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ANALYSIS OF EXCITATORY SYNAPSES IN THE GUINEA PIG INFERIOR COLLICULUS: A STUDY USING ELECTRON MICROSCOPY AND GABA IMMUNOCYTOCHEMISTRY

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Abstract—The inferior colliculus (IC) integrates ascending auditory input from the lower brainstem and descending input from the auditory cortex. Understanding how IC cells integrate these inputs requires identification of their synaptic arrangements. We describe excitatory synapses in the dorsal cortex, central nucleus, and lateral cortex of the IC (ICd, ICc and IClc) in guinea pigs. We used electron microscopy (EM) and post-embedding anti-GABA immunogold histochemistry on aldehyde-fixed tissue from pigmented adult guinea pigs. Excitatory synapses were identified by round vesicles, asymmetric synaptic junctions, and gamma-aminobutyric acid-immunonegative (GABA-negative) presynaptic boutons. Excitatory synapses constitute ~60% of the synapses in each IC subdivision. Three types can be distinguished by presynaptic profile area and number of mitochondrial profiles. Large excitatory (LE) boutons are more than 2 μm^2 in area and usually contain five or more mitochondrial profiles. Small excitatory (SE) boutons are usually less than 0.7 μm^2 in area and usually contain 0 or 1 mitochondria. Medium excitatory (ME) boutons are intermediate in size and usually contain 2 to 4 mitochondria. LE boutons are mostly confined to the ICc, while the other two types are present throughout the IC. Dendritic spines are the most common target of excitatory boutons in the IC dorsal cortex, whereas dendritic shafts are the most common target in other IC subdivisions. Finally, each bouton type terminates on both gamma-aminobutyric acid-immunopositive (GABA+) and GABA-negative (i.e., glutamatergic) targets, with terminations on GABA-negative profiles being much more frequent. The ultrastructural differences between the three types of boutons presumably reflect different origins and may indicate differences in postsynaptic effect. Despite such differences in origins, each of the bouton types contact both GABAergic and non-GABAergic IC cells, and could be expected to activate both excitatory and inhibitory IC circuits.

Key words: auditory, dendritic spines, gamma-aminobutyric acid, ultrastructure, inhibition, circuit.

INTRODUCTION

The inferior colliculus (IC) processes the majority of ascending auditory information and thus is associated with most aspects of auditory perception. The IC also connects with motor centers such as the superior colliculus (SC) and pontine nuclei (PN), and thus contributes to orienting behavior and acoustic guidance of movement (Huffman and Henson, 1990; Thompson, 2005). Finally, the IC is both a target and a source of descending auditory pathways. Auditory feedback to the IC may play a role in detection of signals in noise, sound localization, and auditory attention (reviewed in Schofield, 2011a; Suga, 2012).

Neurons in the IC show a diverse range of responses to sounds. The diversity arises from variation in intrinsic properties across different IC cell types and from convergence of inputs from different origins (Sivaramakrishnan and Oliver, 2001; Kelly and Caspary, 2005; Oliver, 2005; Saldaña and Merchán, 2005; Schofield, 2005; Wu, 2005; Cant and Benson, 2006). Despite the large number of inputs to the IC, both anatomical and physiological studies suggest that the responses of many IC cells depend on a relatively small subset of the ascending auditory inputs (Brunso-Bechtold et al., 1981; Davis, 2002; Pollak et al., 2003; Oliver, 2005; Cant and Benson, 2006; Loftus et al., 2010). However, there remain many questions about the exact inputs that converge onto specific types of IC cells. Solving this puzzle – identifying the circuits of IC cells – is fundamental to understanding how IC cells integrate auditory information and serve the multitude of functions attributed to the IC.

An important step in understanding circuits is identifying their synaptic connections. Ultrastructural studies with the electron microscope (EM) provide the

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Abbreviations: D, dorsal; M, mitochondria; pa, punctum adherens; R, rostral; EM, electron microscopy; GABA-negative, gamma-aminobutyric acid immunonegative; GABA+, gamma-aminobutyric acid immunopositive; IC, inferior colliculus; ICc, central nucleus of the inferior colliculus; ICd, dorsal cortex of the inferior colliculus; IClc, lateral cortex of the inferior colliculus; LE, large excitatory bouton type; LSO, lateral superior olive; ME, medium excitatory bouton type; Mo5, motor trigeminal nucleus; PB, phosphate buffer; PN, pontine nuclei; SC, superior colliculus; scp, superior cerebellar peduncle; SE, small excitatory bouton type; SN, substantia nigra; TBS, tris-buffered saline with 0.1% Triton X-100; VLL, ventral nucleus of the lateral lemniscus; VNTB, ventral nucleus of the trapezoid body.

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necessary resolution to identify synaptic relationships. A common starting point is identifying synaptic features that distinguish potentially excitatory or inhibitory synapses. Details of the presynaptic profiles may allow particular bouton types to be related to their origins. In addition, identifying the postsynaptic target (dendritic spine, dendritic shaft, soma) of a synapse provides insight into integration of inputs by the postsynaptic cell. While the resolution of EM provides detailed information about synapses, its high demands in labor and generally small sample sizes have limited the available information. Much of the information on ultrastructure of the IC is from the central nucleus of the IC (ICc), the major target of ascending lemniscal afferents. Much less information is available for the dorsal cortex (ICd) and the lateral cortex (IClc). Given that the three IC subdivisions are functionally distinct, there will be a need to understand the circuitry of each area (Aitkin, 1986; Irvine, 1986; Huffman and Henson, 1990; Rouiller, 1997; Malmierca and Hackett, 2010).

The available information on the synaptic organization of the IC is also limited with respect to species; most studies have been in cats, rats or mice (Rockel and Jones, 1973; Granstrem, 1984; Roberts and Ribak, 1987; Paloff and Usunoff, 1992; Oliver et al., 1995; Saldaña et al., 1996; Gaza and Ribak, 1997; Kazee and West, 1999; Liu et al., 1999; Mei et al., 1999). We have begun a series of studies to examine the synaptic organization of the IC in guinea pigs. This species is widely used in auditory research but, to our knowledge, there are no descriptions of the guinea pig IC ultrastructure.

This report focuses on presumptive excitatory synapses. We identified ultrastructural features that distinguish three types of excitatory synapse. Two types are present throughout the IC, whereas the third type is largely confined to the ICc. The three types probably arise from different sources and also differ in the relative targeting of spines versus dendritic shafts, implying that they may have different postsynaptic effects. All three types terminate on both GABAergic and non-GABAergic (i.e., glutamatergic) targets, suggesting that inputs associated with each of the excitatory synapse types are likely to activate both excitatory and inhibitory IC cells.

EXPERIMENTAL PROCEDURES

Experiments were performed on six adult pigmented guinea pigs of either gender weighing 400 to 900 g (Elm Hill Breeding Laboratories, Chelmsford, MA, USA). All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) and administered following the National Institutes of Health guidelines for the care and use of laboratory animals. In accordance with these guidelines, all efforts were made to minimize the number of animals used and their suffering. Five of the six animals used for this study underwent survival surgery for injection of anatomical tracers into the auditory cortex prior to perfusion. The procedures for the tracer injections were similar to previous descriptions from this laboratory (e.g., Schofield, 2011b) and were approved by the IACUC. The use of tissue from these animals allowed us to minimize the number of animals needed for the present study. The results of the tracer injections will be described in a separate report.

Perfusion and sectioning

Each animal was sacrificed by overdose with sodium pentobarbital (440 mg/kg; i.p., Euthasol, Virbac Inc., Fort Worth, TX, USA) or isoflurane (inhalation until cessation of breathing; Aerrane, Baxter, Deerfield, IL, USA). The animal was perfused through the aorta with Tyrode's solution, followed by 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4). The brain was then removed and stored overnight at 4 °C in 2% paraformaldehyde and 2% glutaraldehyde in PB. The following day, tissue blocks containing the IC were cut into 50 μm parasagittal sections with a Vibratome. Sections were collected in six series and processed as described below or placed in freezing buffer and stored for future processing.

Identification of IC subdivisions

One series of sections from each animal was stained for NADPH-diaphorase activity (Dawson et al., 1991). The stained sections were mounted on slides, dried overnight and then coverslipped with DPX (Aldrich Chemical Company, Inc., Milwaukee, WI, USA). The NADPH stain reflects the distribution of neuronal nitric oxide synthase and can be used to distinguish IC subdivisions in guinea pigs (Coote and Rees, 2008). In general, the ICc stains very lightly for NADPH whereas the other IC areas are more darkly stained. The borders between the ICc and the surrounding regions can be difficult to specify, particularly in some areas (e.g., the border between the ICc and the dorsomedially adjacent ICd). The ICc appears lightly stained overall because of very limited neuropil staining. The ICd has darker neuropil, but the transition is gradual. The uncertainty can be reduced by looking at the sections under high magnification to allow identification of individually stained cells, which appear in the ICd and also in part of the ICc. In some cases, it is possible to identify cells with dendritic trees that are elongated along a dorsomedial to ventro-lateral axis, consistent with the description of so-called ''flat'' cells that characterize the ICc. We used such staining to refine our estimation of the borders. As discussed by Coote and Rees (2008), this approach leads to a slightly larger ICc than would otherwise be drawn.

Processing for EM

One or more additional series of sections (not stained for NADPH) were post-fixed for 1 h in 2% osmium tetroxide in PB, dehydrated in an alcohol series, embedded in Durcupan resin (Electron Microscopy Sciences, Fort Washington, PA, USA) and flat-mounted between sheets of Aclar Embedding Film (Ted Pella, Inc., Redding, CA, USA). The sections were then examined in a light microscope and compared to NADPH-stained sections in order to determine the boundaries of the IC subdivisions. An area up to 1 mm on a side and located completely within a particular IC subdivision was trimmed from the section with a scalpel and glued onto a resin base with cyanoacrylate (KrazyGlue, Columbus, OH, USA). The positions of the tissue blocks were plotted and superimposed on a series of NADPH-stained sections (Fig. 1), using a Zeiss Axioplan 2 microscope with a Neurolucida system (MBF Bioscience, Williston, VT). For samples from the ICc, we selected regions with low NADPH staining, well within the borders of the ICc as described in the paragraph above. The locations of the samples are shown in Fig. 1 (sections 1300 and 1600). For analysis of the IClc, we took three samples from regions associated with strong NADPH staining and located in very lateral sections in the IC (Fig. 1). Finally, for analysis of the