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Research Paper

Diversity of bilateral synaptic assemblies for binaural computation in midbrain single neurons

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ABSTRACT

Binaural hearing confers many beneficial functions but our understanding of its underlying neural substrates is limited. This study examines the bilateral synaptic assemblies and binaural computation (or integration) in the central nucleus of the inferior colliculus (ICc) of the auditory midbrain, a key convergent center. Using in-vivo whole-cell patch-clamp, the excitatory and inhibitory postsynaptic potentials (EPSPs/IPSPs) of single ICc neurons to contralateral, ipsilateral and bilateral stimulation were recorded. According to the contralateral and ipsilateral EPSP/IPSP, 7 types of bilateral synaptic assemblies were identified. These include EPSP-EPSP (EE), E-IPSP (EI), E-no response (EO), II, IE, IO and complex-mode (CM) neurons. The CM neurons showed frequency- and/or amplitude-dependent EPSPs/IPSPs to contralateral or ipsilateral stimulation. Bilateral stimulation induced EPSPs/IPSPs that could be larger than (facilitation), similar to (ineffectiveness) or smaller than (suppression) those induced by contralateral stimulation. Our findings have allowed our group to characterize novel neural circuitry for binaural computation in the midbrain.

1. Introduction

The central auditory system is capable of integrating information from both ears. Similarly, in binocular vision, this fundamental capacity enables the creation of three-dimensional acoustic images through the comparison of binaural signals (Bishop and Pettigrew, 1986; Moore, 1991; Pettigrew, 1993). Neural computation is therefore fundamental for understanding many benefits of binaural hearing such as sound localization, redundancy, head shadow effect, squelch and listening comprehension (Avan et al., 2015; Carhart, 1965; Ching et al., 2005; Cox and Bisset, 1984; Yin and May, 2005).

The inferior colliculus of the midbrain holds a central position in the central auditory system. This nucleus serves as a convergent center owing to its multiple inputs from bilateral subcollicular auditory nuclei (i.e., the cochlear nucleus, the superior olivary complex and lateral lemniscus) and also due to its single output to the ipsilateral auditory thalamus along the auditory ascending pathway (Oliver, 2005; Loftus et al., 2008; Malmierca et al., 2011; Ono and Ito, 2015). Notably, most subcollicular ascending fibers terminate at the central nucleus of the inferior colliculus (ICc). The flattened dendritic trees of ICc neurons, together with the plexuses of inputting fibers, are oriented into iso-frequency laminar layers (Malmierca et al., 1993; Oliver and Morest, 1984). Each layer has specific functional segments that receive different sets of bilateral inputs (Cant and Benson, 2006; Loftus et al., 2010; Oliver, 2005). All of these properties strongly suggest that the ICc is wired for the integration or computation of the auditory information from two ears, a concept that is supported by several physiological studies (Li and Kelly, 1992; Park and Pollak, 1993; Pollak et al., 2011).

Our understanding of the neural computation of binaural information mostly derives from the investigation of neuronal

Abbreviations: BF, best frequency; CM, complex-mode; EE, contralateral and ipsilateral EPSPs; EI, contralateral EPSP and ipsilateral IPSP; EO, contralateral EPSP and ipsilateral no response; EPSP, excitatory postsynaptic potentials; FA-scan, stimulation by using tones of various frequencies and amplitudes; ICc, central nucleus of the inferior colliculus; IE, contralateral IPSP and ipsilateral EPSP; II, contralateral and ipsilateral IPSPs; IO, contralateral IPSP and ipsilateral no response; IPSP, inhibitory postsynaptic potentials; MT, minimum threshold; PSP, postsynaptic potential

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interaural tunings for sound localization. The responses of binaural neurons in the superior olivary complex, lateral lemniscus and ICc to contralateral stimulation are subject to the modulation of ipsilateral stimulation depending on interaural time and level differences (Ashida and Carr, 2011; Grothe et al., 2010). It is believed that the interaural tunings are primarily created in the medial nucleus (Brand et al., 2002; Goldberg and Brown, 1969; van der Heijden et al., 2013; Yin and Chan, 1990) and the lateral nucleus of the superior olivary complex (Boudreau and Tsuchitani, 1968; Park et al., 2004; Tollin, 2003). However, both the monaural and binaural information from the cochlear nucleus, superior olivary complex and lateral lemniscus are further integrated and processed in the ICc (Li and Kelly, 1992; Malmierca et al., 1998, 2005b; Ono and Ito, 2015; Yin and May 2005). Recent studies using in vivo whole-cell patch recording show similar interaural tunings of tone-evoked excitatory postsynaptic potentials (EPSPs) of ICc neurons (Li and Pollak, 2013; Li et al., 2010). These findings highlight the computation of interaural time/level differences at the synaptic level. To date, the neural mechanisms of binaural information processing, beyond those for sound localization, remain poorly understood. Due to the several tasks required of binaural hearing, an immediate query is how many types of bilateral synaptic assemblies, i.e., inputs, are incorporated in individual single ICc neurons. A subsequent area of investigation concerns the computational abilities of given bilateral assemblies at the synaptic level.

Our study examined these two fundamental issues using in vivo whole-cell current-clamp recording. We found that the membrane potentials of single ICc neurons could be depolarized (EPSP), hyperpolarized (inhibitory postsynaptic potential, IPSP) or not changed in response to either contralateral or ipsilateral tone stimulation. Combinations of contralateral and ipsilateral responses exhibited 7 sets of bilateral synaptic assemblies of single ICc neurons and each set could produce various forms of binaural computation.

2. Materials and methods

Forty-eight female C57 mice aged 3–6 weeks and weighing 15–21 g (Charles River Laboratories, Montréal, Québec, Canada) were used in this study. Animal use was in accordance with the Canadian Council on Animal Care, and was approved by the Animal Care Committee of the University of Calgary (protocol number: AC12-0203).

2.1. Animal preparation and surgery

Mice were initially anesthetized with an intraperitoneal injection of a mixture of ketamine (85 mg/kg, Bimeda-MTC Animal Health Inc., Canada) and xylazine (15 mg/kg, Bimeda-MTC Animal Health Inc., Canada). The anesthetic level was monitored every 40–60 min by pinching the mouse tail. Additional dosages of ketamine (17 mg/kg) and xylazine (3 mg/kg) were given when the mouse showed any response to the tail pinch test to maintain the anesthetic level throughout the experiments. Under anesthesia, the mouse was mounted on a custom-made head holder that was placed in a soundproof chamber. The mouse head was immobilized by rigidly clamping the palate/nasal/frontal bones and positioned to align the bregma and lambda at one horizontal level. A feedback-controlled heating pad was placed underneath the mouse to maintain the body temperature at 37 °C. With the help of a surgical microscope and a dental drill, an opening of ~1.5 mm in diameter was made on the interparietal bone over the left inferior colliculus (0.5–2 mm posterior to the lambda and 0.5–2 mm left to the midline) for electrode placement. The dura was gently removed and the brain surface was cleaned and treated with saline. All electrophysiological experiments were performed in the soundproof chamber.

2.2. Acoustic stimulation

Two pure tone bursts (5-ms duration, 0.2-ms rise-fall times) were digitally generated and converted to analog signals in separate channels using a RZ6 Multi I/O processor (Tucker-Davis Technologies., Gainesville, FL, USA); each channel was connected to a multi-Field Magnetic Speaker (MF1, Tucker-Davis Technologies., Gainesville, FL, USA) through a digital attenuator (PA5, Tucker-Davis Technologies., Gainesville, FL, USA). The tone bursts were delivered to the left and/or right ears of the mouse through separated close-field adapters and 10-cm-long polyethylene tubes; one end of a tube was connected to the adapter and the other end was inserted into the external meatus. The tone amplitudes were expressed as dB SPL (ref. to 20 μPa) and calibrated at the ear-end of polyethylene tube using a Larson–Davis condenser microphone (Model 2520) and a microphone preamplifier (Model 2200C). The outputs (dB SPL) of two speakers across frequencies were equalized by the adjustment of the peak voltages of sinusoid waves that were sent to speakers. During the electrophysiological experiments, tone frequencies and amplitudes to both ears were independently controlled either manually or digitally by BrainWare data acquisition software (TDT). A frequency-amplitude scan (FA-scan) was used to sample the receptive field (frequency threshold tuning curve) of a given neuron. One FA-scan consisted of 527 tones with various frequencies and amplitudes in which the frequency ranged from 5 to 35 kHz with an increment of 1 kHz and the amplitude ranged from 7 to 87 dB SPL with an increment of 5 dB. To sample the reliable receptive field of a single ICc neuron, the FA-scan was repeated 3 times and the frequency/amplitude of tone in each FA-scan were randomly altered using BrainWare software.

2.3. Whole-cell recordings in the ICc

Glass pipettes (1.2 mm OD, 0.69 mm ID, #BF120-69-7.5, Sutter Instrument, Novato, USA) were pulled to construct a glass electrode with a tip of ~1 μm in diameter and a tip impedance of 7–10 MΩ. The electrode was filled with an "intracellular" solution composed of (in mM) 125 K-gluconate, 20 KCl, 10 Na2phosphocreatine, 4 MgATP, 0.3 Na2GTP, 0.5 EGTA and 10 HEPES (7.25 pH and 290 mosM). Biocytin (0.5%) was also added to the intracellular solution for the purpose of labelling the IC neurons around the recording site. The electrode was then connected with a silver wire to the headstage of a MultiClamp 700B amplifier. The MultiClamp 700B amplifier was controlled or operated by computer through the DigiData 1550 (Molecular Device, Sunnyvale, USA).

Our procedure for patching neurons followed standard protocols (Margrie et al., 2002). The glass electrodes were perpendicularly positioned on the surface of the left IC. The electrode tip was advanced by 350 μm without interruption using a digital manipulator; this depth ensured that the electrode tip was positioned in the central nucleus of the IC. At this time, the intraelectrode pressure was set at 200–300 mbar and the MultiClamp 700B was set at voltage-clamp mode. The intraelectrode pressure was then adjusted to ~30 mbar and advanced stepwise (0.5 μm per step). The electrode tip resistance was continuously monitored by delivering a square electrical pulse (10 mV, 10 ms). When the electrode tip resistance sharply increased by >20% and lasted longer than 10 s, the positive intraelectrode pressure was released. This operation commonly achieved a giga-ohm tip resistance that indicated a complete seal between the electrode tip and cell membrane; a supplement with gentle intraelectrode negative pressure was sometimes required. To establish the whole-cell configuration, a

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