RESPONSES OF COCHLEAR NUCLEUS CELLS AND PROJECTIONS OF THEIR AXONS

Philip H. Smith, Philip X. Joris, Matthew I. Banks and Tom C.T. Yin

Department of Neurophysiology, University of Wisconsin, Madison, Wisconsin, USA

The advent of intracellular recording using horseradish peroxidase-filled glass electrodes offered a new and exciting approach to the *in vivo* cochlear nucleus (CN) preparation. Sharp glass microelectrodes, filled with a standard salt solution containing positively charged HRP molecules, made it feasible to record and characterize supra- and subthreshold intracellular responses of individual neurons to auditory stimuli, inject and subsequently recover the same HRP-labeled cell and ask the following basic questions; 1) Do morphologically defined cell types in the cochlear nucleus respond in a certain way to simple auditory stimuli at both the sub- and suprathreshold level? 2) Are the synaptic inputs of different cell types different, in terms of location, type and concentration, and are they arranged in ways that might help to explain the cell type's unique responsiveness? 3) What is the projection pattern of individual axons originating from a given cell type, what are the shapes of vesicles within the terminals of these axons and can we make educated guesses about their influence on other cell populations based on this information?

Although fruitful, the method has proven to be difficult for many reasons including the surgically difficult location of the CN, the respiratory and cardiac-induced pulsations that are prevalent in the brainstem, susceptibility of the nucleus to edema and electrode "plugging" during penetrations deep into the nucleus. As a consequence, the number of cells recorded from and recovered after injection is small. Never-the-less, results from this lab, using this method in the cat CN (Rhode et al., '83; Rhode et al., '83; Smith and Rhode, '85; Smith and Rhode, '87; Smith and Rhode, '89; Smith et al., '91) have indicated that there is a good correlation between certain unique physiological features and a characteristic set of anatomical traits displayed by a given principal cell type.

One of the other shortcomings of this method, when employed on cells or their axons within the cochlear nucleus, is a failure to fill the axon sufficiently such that its course and termination sites outside the CN complex can be identified. For this reason, our recent approach has been to expose a major fiber tract emanating from the CN, penetrate an axon in that tract, record intra-axonally, characterize the response to simple stimulus paradigms and inject with tracer. This method eliminates the possibility of studying synaptic events seen in cell body or proximal axonal recordings but is advantageous in that the spike output of the cell can be monitored more stably, alteration of response behaviors due to injury is less likely, and more of the axon can be filled. To further characterize the influence of some of these CN axons on third order cells we have begun to look at the synaptic responses of cells

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in other auditory brainstem nuclei, using the *in vitro* brain slice method in rats, when an output pathway of the CN is electrically stimulated (Banks and Smith, '90). In addition, we have begun using the biotin/lysine conjugate known as neurobiotin (Vector Labs; Horikawa and Armstrong, '88) as the intracellular marker in both the *in vivo* and *in vitro* experiments. Its advantages over HRP include improved electrode recording characteristics, ease of injection from unbeveled electrodes, and improved filling of the injected neuron. Our initial attempts at strial recordings have focused on the ventral acoustic stria (VAS) or trapezoid body (TB) and it is only recently that we have initiated work on the dorsal and intermediate acoustic striae (DAS and IAS). As a consequence, the data presented here will deal primarily with cells whose axons use the VAS.

Bushy Cells

As the name implies, bushy cells are distinguished by the bushy nature of their dendritic tree (Brawer et al., '74) and are subdivided into spherical (SBC) and globular bushy cell (GBC) categories. Their axons are the primary component of the VAS, the disruption of which affects sound localization, (Casseday and Neff, '75; Jenkins and Masterton, '82; Masterton, et al., '81; Moore et al., '74; Neff, '62) the startle response (Davis et al., '82) and pinna reflexive behavior (Cassella and Davis, '86).

Globular bushy cells show unique physiological features. Intracellular records from GBC somata or the proximal axon (Smith and Rhode, '87) reveal what appears to be very large and very fast excitatory synaptic events that do not necessarily elicit a spike. Subsequent work by others, in mouse brain slice (Wu and Oertel, '84) and the dissociated cell preparation, (Manis and Marx, '92) has shown that the speed of repolarization after a synaptic event is due to a potassium conductance active around the resting potential. In the cat, short tones at the cell's characteristic frequency (STCF, with CF being the frequency which elicits an increase in spike output at the lowest stimulus intensity) generate characteristic peristimulus time histogram (PSTH) patterns (Fig. 1). PSTHs of globular bushy cells with CFs above 3 kHz resemble the auditory nerve (AN) PSTH but differ at higher stimulus intensities in that the PSTH patterns show a precisely timed onset peak which may be followed by a brief depression of activity and then a resumption of sustained activity. This PST pattern is called primary-like-with-notch (PL_N) if the notch is prominent or O_L (onset with low sustained activity) if the notch is not as obvious and the sustained rate not as high. Our more recent experiments (Smith et al., '91), recording from high CF globular bushy axons in the TB, have confirmed these results. In addition, a number of axons of GBCs with CFs $<$ 3 kHz were labeled. Those with CFs between 1 and 3 kHz also show the PL_N response with spikes in the sustained portion of the response phase-locked (spikes occurring at a particular phase angle of a sinusoidal stimulus) albeit not as well as eighth nerve (AN) fibers with the same CF (Johnson, '80). Curiously, these same 1-3 kHz GBCs will often phase-lock very accurately with vector strengths (a measure of phase-locking, Goldberg and Brown, '69) >0.9 when driven by a low frequency tone (less than 1 kHz). GBCs with CFs below 1 kHz also phase-lock better than AN fibers with similar CFs, both in terms of their vector strengths (which were above 0.9) and the degree to which they entrain (respond to every cycle) to the stimulus. The above observations imply that the large, fast (yet sometimes subthreshold) synaptic events generated by AN inputs can, under appropriate stimulus conditions, generate globular bushy cell outputs with enhanced onset components and/or enhanced phase-locking. In addition, the spontaneous spike rates (SR) of our labeled GBCs with CFs below 2-3 kHz were usually lower than AN fibers (less than 2 spks/sec.). When taken with the observation of Liberman (presentation at this meeting), that GBC-AN input is dominated by high SR units, this feature strongly suggests that AN fiber-evoked synaptic events can be subthreshold in the GBC population.