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Divergent response properties of layer-V neurons in rat primary auditory cortex

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Abstract

Layer-V pyramidal cells comprise a major output of primary auditory cortex (A1). At least two cell types displaying different morphology, projections and in vitro physiology have been previously identified in layer-V. The focus of the present study was to characterize extracellular receptive field properties of layer-V neurons to determine whether a similar breakdown of responses can be found in vivo. Recordings from 105 layer-V neurons revealed two predominant receptive field types. Thirty-two percent displayed strong excitatory V/U-shaped receptive field maps and spiking patterns with shorter stimulus-driven interspike intervals (ISIs), reminiscent of the bursting cells discussed in the in vitro literature. V/U-shaped maps remained relatively unchanged across the three sequential repetitions of the map run on each neuron. Neurons with V/U-shaped maps were also easily depolarized with extracellular current pulse stimulation. In contrast, 47% of the neurons displayed Complex receptive field maps characterized by weak and/or inconsistent excitatory regions and were difficult to depolarize with current pulses. These findings suggest that V/U-shaped receptive fields could correspond to previously described intrinsic bursting (IB) cells with corticotectal projections, and that neurons with Complex receptive fields might represent the regular spiking (RS) cells with their greater inhibitory input and corticocortical/corticostriatal projection pattern.

Keywords: Layer V; Rat; Primary auditory cortex; Pyramidal; Extracellular; Receptive field; Juxtacellular

1. Introduction

Layer V comprises a major projection of primary auditory cortex (A1) to other auditory areas in the cortex (Budinger et al., 2000a; Code and Winer, 1986; Winguth and Winer, 1986), subcortically to the thalamus, inferior colliculus, sagulum, superior olivary complex and cochlear nucleus (Aitkin et al., 1981; Beneyto et al., 1998; Budinger et al., 2000b; Coleman and Clerici, 1987; Colwell, 1975; Coomes and Schofield, 2004; Doucet et al., 2002; Faye-Lund, 1985; Games and Winer, 1988; Herbert et al., 1991; Huffman and Henson, 1990; Saldana and Merchan, 1992; Weedman and Ryugo, 1996a,b; Winer, 1992; Winer et al., 1998), and even to non-auditory areas such as the striatum (Moriizumi and Hattori, 1991; Ojima et al., 1992; Reale and Imig, 1983). Layer-V consists of a number of different cell types (Winer and Prieto, 2001); among them are two pyramidal cell types that have been distinguished by their morphology, connectivity and in vitro electrophysiology. Fibers from large layer-V pyramidal neurons appear to make up the corticotectal system while a second group of smaller pyramidal neurons likely comprise the corticocortical (Ruttgers et al., 1990; Vaughan, 1983;

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Winer and Prieto, 2001) and corticostriatal projections (Ojima et al., 1992).

Recent electrophysiological studies also described two distinct layer-V pyramidal types possessing different properties (Hefti and Smith, 2000, 2003). Using in vitro techniques, the intrinsic burster (IB) neurons exhibited robust excitatory bursting responses to electrical and synaptic stimulation, large pyramidal cell bodies and thick apical dendrites frequently extending into layer I. IB neurons demonstrated only modest inhibitory synaptic currents, modest responses to GABAergic blockade and most of their active GABAergic inhibitory synapses were found on distal dendrites. In contrast, regular spiking (RS) neurons were characterized by strong inhibitory influences (Hefti and Smith, 2000, 2003). GABAergic synapses on these cells were commonly found on proximal dendrites and somata. These neurons exhibited dramatically increased excitation upon $GABA_A$ receptor blockade. Morphologically, RS neurons were smaller and possessed finer apical dendrites which extended only to layers II or III.

Collectively, the data suggests there are at least two types of A1 layer-V neurons which differ in morphology, connectivity and the degree of inhibitory influence on them. A similar breakdown of IB and RS cells differing in intrinsic firing characteristics, degree of inhibitory influence, cell morphology and projection patterns have been observed for layer-V in other sensory and motor systems and might be a common organizing feature of layer-V pyramidal neurons (Agmon and Connors, 1989; Chagnac-Amitai et al., 1990; Connors and Gutnick, 1990; Deschenes et al., 1994; Nicoll et al., 1996; Zhu and Connors, 1999).

The focus of the present in vivo study was to characterize extracellular receptive field properties of layer-V neurons to determine whether a similar bimodal parsing of responses can be found in vivo in young adult animals in response to acoustic and current pulse stimuli. Outlining the receptive field properties of layer-V neurons in A1, in combination with previous morphological, connectivity, and in vitro work, should allow for a refinement of the putative models of coding along the auditory neuraxis.

2. Materials and methods

2.1. Subjects

Eleven young-adult (4–6 mos) Fischer Brown Norway (FBN) rats were used to collect data from presumptive layer-V neurons. All experiments were conducted in an International Acoustics Corporation sound-attenuating booth under a protocol approved by the Southern Illinois University School of Medicine Laboratory Animal Care and Use Committee. Experimentation was conducted in accordance with the Society for Neuroscience's Policy on the Use of Animals in Neuroscience Research.

2.2. Surgical protocol

Animals were initially anesthetized with a 1.4 ml/kg dose of a 3:1 mixture of ketamine (100 mg/ml):xylazine (20 mg/ml) and maintained for the remainder of the experiment (typically 10–12 h) on booster doses of urethane (500 mg/kg every 3–4 h, or as needed). Urethane (ethyl carbamate, Sigma) was selected following a series of pilot recordings from A1 neurons in 10 FBN rats. A1 neurons recorded from urethane anesthetized animals were more responsive to acoustic stimuli than cells under ketamine/xylazine or barbiturate (unpublished personal observations). Urethane was also reported to have relatively minor affects on the GABAergic systems (Hara and Harris, 2002; Maggi and Meli, 1986). Animals were placed on a homeothermic blanket with body temperature monitored via a rectal probe and maintained at 37° C. A metal plate was attached to the dorsal skull with ethyl cyanoacrylate (Krazy Glue) and dental acrylic. The skin was reflected from the lateral aspect of the skull, the temporalis muscle retracted and a small craniotomy centered 5 mm caudal and 4 mm ventral to Bregma was performed to expose the left A1. A 3 mm diameter ovoid craniotomy exposed the projected center of A1 as described by Doron et al. (2002), Games and Winer (1988), Paxinos and Watson (1986, 1998), Sally and Kelly (1988) and Zilles and Wree (1995). The dura was then carefully removed and the surface of A1 kept moist with mineral oil.

The center of the recording field was always 5 mm caudal and 4 mm ventral to Bregma. The operational criteria for recording layer-V neurons included correct stereotaxic coordinates, presence of a robust acoustically evoked "slow-wave" and units located between 600 and 950 μ from the pial surface (Games and Winer, 1988) as measured by a Burleigh 6000 series microdrive (Fishers, NY). Generally, one (sometimes two) neuron could be isolated for each penetration (usually >1 mV above background noise). A typical recording session for each animal consisted of more than six electrode penetrations spaced within about 0.5 mm in all surface directions of the stereotaxic middle of A1 (5 mm caudal and 4 mm ventral from Bregma). Recent work suggests that the best estimate for A1 might not be vascular landmarks but a combination of stereotaxic coordinates relative to Bregma and physiological responses (Doron et al., 2002). Using these criteria, we are relatively confident that our recordings, which spanned only 0.5 mm in each direction from the projected center of A1, were likely obtained from A1. All successful Neurobiotin and HRP marks, and electrode tracts which could be