Feldman and Kruger, 1980; Aitkin et al., 1981; Tokunaga et al., 1984). Inputs from the deep superior colliculus, parabrachial nucleus, and substantia nigra par lateralis, globus pallidus and the posterior hypothalamus have also been noted (Willard and Ryugo, 1979; Aitkin et al., 1981; Morest and Oliver, 1984; Tokunaga et al., 1984; Coleman and Clerici, 1987; Inagaki et al., 1990; Yasui et al., 1990). The ICx projects to the medial division of the medial geniculate body (MGB), the superior colliculus, the cerebellum and the pontine gray matter (Edwards et al., 1979; Hashikawa, 1983; Hashikawa and Kawamura, 1983). This lends further support to the notion that the ICx is a multisensory integrative nucleus.

Aitkin et al. (1978) were able to show from *in vivo* recordings in anesthetized cats, that roughly half of the

0306-4522/07$30.00+0.00
doi:10.1016/j.neuroscience.2006.12.031

recorded ICx cells responded to both somatosensory and acoustic stimuli. The remaining portion of neurons recorded was influenced by only one type of stimulation, either auditory or somatosensory. Some neurons that were activated by both modes of stimuli were excited by acoustic stimuli and inhibited by tactile stimuli. This and other studies have shown that the neurons of ICx are poorly driven by acoustic stimuli, display broad irregular tuning curves, and habituate rapidly to presented tones (Aitkin et al., 1978; Syka et al., 2000). These *in vivo* electrophysiological results further support the suggestion that ICx neurons are involved in general sensory signaling, processing both auditory and somatosensory information.

The functional role of ICx remains unclear. One possibility may be to present the position of the body with respect to sound sources in extrapersonal space (Winer and Schreiner, 2005). Also, ICx is thought to contribute to the visceral or emotional responses to acoustic stimuli (Iwada et al., 1986; LeDoux et al., 1988). ICx may therefore play an integrative function which is imperative for coordinated movements of the head, eyes and ears toward a sound source, which may be modulated by emotion.

Previously researchers have examined the neurophysiology of cells either in the whole IC irrespective of individual subnuclei, or place their focus on ICc. Few studies have attempted to systematically compare the various properties of the neurons within the cortical area of the IC including ICx and ICd (Aitkin et al., 1975, 1981; Smith, 1992; Li et al., 1998, 1999; Syka et al., 2000). Smith (1992) made intracellular recordings from rat IC cortex of brain slices but did not differentiate between ICx and ICd. Li et al. (1998, 1999) examined membrane characteristics and synaptic response patterns of neurons in three subdivisions of IC. The study however utilized the CoIC as its stimulation source for synaptic responses.

The purpose of the current study was to systematically investigate the cellular neurophysiology of ICx. A comprehensive analysis of the intrinsic membrane properties of ICx neurons was assembled, including classification of physiological cell types based on firing patterns, characterization of action potentials (APs), examination of ionic conductance, firing ability and responses to membrane hyperpolarization, and the mediation of membrane properties by Ca^{2+}. Synaptic response properties were also examined for ICx neurons. One significant distinction between this study and previous studies lies in the use of ICc as the stimulation source. To our knowledge, no other physiological study has examined the synaptic projections from ICc to ICx. Stimulating the projections from the ipsilateral ICc to ICx would mostly activate the input that ICx neurons normally receive from the lower auditory brainstem. This allows for a focused, localized study of lower auditory brainstem inputs to ICx via ICc. Taken together, these results will further our limited understanding of the role of ICx in gating and processing auditory information.

EXPERIMENTAL PROCEDURES

Brain slice preparation

All animal procedures were carried out in accordance with the Canadian Council on Animal Care and approved by the Carleton University Animal Care Committee. All attempts were made to minimize the pain and suffering of animals during surgery. Brain slices were obtained from young albino rats (Wistar strain, Charles River Co., Saint-Constant, Quebec, Canada) during postnatal days 8–12 using the procedure as previously described (Ma et al., 2002). Briefly, animals were killed by decapitation, and brains were removed and then placed in heated oxygenated artificial cerebrospinal fluid (ACSF) at a temperature range of 31–33 °C. The ACSF consisted of (in mM) 129 NaCl, 3 KCl, 1.2 KH_2PO_4, 2.4 $CaCl_2$, 1.3 $MgSO_4$, 20 $NaHCO_3$, 3 Hepes, and 10 glucose in de-ionized water saturated with 95% O_2–5% CO_2. The pH was adjusted to approximately 7.4 after complete saturation with O_2–CO_2 mixture. Brain slices were cut at 200 μm in the frontal plane through the auditory midbrain with a tissue slicer, and placed in a small recording chamber. The brain slice was continuously perfused with ACSF at the flow rate of between 6 and 8 ml/min. The temperature of the saline was monitored and maintained between 30 and 32 °C. The brain slice was housed in the recording chamber for approximately 30 min before recordings were made.

The brain slice chamber was illuminated from below by light passed through a Hoffman modulation contrast system into a Zeiss Axioskop microscope (Carl Zeiss, Thornwood, NY, USA). The slice was oriented as to clearly display the structures of the IC. A bipolar tungsten stimulating electrode was placed in ICc in close proximity (~0.5 mm lateral medial) to the intended recording site in ICx (Fig. 1).

The patch pipettes were produced using a vertical puller (PP-830, Narishige, Tokyo, Japan), from 1.1 mm OD and 0.8 mm ID thin-walled glass tubing (Kimax-51, Kimble, Vineland, NJ, USA). The glass microelectrodes were filled with an internal solution of the following composition (in mM): 130 K gluconate, 2 $MgCl_2$, 5 KCl, 0.3 GTP, 2 ATP, 0.6 EGTA, 10 Hepes. The internal solution had a pH range of 7.20–7.25. The impedance of the electrodes was between 4 and 6 MΩ. An EPC 8 patch clamp amplifier (HEKA, Lambrecht, Germany) was used in conjunction with the microscope to achieve visualized whole-cell patch-clamp recordings. The series resistance compensation was adjusted to a maximal value of at least 75% with a lag of 20 μs. The series resistance was recorded for each cell and had a range of 10–20 MΩ. The data were acquired in current clamp mode, initially filtered by the amplifier at 5 kHz, then digitized and analyzed at a sample rate of 10 kHz using pClamp 6.0.5 and 8.0 software respectively (Axon Instruments Inc., Union City, CA, USA) on an IBM-compatible PC.

The "test seal" function was constantly monitored to ensure that the seal and its transients were relatively stable with little fluctuation during the period of recording. Furthermore, if the series resistance exceeded more than 20 MΩ, or the resting membrane potential (RMP) of the cell was −45 mV or less negative at any point during the recording, the recording procedure was aborted.

Membrane properties

The cell's intrinsic membrane properties including current–voltage (IV) relationships and firing patterns were examined for each of the neurons in response to current injection over the range of −300–1000 pA, applied in 10 pA or 20 pA increments, with a duration range of 100–300 ms. To study the possible diversity of firing patterns, neurons were also subjected to a prehyperpolarizing current injection of −200 pA for a duration of 250 ms prior to a depolarizing current injection range of 0–200 pA, applied in increments of 10 pA for 300 ms. A second prehyperpolarizing