Hearing Research 341 (2016) 79–90

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Research Paper

Differences in synaptic and intrinsic properties result in topographic heterogeneity of temporal processing of neurons within the inferior colliculus

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ARTICLE INFO

Article history:
Received 8 February 2016
Received in revised form
15 August 2016
Accepted 16 August 2016
Available online 17 August 2016

Keywords:
Auditory midbrain
Topography
Balanced excitation and inhibition
Intrinsic connectivity
Synaptic integration
Functional mapping

ABSTRACT

The identification and characterization of organization principals is essential for the understanding of neural function of brain areas. The inferior colliculus (IC) represents a midbrain nexus involved in numerous aspects of auditory processing. Likewise, neurons throughout the IC are tuned to a diverse range of specific stimulus features. Yet beyond a topographic arrangement of the cochlea-inherited frequency tuning, the functional organization of the IC is not well understood. Particularly, a common principle that links the diverse tuning characteristics is unknown. Here we used *in vitro* patch clamp recordings combined with laser-uncaging, and *in vivo* single cell recordings to study the spatial and functional organization principles of the central IC. We identified a topographic bias of ascending synaptic input timing that is balanced between inhibition and excitation and co-varies with *in vivo* first −spike latency. This bias was paralleled post-synaptically by differences in biophysical membrane properties and firing patterns, with integrating neurons predominantly found in the dorso-medial part, and coincidence-detector neurons biased to the ventro-lateral IC. Importantly, these cellular and network features translated into distinct temporal processing capabilities irrespectively of the neurons' characteristic frequency. Our data therefore imply that heterogeneity of synaptic inputs, intrinsic properties and temporal processing are functional principles that underlie the spatial organization of the central IC.

1. Introduction

The central nucleus of the inferior colliculus (ICC) receives input from most afferent auditory nuclei, making it a major processing center in the auditory pathway (Davis, 2005; Pollak, 2011; Wenstrup and Portfors, 2011). ICC neurons are heterogeneous in functional properties, as a wide variety has been reported for the shape of receptive fields, temporal response patterns (Ehret and Merzenich, 1988; Escabi and Schreiner, 2002; Lesica and Grothe, 2008; Palmer et al., 2013; Meyer et al., 2014), cell morphology (Rockel and Jones, 1973; Oliver and Morest, 1984; Malmierca et al., 2011) and receptor composition (Adams and Wenthold, 1979; Glendenning and Baker, 1988; Klepper and Herbert, 1991; Fubara et al., 1996; Merchan et al., 2005). This vast heterogeneity has obscured the understanding of a functional organizational principle of the ICC beyond the well documented tonotopy (Clopton and Winfield, 1973; Merzenich and Reid, 1974; Romand and Ehret, 1990; Bruckner and Rubsamen, 1995; Malmierca et al., 2008).

Structurally, a gradient of different receptor distributions in the ICC has been reported to stretch orthogonally to the tonotopy from the ventro-lateral to the dorso-medial region (Adams and Wenthold, 1979; Glendenning and Baker, 1988; Klepper and Herbert, 1991; Fubara et al., 1996; Merchan et al., 2005), indicating more prominent neuro-modulation in the dorso-medial ICC region. In alignment with the biochemical gradient, *in vivo* (Schreiner and Langner, 1988; Hattori and Suga, 1997) and *in vitro* (Reetz and Ehret, 1999; Chandrasekaran et al., 2013) recordings showed a latency gradient of evoked responses in the ICC. Neurons in the ventro-lateral ICC region respond primarily with short

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http://dx.doi.org/10.1016/j.heares.2016.08.005
0378-5955/

latencies to stimulations, whereas neurons in the dorso-medial ICC respond rather with long latencies. This latency difference results either from intrinsic cascaded ICC feed-forward signaling from the ventro-medial to the dorso-medial ICC (Reetz and Ehret, 1999; Miller et al., 2005) or on adaptations in the axonal conduction velocities of the afferent fibers (Hattori and Suga, 1997). Furthermore, auditory response thresholds appear arranged diagonally to tonotopy, with lowest thresholds at central ICC locations (Stiebler and Ehret, 1985). There are also a number of studies on the organization of neuronal sensitivity to amplitude modulations within the ICC. Schreiner and Langner (1988) were the first to report an increase of preferred amplitude modulation frequencies from dorsal to ventral regions of ICC in the cat. Using function magnetic resonance imaging, Baumann et al. (2011) discovered a topographic representation of preferred amplitude modulation frequencies that stretched orthogonal to the tonotopic gradient in the rhesus monkey IC. *In vivo* single cell data also suggested a systematic change in temporal preference in cat IC, however along the tonotopic axis (Rodriguez et al., 2010a). Recently, it was reported that in the gerbil, temporal sensitivity is not distributed gradually, but by local clustering within the ICC (Schnupp et al., 2015). In summary, the spatially arranged biochemical, latency and tuning differences suggest additional organizational principles beyond tonotopy in the ICC. However, it remains unclear whether the spatial distribution of these various properties represents independent organization features or a product of a common processing principle.

Using *in vitro* and *in vivo* methods, we provide evidence that physiological relevant latency differences between ventro-lateral to dorso-medial regions of the ICC are the result of a combination of region-specific specializations of cellular properties and the complexity of the local ICC circuitry. We explain that these region-specific specializations shape the temporal processing capabilities of the respective ICC neurons, and thus contribute to a common processing motif. In summary our data manifests a functional organization principle based on heterogeneity of synaptic inputs, intrinsic properties and resulting temporal processing within the ICC.

2. Materials and methods

2.1. In vitro slice preparation

Slices were prepared from Mongolian gerbils (*Meriones unguiculatus*) of both sexes from postnatal day (P) 14–17 according to the guidelines of the Regierung of Oberbayern and the Deutsches Tierschutzgesetz (AZ 55.2-1-54.2531.8-211-10). Animals were decapitated and brains were removed in dissection solution containing (in mM) 50 sucrose, 25 NaCl, 25 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 3 $MgCl_2$, 0.1 $CaCl_2$, 25 glucose, 0.4 ascorbic acid, 3 *myo*-inositol and 2 Na-pyruvate (pH 7.4 when bubbled with 95% O_2 and 5% CO_2). Midbrains were trimmed caudo-rostrally at ~45° and 250–300 μm slices were taken with a VT1200S vibratome (Leica). The most caudal slices were not used, since they contained no visually detected lateral lemniscus fibers. Slices were incubated in recording solution (same as slice solution but with 125 mM NaCl, no sucrose and 2 mM $CaCl_2$ and 1 mM $MgCl_2$) at 36 °C for 15–45 min, bubbled with 5% CO_2 and 95% O_2.

2.2. In vitro electrophysiology

Slices were transferred to a recording chamber attached to a microscope (BX50WI; Olympus) equipped with gradient contrast illumination (Luigs and Neumann) and continuously perfused with recording solution. All recordings were performed at 33–35 °C using an EPC10/2 amplifier (HEKA electronic). Access resistance was compensated to a residual of 2.5–3 MOhm; electrophysiological patch-clamp data were acquired at 20–50 kHz and filtered at 3 kHz. Synaptic responses were evoked with a concentric bipolar electrode placed in the lateral lemniscus bundles between the IC and the dorsal nucleus of the lateral lemniscus while cells were held in voltage clamp at −60 mV. Biphasic stimulations of 200 μs total length with stimulus amplitudes of 80 V were delivered by an A-M systems stimulator Model 2100. This stimulation strength appeared best suited to evoke a maximal synaptic response. Excitatory post synaptic currents (EPSC), potentials (EPSP) and evoked action potentials were recorded in the presence of SR95531 (SR; 10 μM) and strychnine (Stry; 0.5 μM). Evoked inhibitory post-synaptic currents (IPSC) were recorded in the presence of DNQX (20 μM), D-APV (50 μM) or *R*-CPP (10 μM). The intracellular solution used to record EPSCs was (in mM): 130 Cs-gluconate, 10 Cs-HEPES, 20 tetraethylammonium (TEA)-Cl, 3.3 $MgCl_2$, 2 Na_2-ATP, 0.3 Na_2-GTP, 3 Na_2-phosphocreatine, 5 Cs-EGTA and 5 QX-314 [*N*-(2,6-dimethylphenylcarbamoylmethyl) triethylammonium bromide] with 50–70 μM Alexa Fluor 488. IPSCs were recorded with an internal solution containing (in mM): 105 Cs-gluconate, 26.7 CsCl, 10 Cs-HEPES, 20 TEA-Cl, 3.3 $MgCl_2$, 2 Na_2-ATP, 0.3 Na-GTP, 3 Na_2-phosphocreatine, 5 Cs-EGTA, and 5 QX-314 with 50–70 μM Alexa Fluor 488. Intracellular voltage-clamp solutions were adjusted to pH 7.2 with CsOH. The internal solution for current-clamp recordings contained (in mM): 145 K-gluconate, 15 HEPES, 5 KCl, 2 Na_2-ATP, 0.3 Na-GTP, 2 K-ATP, 5 Na_2-phosphocreatine, 5 K-EGTA, with 50–70 μM Alexa Fluor 568, Intracellular solutions were adjusted to pH 7.2 by KOH. No correction for liquid junction potential was carried out.

2.3. Inter ICC input mapping

Input mapping was carried out at room temperature (22–25 °C), according to (Bendels et al., 2008; Couchman et al., 2012). After incubation, slices were transferred to a recording chamber attached to a microscope (BX51W1, Olympus) equipped with gradient contrast illumination (Luigs and Neumann). 10 ml of recording solution containing 0.25 mM MNI-caged-L-glutamate were recirculated throughout the experiment. Cells were dye filled via the recording pipette, visualized and imaged with a TILL Photonics IR camera (VX 55) with fluorescence lamp (Xcite, Olympus) under the control of an acquisition and microscope control software (Morgentau M1, Morgentau Solutions, Munich, Germany). Input mapping started after the slice was incubated for 25 min in MNI-caged-L-glutamate containing solution and was carried out for maximally 70 min. The focus of the double-pumped solid state UV laser (DPSL-355/1000 Rapp Optoelectronics), coupled to the microscope with a 50 μm quartz light guide was placed between 30 and 50 μm below the slice surface. Optical stimulation of caged-glutamate was carried out with 4 ms laser pulses at a 0.25 W. For these stimulation parameters we estimated the radius at which the light stimulus activates glutamate receptors (uncaging spot size) by voltage clamp recordings while the stimulation site was successively moved away from a proximal dendrite of an ICC neuron. The uncaging spot size was then computed as the distance at which the current amplitude decreased by 50% using a Gaussian fit. The resulting full width at half maximum was ~13.5 μm ($n = 5$). To calibrate the scanning resolution the direct optical activation of action potentials by glutamate uncaging was determined in current-clamp, while blocking synaptic transmission with DNQX, D-AP5, Strychnine and SR95531. From these experiments ($n = 20$) an effective radius of 35 μm in which the cell could be optically driven suprathreshold was derived. For input mapping the laser beam was directed at multiple predefined stimulation sites organized as a hexagonal grid with node distance of 35 μm and liberated