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AUDITORY NERVE INPUTS TO COCHLEAR NUCLEUS NEURONS STUDIED WITH CROSS-CORRELATION

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Abstract—The strength of synapses between auditory nerve (AN) fibers and ventral cochlear nucleus (VCN) neurons is an important factor in determining the nature of neural integration in VCN neurons of different response types. Synaptic strength was analyzed using cross-correlation of spike trains recorded simultaneously from an AN fiber and a VCN neuron in anesthetized cats. VCN neurons were classified as chopper, primarylike, and onset using previously defined criteria, although onset neurons usually were not analyzed because of their low discharge rates. The correlograms showed an excitatory peak (EP), consistent with monosynaptic excitation, in AN–VCN pairs with similar best frequencies (49% 24/49 of pairs with best frequencies within ±5%). Chopper and primarylike neurons showed similar EPs, except that the primarylike neurons had shorter latencies and shorter-duration EPs. Large EPs consistent with end bulb terminals on spherical bushy cells were not observed, probably because of the low probability of recording from one. The small EPs observed in primarylike neurons, presumably spherical bushy cells, could be derived from small terminals that accompany end bulbs on these cells. EPs on chopper or primarylike-with-notch neurons were consistent with the smaller synaptic terminals on multipolar and globular bushy cells. Unexpectedly, EPs were observed only at sound levels within about 20 dB of threshold, showing that VCN responses to steady tones shift from a 1:1 relationship between AN and VCN spikes at low sound levels to a more autonomous mode of firing at high levels. In the high level mode, the pattern of output spikes seems to be determined by the properties of the postsynaptic spike generator rather than the input spike patterns. The EP amplitudes did not change significantly when the presynaptic spike was preceded by either a short or long interspike interval, suggesting that synaptic depression and facilitation have little effect under the conditions studied here.

Key words: cross-correlation, ventral cochlear nucleus, synaptic strength.

The definition of neuron types in the cochlear nucleus by Osen (1969) was a seminal observation for research on this nucleus. In the ventral cochlear nucleus (VCN), the differentiation of spherical and globular bushy cells and their distinction from a heterogeneous population of multipolar cells provided the basic model for the VCN that was elaborated over subsequent years. In this model, the VCN consists of parallel systems of different neuron types, each innervated by auditory nerve (AN) fibers of all best frequencies (BFs) (Osen, 1970b).

As knowledge of the differences in innervation patterns (Osen, 1970a; Cant, 1992) and postsynaptic membrane properties (Oertel, 1983; Manis and Marx, 1991) of VCN neurons accumulated, functional models of neural integration in each parallel pathway were developed. Bushy cells participate in secure synapses with AN fibers so as to provide precise temporal information about AN spike times for the analysis of interaural time difference in the superior olivary complex (e.g. Joris and Yin, 2007; Joris and Smith, in press). Multipolar neurons temporally integrate the activity of AN fibers to provide a stable and robust representation of stimulus spectrum (e.g. Blackburn and Sachs, 1990). Critical to these models was the identification of primarylike (pri) neural response patterns with bushy cells and chopper response patterns with multipolar cells (Rhode et al., 1983; Smith and Rhode, 1987, 1989; Ostapoff et al., 1994; Rhode, in press).

Computational studies identified the strength of the synapses made by AN fibers on VCN neurons as an important variable in determining their response properties (Molnar and Pfeiffer, 1968; Rothman et al., 1993; Hewitt and Meddis, 1993; Joris et al., 1994). Although postsynaptic properties determine the basic features of VCN responses (Banks and Sachs, 1991; Arle and Kim, 1991; Wang and Sachs, 1995; Rothman and Manis, 2003) including phase locking, regularity, and peri-stimulus time (PST) histograms, these properties are strongly modulated by the degree of convergence of AN inputs and their synaptic strengths.

There has been no direct study of the functional strength of synapses between AN fibers and VCN neurons *in vivo*. This information can be obtained from studies of the cross-correlation of spike trains of simultaneously recorded AN fibers and VCN neurons, which is reported here. Cross-correlation has been used in the auditory system to work out neural circuits (Voigt and Young, 1990), to analyze the ensemble representation of stimuli (deCharms and Merzenich, 1996; Eggermont, 2006), to analyze temporal features of the responses to sound (Louage et al., 2005), and to analyze the organization of projections from one level of the system to the next (Miller et al., 2001). Because the organization of AN synapses on CN neurons is relatively simple and much is known about it, it is pos-

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Abbreviations: AN, auditory nerve; BF, best frequency; CM, central mound; DCN, dorsal cochlear nucleus; EP, excitatory peak; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; pri, primarylike; pri-N, primarylike-with-notch; PST, peri-stimulus time; VCN, ventral cochlear nucleus.

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sible to interpret the results of cross-correlation at this synapse with some certainty, as opposed to the difficulties posed by unknown circuits in other parts of the auditory system.

Here we show that the cross-correlograms of AN fibers and VCN neurons have the expected properties of short duration, short latency excitatory effects that are tonotopic. Little effect of short-term plasticity was observed. However, cross-correlation was observed only at low sound levels, which raises new questions about the modes of integration of AN activity by VCN neurons at high sound levels.

EXPERIMENTAL PROCEDURES

Two types of experiment were done using similar preparations: 1) simultaneous recordings from pairs of AN fibers (AN/AN experiments, four cats); 2) simultaneous recordings from AN fibers and VCN neurons (AN/VCN experiments, 14 cats). All surgical and experimental procedures were approved by the Johns Hopkins Animal Care and Use Committee; procedures are in compliance with the U.S. Animal Welfare Act and with Public Health Service policies. Every effort was made to minimize the number of animals used and their suffering.

Surgical preparation and electrodes

Cats were given atropine (0.03 mg/kg i.m.) to control secretions and anesthetized with ketamine (30–40 mg/kg i.m.). A tracheotomy was performed and a venous cannula was inserted. Anesthesia was maintained by sodium pentobarbital (≈3 mg/kg/h i.v., as necessary to maintain areflexia). The cat's temperature was maintained at 39 °C with a feedback-controlled heating pad. Lactated Ringer's was injected i.v. to prevent dehydration. The bulla was vented with a length of small-bore polyethylene tubing to prevent buildup of static pressure in the middle ear.

The skull over the cerebellum was removed and retraction and aspiration of the cerebellum were used to expose the cochlear nucleus. Gentle traction on the cerebellum and small cotton balls placed against the dorsal cochlear nucleus (DCN) were used to expose the AN at the internal meatus. For AN recording, micropipettes filled with 3 M NaCl were used (10–30 MΩ impedance); for VCN recording, platinum–iridium electrodes were used. Only well-isolated single neurons were studied. In the AN/AN experiments, two micropipettes were placed in the AN in close proximity (<1 mm) in a medial–lateral orientation, so that they might intercept the same bundles of fibers and thus record from fibers with similar BFs. In the AN/VCN experiments the pipette was placed in the nerve and the metal electrode in the VCN. Histology was done in nine of the AN/VCN experiments to locate the electrode tracks. All were in the VCN.

For recording, the cat was placed in a soundproofed chamber. The closed acoustic system was coupled to the ear through a 13 cm ear bar and was calibrated *in situ* with a probe tube placed at the mouth of the ear bar.

Data acquisition

In experiments involving the VCN, a neuron was isolated on the CN electrode and held while a succession of AN fibers was isolated and studied. Generally it was possible to hold CN neurons for periods of up to an hour or two, whereas AN isolation lasted typically <15 min. We attempted to find fibers with BFs similar to the VCN neuron. The AN/AN experiments were conducted similarly, except that both neurons were in the AN. When a neuron was isolated on one electrode, its BF and threshold were determined manually, as the frequency at which a rate response could be detected at the lowest sound level. The spontaneous rate class (low, medium, or high) of AN fibers was determined by counting spontaneous spikes over a period of a few s; low spontaneous rates are below 1/s, medium are between 1 and 20/s, and high are above 20/s. For VCN neurons, data for a PST histogram of responses to BF tones at 20–30 dB above threshold were taken (50 ms bursts, 1.6 ms rise/fall, presented once per 500 ms, 200–300 repetitions).

Cross-correlation data consisted of 50 s long periods of spontaneous discharge or discharge driven by a steady tone. Because stimulus-driven rate fluctuations produce artifacts in cross-correlation analysis, as discussed below, the emphasis was on obtaining data at steady discharge rates. Spontaneous activity was used if the rate was sufficient to give enough spikes for the analysis. If a stimulus was presented, it was turned on 10 s before the 50 s data acquisition period was started, to minimize the amount of rate adaptation. If a tone was presented, its frequency was usually set to the BF of the AN fiber in order to emphasize that fiber's contribution to the VCN neuron. As is shown below, significant correlation was only seen at sound levels near threshold, so stimuli were first set a few dB above threshold. If the pair of neurons was held long enough, the stimulus level was raised in 2–10 dB steps until no cross-correlation was seen in an online cross-correlogram. The 50 s data acquisition periods were repeated for each stimulus until the presence or absence of a significant feature in the cross-correlogram was clear (typically 1000–10,000 spikes in both neurons).

In a few cases, broadband frozen (i.e. periodic) noise was used as the stimulus. The noise had an approximately flat spectrum with a bandwidth of 50 kHz and a period of 1 s.

Cross-correlation analysis

The cross-correlogram is an estimate of the rate of spiking in the presumed postsynaptic neuron (the VCN neuron) as a function of time preceding and following spikes in the presumed presynaptic neuron (the AN fiber), called the *reference neuron* below. Here, spike times were recorded with a time resolution $h=0.1$ ms, which sets the basic binning of the data. The cross-correlogram $x(k)$ was computed with a bin resolution $b=3$, corresponding to an actual bin width $bh=0.3$ ms, as follows:

$$x(k)=\frac{\#[\text{A-spike in bin } i, \text{ B-spike in bins } i+kb \text{ through } i+kb+b-1]}{N_A bh} \quad (1)$$

where the notation #[·] means the number of events of the type described within the brackets and a spike is "in bin i" if the spike occurs at a time in $(ih, (i+1)h)$. A-spikes are spikes of the reference neuron, B-spikes belong to the other neuron, and N_A is the number of A-spikes. Essentially Eqn. 1 corresponds to computing a series of PST histograms for the B neuron centered on the A-spikes and then averaging those histograms. The units of $x(k)$ are spikes/s.

If A and B discharge independently, $x(k)$ should fluctuate around R_B, the average discharge rate of the B neuron. However, rate trends in the data, caused usually by adaptation to an acoustic stimulus, cause the mean value of $x(k)$ to deviate from R_B. The effect is small here because stimuli were close to threshold. Because the rate trend is very slow compared with the important features of correlograms (described below), it produces a broad elevation in the correlogram, which appears as a constant offset over the time interval analyzed. For this reason, the mean value of $x(k)$ is used here as the null value of the correlogram instead of R_B.

For analysis, *significant features* are considered to be sequences of two or more successive bins of $x(k)$ that deviate from the mean value by ≥2 standard deviations. For computing the mean and standard deviation, an iterative process was used in which statistics were computed for bins lying outside features