Electrically Evoked Responses in Onset Chopper Neurons in Guinea Pig Cochlear Nucleus

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Mulders WH, Harvey AR, Robertson D. Electrically evoked responses in onset chopper neurons in guinea pig cochlear nucleus. J Neurophysiol 97: 3288–3297, 2007. First published February 28, 2007; doi:10.1152/jn.01148.2006. Extracellular recordings were obtained from single cochlear nucleus neurons in guinea pigs anesthetized with Nembutal and Hypnorm. Neurons were classified by their spontaneous firing rates and responses to acoustic stimuli. In addition, electrical shocks were applied to the midline at the level of the IVth ventricle and spike responses were recorded. Spikes were evoked by shocks only in neurons that were classified as onset choppers (Oc). The shock-evoked spikes could be extinguished by acoustically evoked action potentials in the same neurons. In roughly 30% of the sample of Oc neurons, quantitative aspects of the timing of this extinction were not compatible with the shock-evoked spike being antidromically conducted from Oc output axons. Together with the presence of temporal jitter at high shock rates, the data suggest the possibility that at least some of the shock-evoked spikes may be generated by excitatory synaptic input to the Oc neurons, most likely from the collaterals of the medial olivocochlear system (MOCS), whose axons pass close to the floor of the IVth ventricle. This excitatory synaptic input may operate to modulate the activity of Oc neurons in addition to MOCS actions in the auditory periphery.

INTRODUCTION

The mammalian cochlear nucleus contains a number of neuronal response types that can be classified on the basis of their responses to sound. One response type, the onset chopper (Oc) neuron (Rhode and Smith 1986; Winter and Palmer 1990, 1995) is interesting for a number of reasons. Oc neurons are thought to correspond to large multipolar glycinergic neurons that exert inhibitory effects on other cochlear nucleus neurons both ipsi- and contralaterally (Alibardi 1998; Doucet et al. 1999; Needham and Paolini 2003; Smith and Rhode 1989; Wenthold 1987). Oc neurons exhibit a wide dynamic range and robust firing to broadband stimuli and this has prompted suggestions that they may have a role in a variety of circuits involved in signal processing in noise and in the detection of spectral cues related to sound localization (Arnott et al. 2004; Nelken and Young 1994; Pressnitzer et al. 2001; Spirou and Young 1991). They were also shown to respond to temporal fine structure in complex acoustic stimuli, making them well suited to detecting the periodic components of common biologically generated sounds such as speech (Winter et al. 2001).

Oc neurons are also believed to be the targets of collaterals of the descending medial olivocochlear system (MOCS) (Brown 1993; Brown et al. 1991), raising the interesting possibility of selective “top-down” modulation of Oc neurons and the circuits in which they participate. However, the action of MOCS collateral inputs on Oc neurons is still uncertain. Available evidence suggests that, in contrast to the peripheral action of the MOCS in suppressing cochlear neural output (Guinan 1996; Mulders and Robertson 2000; Mulders et al. 2002; Warr and Guinan 1979), the MOCS collateral effects in cochlear nucleus may be excitatory, although this evidence is either indirect or inconclusive. Electron microscopy shows presynaptic features on large multipolar neurons consistent with excitatory input from MOCS collaterals (Benson and Brown 1990; Benson et al. 1996). Physiological recordings have varied in their approaches to elucidate the action of MOCS collaterals. Using extracellular recordings in guinea pigs, Mulders et al. (2002) reported that acoustically driven firing rates in onset neurons (not fully characterized as Oc neurons) were higher after shock trains to the MOCS. Intracellular recordings in rat cochlear nucleus (Mulders et al. 2003) showed evidence for excitatory synaptic potentials in onset neurons evoked by single shocks to the midline of the IVth ventricle, but activation of the MOCS was not independently confirmed in this study and the relationship between the neuronal type studied and the Oc neurons in guinea pig was uncertain.

The goal of the present work was therefore to further investigate the properties of Oc neurons, with a focus on the nature of MOCS collateral inputs. We used extracellular recording from cochlear nucleus neurons to obtain detailed characterization of neuronal type and combined this with midline electrical stimulation at a location known to activate MOCS axons. The nature of spikes evoked in cochlear nucleus neurons by such electrical stimulation was investigated using an extension of the classical collision technique (Bishop et al. 1962; Harvey 1980).

METHODS

General techniques

Experiments were performed on 21 pigmented guinea pigs (360–420 g) of either sex. All details of animal preparation and surgery were published previously (Mulders and Robertson 2000). Animals initially received Nembutal (pentobarbitone sodium, initial dose 30 mg/kg, intraperitoneal) followed 10–15 min later by 0.15-ml intramuscular injection of Hypnorm (fentanyl citrate 0.315 mg/ml and
fluani sone 10 mg/ml), which induced deep anesthesia and analgesia with no reaction to painful stimuli. The depth of anesthesia was maintained by hourly administration of full doses of Hypnorm and half doses of Nembutal every 2 h. Animals were artificially ventilated by a tracheostomy. Neuromuscular paralysis was induced by intramuscular injection of 0.1 ml Pancuronium (pancuronium bromide 2 mg/ml) after all surgery and electrode placements were completed. Heart rate was continuously monitored by ECG recording and did not rise above preparalysis rates at any time during the experiment. Animals were killed at the end of the experiment by injection of 0.2 ml of Lethabarb (sodium pentabarbitone 325 mg/ml). All procedures conformed to the Code of Practice of the National Health and Medical Research Council of Australia and were approved by the Animal Ethics Committee of The University of Western Australia.

For initial evaluation of the cochlear condition, the tympanic bulla was opened by a dorsolateral approach, a silver wire hook electrode was placed on the bony shelf near the round window, and a silver wire reference electrode was inserted into the animal’s neck muscles. The threshold of the compound auditory nerve action potential (CAP) was measured at tone-burst frequencies ranging from 4 to 20 kHz (Johnstone et al. 1979). The cochlear nucleus was approached by a posterior craniotomy. Part of the cerebellum overlying the dorsal cochlear nucleus was aspirated and glass-insulated metal electrodes were introduced into the cochlear nucleus at an angle of about 45° from the vertical plane. Agar (4%) in saline (0.9%) was used to fill the craniotomy and reduce pulsations of the brain stem related to respiratory movements. The electrode was advanced using a stepping-motor microdrive and a broadband acoustic search stimulus was delivered to the animal. Single-neuron discharges were acquired and partly analyzed on-line using custom-designed software (Neurosound, MI Lloyd), which also delivered all acoustic stimuli. Further aspects of analysis were carried out off-line.

For all neurons encountered, the characteristic frequency (CF) was determined and the neuron response type was classified using a variety of measures as follows. Spontaneous firing rate was measured using a 10 s sample. Peristimulus time histograms (PSTHs) were measured using 250 repetitions (4/s) of a 50-ms CF tone using at least two intensities (20 dB above threshold and higher). Regularity analysis was also performed (Young et al. 1988) and input–output curves were measured to CF tones and to broadband noise (25 Hz to 25 kHz). To allow direct comparison of input–output curves to tones and noise, all sound intensities were expressed as decibel attenuation relative to the maximum output from the computer soundcard. Neurons were classified as sustained choppers (Cc) if regularity analysis revealed a coefficient of variation (CV) <0.3. Transient choppers (Ct) had a CV between 0.3 and 0.5 and primary-like (Pl) neurons >0.5. Onset neurons had an onset peak in their PSTH greater than tenfold the steady state. Oi and Ol neurons were grouped together and differed from Oi neurons in only showing a single onset peak even at high intensities and unlike Oi neurons did not respond robustly to broadband noise.

**Electrical stimulation**

For electrical stimulation of fiber tracts, the floor of the IVth ventricle was exposed by aspiration of part of the cerebellum and shocks were delivered to the midline of the brain stem using bipolar Araldite-insulated tungsten electrodes connected to an isolated stimulator output (AM Systems Model 2100). The electrodes were positioned under direct visual control at a point where the threshold stimulus required to evoke a whisker twitch was lowest. This corresponds closely to the point of decussation of the fibers of the MOCS (K Selukumaran, unpublished data). The animals then received an intramuscular dose (0.1 ml) of Pancuronium to eliminate muscle contractions during shock delivery. When paralysis was complete the proper placement of the stimulating electrodes on the olivocochlear axons was confirmed by measuring suppression of the cochlear action potential and increase in cochlear microphonic receptor potential produced by 100-ms shock trains at a rate of 300 Hz (bipolar stimuli, width of each phase 0.1 ms), repeated at a rate of 1/s. Shock strength was adjusted to produce suppression of the CAP to a 10-kHz tone by an amount equivalent to a reduction in sound pressure of 15–20 dB, ensuring maximal or near-maximal activation of the MOCS (Rajan 1988). Shock strength varied from 2 to 6 V (equivalent to 200–600 μA) with a mean of 3.5 V. In three animals we confirmed histologically the location of the stimulating electrodes. At the end of the experiments, electrolytic lesions were made at the point where whisker twitch thresholds were lowest and at depths of 0.5 and 1 mm more ventrally. Animals were then fixed by intracardiac perfusion of phosphate-buffered glutaraldehyde (2.5%) and frozen sections were stained with toluidine blue. Figure 1 confirms that the stimulating electrodes were located correctly between the facial genua.

All cochlear nucleus neurons were tested for the presence of spikes evoked by single shocks of varying strengths applied to the floor of the IVth ventricle. In a limited number of cases, the position of the stimulating electrode was altered (depth relative to floor of IVth ventricle) while still maintaining contact with a single neuron in the cochlear nucleus. These experiments took advantage of the fact that MOCS axons pass in a bundle superficially at the floor of the IVth ventricle, whereas the ascending axons of cochlear nucleus neurons pass more ventrally in the brain stem (Adams and Warr 1976; Oliver...
et al. 1999; Smith et al. 2005). In a subset of experiments, the ability of shock-evoked spikes to follow high-frequency trains of shocks (50–250 Hz) was also investigated.

Collision experiments

In the present experiments, collision of action potentials was produced by using acoustic stimuli to generate a spike in the cell. A shock-evoked spike was generated after this spike and the maximum delay between the acoustically evoked spike and the shock that gave rise to extinction of the shock-evoked spike was measured. This was achieved by systematically varying the shock delay relative to the start of each acoustic stimulus period. Acoustic stimuli were short CF tones whose intensity and duration were adjusted to produce a single onset spike from the neuron. These acoustic stimuli were such that the normal physiological fluctuations in the timing of the acoustically driven initial spike provided a convenient method for “fine-grain” investigation of a range of delays. All spike recordings from collision experiments were recorded using a sampling rate of 100 kHz (Scope software, ADI Instruments). Measurements of delays were performed off-line.

Collision has traditionally been used to confirm that a shock-evoked spike is antidromically conducted to the cell body (for explanation see Bishop et al. 1962; Harvey 1980). This situation is illustrated in Fig. 2A. An antidromic spike traveling toward the soma can be extinguished by collision with a preceding spike in the same cell that travels from the soma. The maximum delay (delay X in Fig. 2C) between an acoustically driven spike and the shock, for which such extinction can be observed, must equal the action potential conduction time from the soma to the point of electrical stimulation on the axon plus the refractory period of the axon. An estimate of the conduction time from the soma to the electrical stimulation site can be obtained from the delay between the shock and the shock-evoked spike (delay Y in Fig. 2C) because there should be no difference in the overall conduction time for antidromic or orthodromic conduction along the axon. Thus when the shock-evoked spike is antidromically conducted, the maximum delay for extinction (X) must be greater than delay Y (Fig. 2, A and C).

However, there is another situation in which collision can occur. In the context of the present experiments, it is hypothesized that synaptically mediated excitation of O$_C$ neurons arises when the stimulating electrodes activate the MOCS axons lying superficially near the floor of the IVth ventricle (Fig. 2B). In this case, collision with another spike traveling along the O$_C$ axon is obviously not possible. However, extinction of the shock-evoked spike can still occur if the electrically evoked spike arrives within the refractory period of the soma caused by a preceding acoustically evoked spike. In this case the longest value of delay X for which extinction can occur corresponds to the refractory period of the soma and it can, in theory, be less than delay Y, a finding that can never be true in the antidromic case.

The test has the following important limitation. Maximum extinction delays (X) that are greater than the shock–spike delay (Y), even though they are consistent with the shock-evoked spike being antidromic, could also be compatible with the shock-evoked spike being generated by synaptic input (as in Fig. 2B) if the soma refractory period happens to be longer than the shock–spike delay. Thus the test may underestimate the number of instances in which a shock-evoked spike is judged to be generated by synaptic input.

Collision experiments were performed on all neurons that exhibited spikes to midline shocks. Because all these neurons showed onset characteristics (see RESULTS) and very low spontaneous firing rates, the intensity and duration of acoustic stimulation at CF could be adjusted to reliably elicit a single onset spike. Shocks, presented at varying delays after the acoustic stimulus, were delivered at a current strength that reliably gave rise to a shock-evoked spike in the absence of preceding acoustic stimulation. Figure 3 shows recordings from a single O$_C$ neuron that illustrate the basic phenomenon of collision. A critical delay exists between the two spikes at which the shock-evoked spike is extinguished (Fig. 3D) because of collision with the preceding action potential. Delay X (Fig. 2C) was measured from the first peak of the acoustically evoked spike to the rising edge of the shock artifact and delay Y (Fig. 2C) from the rising edge of the shock artifact to the first peak of the shock-evoked spike. In the example shown, collision clearly occurs when delay X is greater than delay Y.

RESULTS

A total of 126 cochlear nucleus neurons were classified and were also tested for the occurrence of spikes evoked by single shocks delivered to the midline at the level of the IVth
ventricle. These recordings were made from the rostral end of the dorsal cochlear nucleus (DCN), from posteroventral cochlear nucleus (PV CN), and the anteroventral cochlear nucleus (AV CN). Recording locations were not routinely confirmed by histological processing. Instead electrode placement on the surface of the cochlear nuclear complex was under direct visual control and location within the different subdivisions was inferred from tonotopic progression, depth, and the cell types encountered. One hundred of the neurons were variously classified as primary-like (36 neurons), sustained choppers (18 neurons), pauser/buildup (17 neurons), transient choppers (18 neurons), and both Onset-I and Onset-L (11 neurons). None of these 100 neurons generated spikes in response to maximum-amplitude (12 V) shocks delivered to the floor of the IVth ventricle in the midline of the brain stem (Table 1). However, in two pauser/buildup neurons and two Onset-L neurons we tested deeper electrode placement and found that we could evoke spikes when the stimulating electrodes were lowered 1.4–2.4 mm deeper than the floor of the IVth ventricle.

Of the remaining 26 neurons, 25 were characterized as definite O$_c$ neurons. These all had low spontaneous firing rates (<1 Hz), PSTHs to CF tones that exhibited at least two distinct onset peaks for the higher intensity of two levels of CF tone stimulation, an onset-to-steady state firing ratio of $\geq$10 and input–output curves that showed high firing rates to broadband noise comparable to or better than those produced by single CF tones. These characteristics are illustrated in two examples in Fig. 4, A–F. The remaining neuron had characteristics of an O$_c$ neuron (low spontaneous rate and PSTH showing two peaks with appropriate onset/steady state ratio at 20 dB above CF threshold) but because of its lack of sensitivity to tones, its PSTH could not be evaluated at a higher intensity.

Of these 26 neurons, 23 generated spikes in response to shocks delivered at the midline of the brain stem at depths ranging from 0 to 4.5 mm below the floor of the IVth ventricle. Three examples of shock-evoked spikes are illustrated in Fig. 5. The shock to the midline generated a large complex shock artifact and a field potential in the cochlear nucleus, but in all cases the shock-evoked spike could be clearly distinguished from these other components of the recordings. In Fig. 5, the shock strength was chosen so that occasional spike failure occurred and this is used to illustrate the clear distinction between the spike and the shock artifact and field potential. The spikes were elicited at shock strengths that ranged in different neurons from 1.2 to 12 V (average 4.8 V). For all shock-evoked spikes, the average latency from the leading edge of the shock artifact to the peak of the spike (value Y in Fig. 2C) was 0.88 ± 0.24 ms.

In all 23 of these neurons, collision experiments were performed (see METHODS). For each neuron, the maximum value of X where collision occurred was compared with the value of Y (see Figs. 2 and 3). In 15 neurons, the maximum value of X for which extinction of the shock-evoked spikes occurred was greater than Y, a result that is consistent with collision of the acoustically evoked spike with an antidromically traveling spike generated by the shock. Two additional examples of this result are shown in Fig. 6, B and D. In eight neurons, the maximum value of X at which collision occurred was <Y (two examples shown in Fig. 6, A and C). As explained in METHODS, such a result can be explained only by the shock-evoked spike arriving at the cell soma by a synaptic input generated along a different axonal path than the neurons’ own axon. Seven of these eight neurons were those classified as definite O$_c$ neurons and the other was the high-threshold putative O$_c$ neuron. In three O$_c$ neurons, it proved possible to carry out a difficult but crucial experiment. In these cases, strikingly different collision results were obtained in the same neuron by altering the location of the stimulating electrode while continuing to maintain contact with the cell in the cochlear nucleus. The traces in Fig. 5 illustrate this result for two of these neurons and the results from all three are presented as scatterplots in Fig. 7.

![Figure 3](http://example.com/figure3.png)

**FIG. 3.** Example of spike collision in a classified O$_c$ neuron. First spike is generated by acoustic stimulation; second spike (solid arrows) is generated by electrical stimulation at floor of IVth ventricle (note stimulus artifact) at varying delays from first spike. Dotted arrow indicates time of application of shock. Extinction of the shock-evoked spike occurs in D.

<table>
<thead>
<tr>
<th>Neuron Type</th>
<th>$n$</th>
<th>Shock-Evoked Spike, %</th>
</tr>
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<tr>
<td>Onset chopper</td>
<td>26</td>
<td>88</td>
</tr>
<tr>
<td>Onset-I and Onset-L</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Transient chopper</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Sustained chopper</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Primary-like</td>
<td>36</td>
<td>0</td>
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<tr>
<td>Pauser</td>
<td>17</td>
<td>0</td>
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**TABLE 1.** Summary of responses to single shocks applied to floor of the IVth ventricle on classified cochlear nucleus neurons.
Stimulation at the level of the floor of the IVth ventricle resulted in spike extinction only when delay X was smaller than delay Y (Figs. 6, A and C and 7, A, F, and H). When the stimulating electrodes were progressively lowered more ventrally into the brain stem, in all three neurons, the shock-evoked spikes at first disappeared and then reappeared when the stimulating electrodes were 1.5–1.8 mm deeper. The latencies of the spikes evoked by shocks at the deeper location were different from those evoked at the floor of the IVth ventricle. Furthermore, when the collision experiment was repeated on these spikes evoked by deeper shocks, the collision result now showed that the shock-evoked spike was extinguished when delay X was clearly longer than delay Y, consistent with the shock-evoked spike now being antidromically conducted (Figs. 6, B and D and 7, B, G, and I). In addition to scatterplots of the extinction phenomenon at the two depths of electrical stimulation, Fig. 7 also shows the PSTH and input–output data for one of these Oc neurons (PSTH and input–output data for the other two neurons were presented earlier in Fig. 4).

**Spike jitter at high shock rates**

In 11 Oc neurons we investigated how reliably the shock-evoked spike could be generated in response to trains of shocks at high rates. Trains of 50-ms duration with shock rates ranging from 50 to 250 Hz were used. Figure 8 shows the results from three cells in which responses to a 200-Hz train are overlaid to show the temporal jitter in latency of the spike. It is apparent that the amount of jitter varies considerably from cell to cell. In some cases, successive spikes are virtually overlaid with very small jitter, whereas in other cells, the latencies vary substantially. In the sample of 11 cells, jitter of spike arrival times ranged from 0.05 to 0.21 ms. The largest jitter was found for a neuron in which the collision data indicated that the shock-evoked spikes were generated by synaptic input (Fig. 8C) and the smallest jitter was found for a neuron in which the collision result was consistent with the spike being antidromically conducted (Fig. 8A).

**DISCUSSION**

In this study, the only cochlear nucleus neurons that exhibited spikes to shocks delivered at the level of the IVth ventricle were either definite Oc neurons (22 of 25) or the one neuron with Oc-like responses that could not be fully classified. This latter neuron is very likely to have been an Oc neuron but its high threshold prevented full classification. Such high-threshold neurons, exhibiting many features of Oc neurons were previously reported (Arnott et al. 2004). It must be noted, however, that MOCS collateral actions on other neuronal types cannot be excluded because anatomical evidence shows that MOCS collaterals contact targets other than large multipolar neurons in the cochlear nucleus (Benson and Brown 1990) and these may evoke subthreshold effects that cannot be revealed by our extracellular recording methods. Nonetheless, we believe that the present data constitute evidence in support of an excitatory action of MOCS collaterals on Oc neurons. Arguments in support of this view follow.

First, in the present experiments, care was taken to place the stimulating electrodes at a location that corresponded to the facial genua and we confirmed the presence of classical MOCS effects in the auditory periphery. The fact that with this
electrode placement only Occoli neurons exhibited spikes is a crucial observation. It supports the notion that these spikes were produced by activation of MOCs collaterals and that we were not activating axons of either the dorsal or the intermediate acoustic striae (Adams and Warr 1976; Arnott et al. 2004; Oliver et al. 1999; Smith et al. 2005) because these contain the output axons of Occoli, Pp, Oci, and Ol neurons and the latter three types never showed shock-evoked spikes in our routine experiments when the stimulating electrodes were at the floor of the IVth ventricle. In the few instances in which we tested deeper stimulations for neurons other than Occoli neurons (Pp and Ol neurons), the occurrence of shock-evoked spikes was consistent with the output axons of these neurons coursing in the more ventrally located dorsal acoustic stria/intermediate acoustic stria. In the two Ol neurons, spike collision was observed and was indeed consistent with antidromic activation. Collision experiments could not be performed on the two Pp neurons because of their high spontaneous firing rates. The fact that activation of these neuronal types was not seen when stimulating at the floor of the IVth ventricle supports the notion that the stimulation was highly localized.

Second, to test the nature of the shock-evoked spikes in Occoli neurons, we investigated details of the collision of the shock-evoked spikes with acoustically evoked spikes. The traditional interpretation of collision is that the shock-evoked spike arises in the axon and is conducted antidromically. However, we believe that the data presented here support the notion that in a significant number of cases the shock-evoked spikes seen in Occoli neurons are generated by excitatory synaptic input from MOCs collaterals, rather than by antidromic activation of the Occoli axons. As argued earlier in METHODS, a collision occurring in the axon (antidromically conducted spike) must be observable at maximum delays X that are greater than delay Y because the most distant point where collision can occur is at the distant axonal stimulation site and the delay to this point must be added to the axon’s refractory period (Fig. 2).

In the cases where collision occurs only at delays that are incompatible with antidromic collision (eight Occoli neurons), we suggest that the only explanation is that the shock-evoked spike is generated by synaptic input by a separate input pathway (the MOCs collaterals) that excites the cell soma during its refractory period. In such a case, because the collision is occurring at the soma and not remotely in the axon, there is not an absolute requirement for the maximum value of delay X where collision can occur, to be greater than delay Y. Strong support for this interpretation of the collision test is provided in particular by the experiments in the three Occoli neurons in which we were able to alter the collision result by lowering the stimulating electrodes more ventrally in the brain stem. The results seen are consistent with the notion that the deeper stimulation activated

Fig. 5. Examples of extracellular spikes recorded from 3 single Occoli neurons in response to shocks applied to the midline at the level of the IVth ventricle. Arrow indicates spikes. Note large stimulus artifact preceding spikes. Dotted arrow indicates time of application of shock. Stimulus strength close to threshold. Occasional failure of spiking (thin traces) illustrates prominent nature of spike relative to artifact and field potential.

Fig. 6. Examples of different collision results for different depths of electrical stimulation in 2 different Occoli neurons (A, B and C, D). In all parts of the figure, first spike is in response to acoustic stimulation. Note clearly visible second spike of single neuron occurring after large shock artifact. Delays X and Y correspond to those shown schematically in Fig. 2C. In all cases, delays between first spike and shock are chosen so that small variations in first spike timing reveal critical delay for extinction of shock-evoked spike (thin trace). A and C: extinction in both cells when X is < Y (stimulation at floor of IVth ventricle). B and D: extinction in both cells when X is > Y (stimulation at >1.5 mm more ventrally).
the ascending axons of the O₃ neurons, generating an antidromic spike, whereas the more superficial stimulation activated MOCS axons, providing synaptically driven excitatory input to the same neurons.

This interpretation of the collision data may seem novel, but is implicit in the early work of Bishop et al. (1962) on lateral geniculate neurons, in which they acknowledge that a transsynaptic shock-evoked spike, to escape from a previous firing of the postsynaptic cell, would need to fall outside the soma refractory period, implying that its extinction would occur for arrival times shorter than this. Such extinction could occur if the soma refractory period were shorter than the shock-spike delay but this would require that the shock was applied before the sound-driven spike so that the initiation of the spike in the soma, generated by the shock, was attempted while the soma was still refractory. The fact that in our data, all extinctions were seen when the shock was applied after the acoustically evoked spike occurred suggests that the extinction occurs in same 3 neurons when X is ≥ Y. When stimulating electrodes are located more ventrally in brain stem (B, G, and I), extinction occurs in some 3 neurons when X is ≥ Y. C and D: PSTHs for neuron in A and B at 10 and 20 dB above CF threshold. E: input–output curves of neuron in A and B, showing robust response to broadband noise and low spontaneous firing rate characteristic of O₃ neurons.
of Oc neurons of 1.67 ms. It is possible that this estimate includes the relative refractory period because, if our hypothesis is true, spikes evoked by an excitatory synaptic input may be prone to failure during the relative refractory period. No direct measurement has yet been made of the refractory period of Oc somata, but an estimate can be derived from the intervals between the two characteristic onset peaks of the PSTHs generated in response to acoustic stimulation. The mean interval between these peaks derived from our own data (12 cells) was 1.4 ms.

Third, the responses to high rate shock trains showed varying degrees of jitter. The presence of jitter in shock-evoked responses was taken as a signature of synaptic drive (for discussion see Rose and Metherate 2001). In the present experiments, the jitter was greatest for Oc neurons in which the collision result indicated a synaptically driven spike.

Finally, it needs to be remembered (see METHODS) that even the collision test results consistent with antidromic conduction of the shock-evoked spike are themselves open to interpretation (Bishop et al. 1962). Collision occurring at delays longer than the shock-spike delay are consistent with but do not prove the antidromic nature of the shock-evoked spike because this result could also arise from a synaptically generated input encountering a soma refractory period longer than the shock-spike delay. The fact that a number of neurons with “antidromic” collision results showed significant jitter suggests that in some of these cases the shock-evoked spike may be the result of excitatory synaptic input, possibly from MOC collaterals. Furthermore, Rose and Metherate (2001) argue that in other parts of the brain, low jitter per se is not conclusive evidence that the responses are not synaptically driven. Thus we may in fact be underestimating the number of Oc neurons that are receiving excitatory MOCs input.

It is possible, however, that despite electrode placement designed to optimize selective activation of MOCs axons, our electrical stimulation did evoke antidromic spikes in a number of Oc neurons. Although the axons of the Oc neurons pass more ventrally below the floor of the IVth ventricle than MOC axons, current spread at higher shock strengths may activate them. There are no data on the axon diameters of Oc neurons, but if they are related to cell soma size, they are likely to be large diameter and thus probably easier to activate than smaller-diameter axons coursing in the same fiber tracts. In addition, several of the neurons tested with high shock rates showed very little jitter, consistent with the spikes being antidromically conducted.

Antidromic spikes evoked in cochlear nucleus neurons could activate pathways intrinsic to the cochlear nucleus, ultimately generating excitatory responses in the recorded Oc neurons. We think this unlikely because the electrode placement at the floor of the IVth ventricle failed to elicit action potentials in any of the other major response classes that constituted the bulk of cochlear nucleus neurons in the present sample. It is also possible that the shocks excite axons projecting from the opposite cochlear nucleus; however, the pathway from the opposite cochlear nucleus arises from Oc neurons and generates inhibitory, not excitatory, effects in the target cochlear nucleus (Needham and Paolini 2003). These inhibitory effects could conceivably be converted into excitation by a process of disinhibition of Oc neurons by inhibition of a second inhibitory interneuron, but the rather short latencies of our shock-evoked spikes in Oc neurons (mean latency 0.88 ms) makes this extremely unlikely.

Excitatory action of MOCs collaterals on Oc neurons is consistent with our earlier in vivo recordings in both rat and guinea pig (Mulders et al. 2002, 2003) and with EM data showing MOC collaterals making excitatory-like synaptic contact with large multipolar neurons (Benson and Brown 1990, 1996). However, these data are at variance with those obtained from slices of mouse cochlear nucleus (Fujino and Oertel 2001). These authors reported that so-called D-stellate neurons were unresponsive to cholinergic agonists applied locally or delivered in the organ bath. Instead, they reported that cholinergic responses were observed in “T-stellate” neurons. On the basis of these findings, Fujino and Oertel suggested that MOC collaterals made excitatory cholinergic connections with T-stellate and T-stellate neurons and there is a substantial body of circumstantial evidence that D-stellate and T-stellate neurons correspond to Oc and sustained/transient chopper neurons, respectively (Arnott et al. 2004; Ferragamo et al. 1998; Oertel et al. 1990; Smith and Rhode 1989; Smith et al. 2005). Note, however, that whereas MOC neurons are indeed cholinergic (White and Warr...
1983), the observed responses in T-stellate neurons in tissue slices could be mediated by receptors associated with other cholinergic inputs from the superior olivary complex (Sherriff and Henderson 1994). Nonetheless, the lack of effect on D-stellate neurons is difficult to explain, assuming that species differences are not important. A difficult in vivo experiment that might resolve this issue would be to test whether putative synaptically driven spikes evoked by midline shocks are blocked by cholinergic antagonists applied directly to individual, physiologically characterized O$_c$ neurons. A further point to consider is that MOC collaterals do not make exclusive contact with large multipolar neurons. Brown and coworkers also reported endings on varicose dendrites of small cells that presumably are neither D- nor T-stellate neurons but whose precise identity is not established.

If MOCs collaterals do provide excitatory input to O$_c$ neurons, then our data suggest that this input has some remarkable features. The overall latency is quite short, especially if a synaptic delay also has to be included. There are no data on the conduction velocity of MOCs axons, although available data in guinea pig suggest they are small- to medium-diameter myelinated fibers ranging in size from 1.2 to 1.4 μm (Brown 1989). Needham and Paolini (2003) reported a delay of 1.4 ms in the rat for inhibitory postsynaptic potentials evoked in cochlear nucleus neurons by electrical stimulation of the contralateral cochlear nucleus. If we assume their conduction path length is approximately twice that in the present study, our latency data are at least comparable. This comparison, however, can be only approximate because of possible differences in axonal conduction velocities and synaptic delays in the two cases. In addition, although in a number of O$_c$ neurons the responses to high rate shock trains do show jitter (Fig. 7), the firing is still remarkably secure and tightly timed, compared with “typical” chemical synapses that have difficulty following input rates of ≥100 Hz. This property might be remarkable in other parts of the nervous system (although see Rose and Metherate 2001), but the auditory pathways provide numerous examples of such secure synaptic drive that is achieved in a variety of ways. In some cases (e.g., bushy cells of the AVCN and principal cells of the MNTB) this is a property of powerful specialized presynaptic terminals with multiple release sites. In others (e.g., the octopus cells of the PVCN) it comes about because of highly convergent multiple synaptic inputs combined with unusual postsynaptic electrical properties (Oertel 1991, 1999). Benson and Brown (1990) and Benson et al. (1996) extensively studied the morphology of MOCs collateral terminals on large multipolar neurons in the mouse cochlear nucleus. They report that each collateral makes numerous contacts with individual proximal dendrites of their postsynaptic targets, which may be consistent with a strong synaptic drive.

The present findings have significant implications for auditory processing. If the O$_c$ neurons have a role in processes such as signal detection in the presence of maskers and/or sound localization based on pinna spectral cues, then an excitatory MOCs collateral system would provide a potentially powerful means of modulating the effectiveness of such a role by top-down activation of the olivocochlear pathway. Our previous report of excitatory effects based on measuring responses to tones presented 5–10 ms after trains of shocks to the MOCs axons (Mulders et al. 2002) may have underestimated the action of the collaterals. By providing evidence for short-latency synaptically driven excitation by single shocks, the present results suggest that the MOCs neurons may be able to operate in at least two modes—one in which single action potentials generate rapid, strong excitatory effects in O$_c$ neurons in the cochlear nucleus, possibly without significant peripheral suppression; and another in which high sustained action potential rates in MOCs neurons also cause peripheral suppressive effects (Rajan 1988). These two modes of firing in MOCs neurons may play quite different roles in modulating the activity of neurons at different stages of the afferent pathway. For example, if O$_c$ neurons supply wideband inhibition crucial for the function of selected circuits (Nelken and Young 1994; Pressnitzer et al. 2001; Spirou and Young 1991), the collateral action would selectively modulate the action of these circuits. Peripheral suppression, on the other hand, by reducing overall cochlear gain will have a more widespread action on many circuits within the cochlear nucleus and beyond.

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