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Monaural interaction of excitation and inhibition in the medial superior olive of the mustached bat: An adaptation for biosonar

(audition/brainstem/neuropharmacology/electrophysiology/neuroanatomy)

BENEDIKT GROTHE*, MARIANNE VATER*†, JOHN H. CASSEDAY‡, AND ELLEN COVEY‡§

*Zoologisches Institut, University of Munich, 8000 Munich, Federal Republic of Germany; and ‡Department of Neurobiology, Duke University Medical Center, Durham, NC 27710

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ABSTRACT **In most mammals, the superior olive is the first stage for binaural interaction. Neurons in the medial superior olive (MSO) receive excitatory input from both ears and are sensitive to interaural time or phase differences of low-frequency sounds. The mustached bat (*Pteronotus parnellii parnellii*), a small echolocating species with high-frequency hearing, probably does not use interaural time or phase differences as cues for sound localization. Although the mustached bat has a large MSO, there is some evidence that it is functionally different from the MSO in nonecholocating mammals. Most MSO neurons in the mustached bat are monaural, excited by a contralateral sound. Their responses are phasic and correlated with either the onset or the offset of a sound. As a first step in determining the origin of these phasic monaural responses, we traced the connections of the MSO by using both retrograde and anterograde transport methods. Excitatory inputs to the MSO originate from spherical cells in the anteroventral cochlear nucleus, almost exclusively from the contralateral side. Glycinergic inhibitory input is relayed from the contralateral cochlear nucleus through the medial nucleus of the trapezoid body. To investigate the interactions of the contralateral excitatory and inhibitory inputs at the level of the MSO cell, we recorded sound-evoked responses and applied glycine or its antagonist by using microiontophoresis. The results show that the phasic response to a contralateral sound is created by interaction of a sustained excitatory input with a sustained inhibitory input, also from the contralateral ear. Whether the response is to the onset or offset of a sound is determined by the relative timing between the excitatory and inhibitory inputs. Thus, in MSO of the mustached bat, the ipsilateral excitatory pathway from the cochlear nucleus seen in animals with low-frequency hearing is virtually absent, and the MSO is adapted for timing analysis by using input from only the contralateral ear.**

The mammalian superior olivary complex is the first stage at which there is convergence of inputs from the two ears. The current view of its function is that it provides two parallel pathways to the auditory midbrain, each of which is specialized to compare sound at the two ears and transmit basic information necessary for sound localization. In this scheme, neurons in the lateral superior olive (LSO) are selective for interaural sound level differences and neurons in the medial superior olive (MSO) are selective for interaural time or phase differences (1, 2).

This view of MSO function is based mainly on data obtained in mammals with large heads and good low-frequency hearing. Species with small heads and high-frequency hearing probably do not have interaural time or phase differences in a range that would be useful for sound localization, and in some cases they appear to lack an MSO (1, 3, 4). However, in other cases, notably in echolocating bats, a large MSO is present despite a small head size and high-frequency hearing range (5, 6). On the basis of its location and cytoarchitecture, the bat MSO appears homologous with the MSO of nonecholocating mammals (6); however, electrophysiological studies have shown that it is functionally quite different (7–10).

Here, we combine electrophysiological, anatomical, and neuropharmacological evidence from one species of bat to investigate the basis for the specialized functional properties of a high-frequency MSO. The species used is the Jamaican mustached bat, *Pteronotus parnellii parnellii*, an insectivorous echolocating bat with a large MSO. We first summarize the data on the response properties of MSO neurons in this species and show that most are excited only by a contralateral stimulus. We then present anatomical data to show that the contralateral excitatory responses characteristic of MSO neurons in the mustached bat are due to the virtual absence of projections from spherical cells in the ipsilateral cochlear nucleus. Finally, we show that the temporal characteristics of the response to a contralateral sound are shaped by glycinergic inhibitory input at the level of the MSO cell. These results are significant from an evolutionary point of view. They suggest that the MSO in some species has undergone major adaptations in response to such factors as head size, range of frequency sensitivity, and ecological niche.

MATERIALS AND METHODS

A total of 16 male *Pteronotus parnellii*, obtained from Jamaica, were used for these studies. To characterize response properties of cells in the MSO, we presented pure tone stimuli binaurally through earphones and recorded the responses of single units. Data were obtained from a total of 321 single units in the MSO. The procedures for stimulus presentation, single-unit recording, and anatomical analysis have been described in detail (8, 9). For surgery, animals were anesthetized with halothane or a mixture of ketanest (10 mg/ml) and 2% Rompun injected subcutaneously [1.5 ml/100 g (body weight)]. A metal post affixed to the skull held the head in a set position relative to stereotaxic coordinates. The animal's body was held in a cushioned restraint device. Binaural stimuli were presented in a closed system through earphones. During recording, bats were awake, but local anesthetic was applied to incisions and pressure points. In some of the same animals used for electrophysiology, we made iontophoretic injections of the retrograde and anterograde tracer wheat

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Abbreviations: AVCN, anteroventral cochlear nucleus; LSO, lateral superior olive; MNTB, medial nucleus of the trapezoid body; MSO, medial superior olive; WGA-HRP, wheat germ agglutinin-conjugated horseradish peroxidase.

†Present address: Institut für Zoologie, Universität Regensburg, Regensburg, Federal Republic of Germany.
§To whom reprint requests should be addressed.

germ agglutinin-conjugated horseradish peroxidase (WGA-HRP).

For the neuropharmacology experiments (10), we used a Medical Systems model MS2B microiontophoresis system; pharmacological agents were delivered through a five-barreled glass micropipette with a collective tip diameter of 5–10 μm. One barrel was filled with glycine (0.5 M, pH 4.0) and one was filled with strychnine (0.01 M, pH 3.5). Another barrel was filled with 1 M sodium acetate and served as the balance barrel. Except during application, a holding current of −15 nA was applied to each barrel. For recording, a "piggy-back" electrode (11) filled with 3 M KCl was glued to the multibarreled electrode so that the tip protruded 5–25 μm. The recording electrodes had impedances of 8–12 MΩ. For iontophoresis, positive or negative current (1–100 nA) could be passed through each barrel independently. Application currents ranged from 1 to 20 nA for glycine and from 20 to 60 nA for strychnine.

RESULTS

Figs. 1 and 2 summarize the general organization and binaural responsiveness of the MSO in the mustache bat. The MSO, like the LSO, contains a cochleotopic frequency representation, with an expansion of the range corresponding to the predominant harmonic in the bat's echolocation call, around 60 kHz (Fig. 1). Otherwise, the tonotopic progression from low to high frequencies follows the typical mammalian pattern.

Although the binaural responses of neurons in the mustached bat's LSO are essentially identical to those found in other mammals, the response properties of MSO neurons are different (Fig. 2). In the dog, cat, and rat, most MSO neurons are binaural, with half or more excited by a sound at either ear and the remainder excited by a sound at one ear and inhibited by a sound at the other (12–17). In *Pteronotus*, about 85% of all MSO units are monaural, excited only by a sound at the contralateral ear and unaffected by a sound at the ipsilateral ear. The remainder are binaural, and the largest single group of binaural neurons are those that are excited by a contralateral sound and inhibited by an ipsilateral sound. In a small sample of neurons obtained in another species of echolocating bat, *Molossus ater*, the largest single group of units in the MSO were those excited by a contralateral sound and inhibited by an ipsilateral sound (7).

The discharge patterns of MSO units in the mustached bat differ from those reported for MSO neurons in nonecholocating mammals. In the dog and cat, MSO units typically respond in a sustained pattern (12, 14) or phase-lock at low frequencies (13). In *Pteronotus*, ≈80% of MSO units respond in a phasic pattern, with one or a few spikes, at the onset (on), at the offset (off), or at both onset and offset of the stimulus (on–off). For some MSO neurons, the response pattern changes from on to on–off or off as frequency is varied (9).

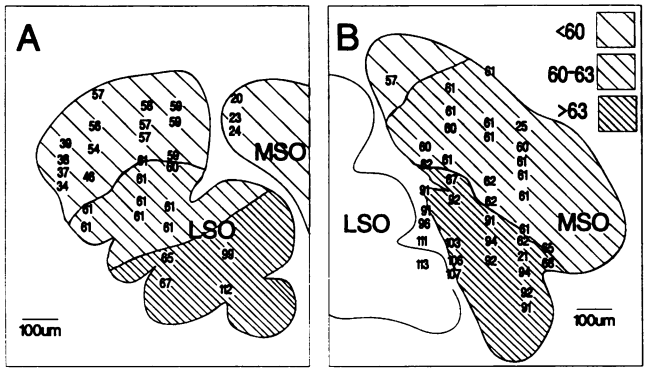

FIG. 1. In *Pteronotus*, the LSO is structurally similar to the LSO in other mammals. The MSO is large and convoluted to form dorsal and ventral limbs. In both the LSO and the MSO, there is a dorsolateral to ventromedial progression from low to high frequencies, in which the representation of the main harmonic of the echolocation call (60–63 kHz) is greatly expanded. Here, the best frequencies of units along representative penetrations are shown in kHz. (*A*) Schematic frontal section through the LSO. (*B*) Schematic frontal section through the MSO. (Bars = 100 μm.)

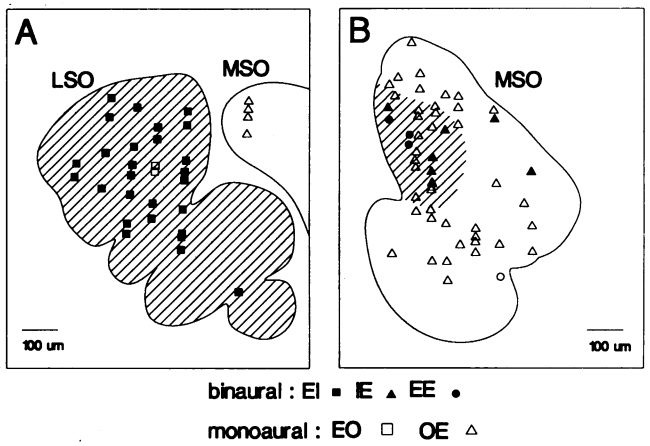

FIG. 2. In the LSO, 93% of all units (N = 55) were excited by an ipsilateral sound and inhibited by a contralateral sound (EI). In the MSO, 85% of all units (N = 321) were monaural and excited by a contralateral sound (OE). Some units around the margins of the MSO were binaural, excited by a contralateral sound and inhibited by an ipsilateral sound. These made up 8% of the total. Very few units, only 4% of the total, were excited by both ears. The hatched areas indicate the location of binaural units. EI, ipsilateral excitatory, contralateral inhibitory; IE, ipsilateral inhibitory, contralateral excitatory; EE, both excitatory; EO, ipsilateral excitatory; OE, contralateral excitatory. (*A*) Schematic frontal section through the LSO. (*B*) Schematic frontal section through the MSO. (Bars = 100 μm.)

There is an anatomical basis for these response properties. Figs. 3 and 4 show the pattern of inputs to the MSO as seen by both retrograde and anterograde transport of WGA-HRP. After an injection in the MSO (Fig. 3), there are many labeled cells in the contralateral anteroventral cochlear nucleus (AVCN) but only a few in the ipsilateral AVCN. The contralateral cells are mostly spherical cells, but the ipsilateral cells appear to be multipolar or stellate cells. This indicates that the spherical cells in AVCN provide excitatory input to the MSO just as they do in other mammals. The stellate cells appear to be one of several possible sources of ipsilateral inhibitory input to the MSO (18). There are a large number of labeled cells in the medial nucleus of the trapezoid body (MNTB). The MNTB receives its input from the contralateral AVCN and is known to provide contralateral glycinergic inhibitory input to LSO cells (19–21). The results in the bat and data from previous studies in the cat (19, 20) indicate that MNTB also provides glycinergic inhibitory input to MSO cells. The MNTB projection in the bat appears to be particularly large compared to other species (19, 20).

Electrophysiological data from two echolocating bat species suggest that at least some units in the MSO receive inhibitory input from the ipsilateral ear (7, 8). Projections from the cochlear nucleus are one possible source of this input. After an injection of WGA-HRP in AVCN (Fig. 4), there is dense terminal-like label in the contralateral MSO. In the ipsilateral MSO, label is sparse and is mostly distributed around the lateral edge of MSO. In *Pteronotus*, the binaural cells are located around the lateral edge of the MSO, in the area that receives projections from the ipsilateral AVCN. This finding suggests that inhibitory input originates in the AVCN, possibly from stellate cells.

The afferent projections to the MSO in the mustached bat are summarized in Fig. 5. In the three species of echolocating

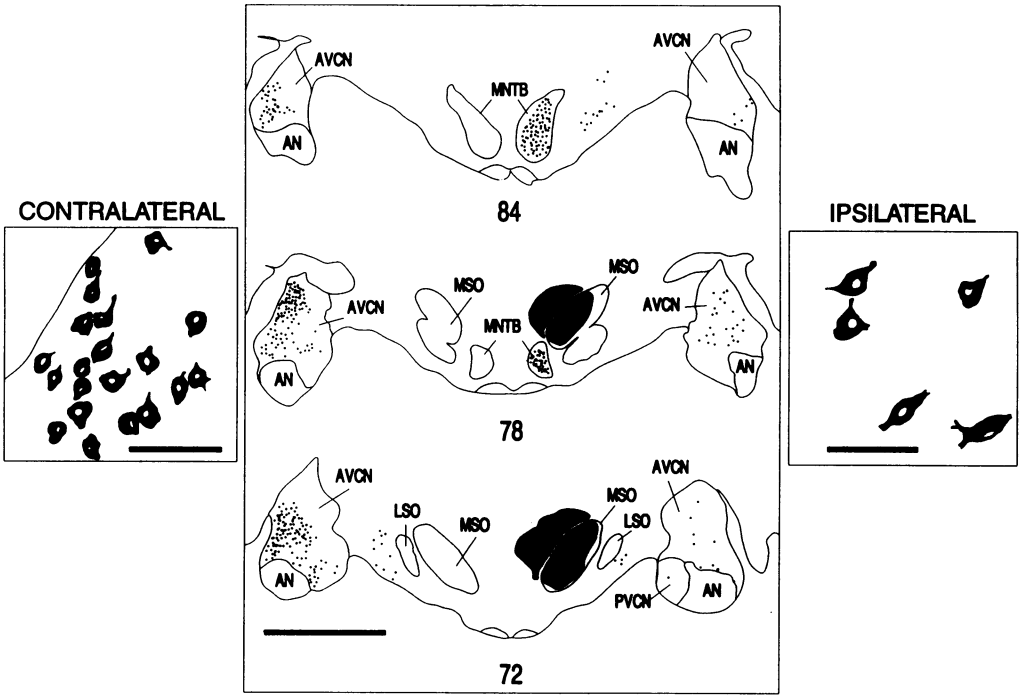

FIG. 3. (*Center*) Retrograde transport from an injection of WGA-HRP centered in the caudal MSO in a region where units had the best frequencies around 61.5 kHz. Cells labeled by retrograde transport are shown as dots. Projections to the MSO are considerably more lateralized than they are in nonecholocating mammals, where the number of cells projecting from the AVCN of both sides is approximately equal (1). Here, the ipsilateral AVCN provides only a minor projection to the MSO; most input to the MSO comes from the contralateral AVCN, either directly or through the ipsilateral MNTB. Drawings of labeled cells in the AVCN on the contralateral (*Left*) and ipsilateral (*Right*) sides are also shown. AN, auditory nerve; PVCN, posteroventral cochlear nucleus. (Bars: *Left* and *Right*, 50 μm; *Center*, 1 mm.)

bats studied so far, *Rhinolophus rouxi* (8, 22), *Pteronotus parnellii* (9), and *Eptesicus fuscus* (E.C. and J.H.C., unpublished data), input from the ipsilateral cochlear nucleus to the MSO is greatly reduced. Thus, *Pteronotus* is not the only echolocating bat species with reduced ipsilateral input to the MSO.

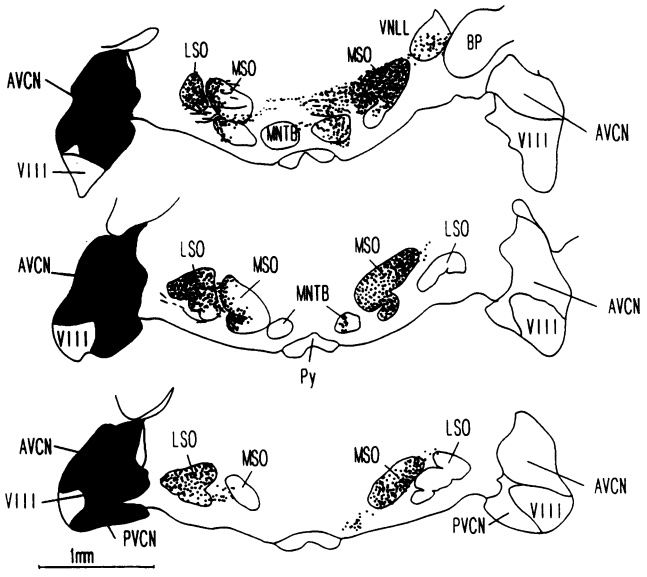

FIG. 4. Anterograde transport from a large injection of WGA-HRP that fills most of the AVCN. The projections to the LSO and MNTB follow the typical mammalian pattern and are consistent with the binaural responsiveness of the LSO cells. The projections to the MSO are mainly to the contralateral side, where terminal-like label is dense throughout the entire MSO. In the MSO ipsilateral to the injection, there is only sparse label around the lateral edge. Most binaural MSO units were found within this marginal region. The injection is shown as a solid area and the anterograde transport is shown as a stippled area. VNLL, ventral nucleus of the lateral lemniscus; BP, brachium pontis; Py, pyramidal body; VIII, auditory nerve.

To investigate interactions between the contralateral excitatory and inhibitory inputs at the level of the MSO cell, the effects of pharmacological agents on the responses of neurons in the MSO were measured (10). While we recorded from single units, we applied glycine, the putative inhibitory neurotransmitter present in the pathway that projects to the MSO by way of the MNTB (20), or strychnine, an antagonist of glycine (21). Application of glycine or strychnine caused changes in response pattern with subsequent full recovery in all 35 MSO neurons tested. Fig. 6 shows examples of how the responses of three MSO units were modified by the presence of these agents. Fig. 6*A* shows the typical phasic on response most commonly seen in bat MSO neurons; this response was abolished by application of glycine but subsequently recov-

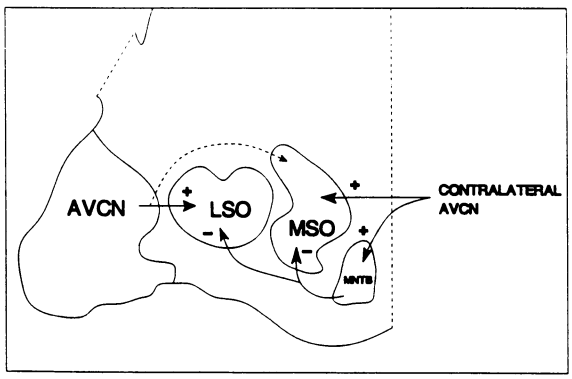

FIG. 5. Diagram to summarize the connections of the superior olivary nuclei in the mustached bat.

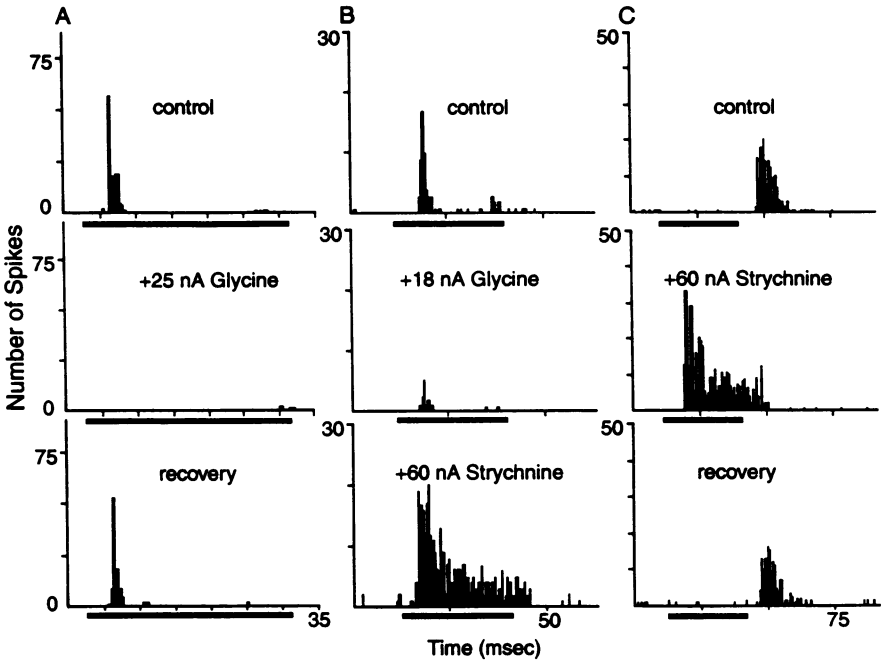

FIG. 6. Responses of three different MSO neurons in *Pteronotus* are modified by iontophoretic application of glycine or strychnine. Each peristimulus time histogram represents responses to 100 identical stimulus presentations. Stimuli were 30-msec pure tones, 10 decibels (dB) above threshold at the unit's best frequency. The bar below each histogram indicates stimulus duration. (*A*) Glycine abolishes phasic on response. (*B*) Strychnine changes phasic on response to a sustained response. (*C*) Strychnine changes phasic off response to sustained on response.

ered. Thus glycine produces inhibition of sound-evoked neural discharges in the MSO. In Fig. 6*B*, a phasic on response was greatly reduced by application of glycine. When strychnine was applied alone, it appeared to block the effects of endogenous glycine and thus showed that glycinergic input is present and functional in the MSO. The phasic on response seen under control conditions was transformed by strychnine to a sustained response. Thus, in the control condition, the endogenous glycinergic inhibition must have been delayed with respect to the excitation because it suppressed the late part of a sustained excitatory response and thus left only an initial discharge that correlated with stimulus onset. Fig. 6*C* shows the response of a unit that discharged only to the offset of sound under control conditions. Application of strychnine transformed this off response to a short latency sustained response correlated with the onset of the stimulus. Thus, in the control condition, the endogenous glycinergic inhibition must have preceded the excitation to suppress the early part of a sustained excitatory response and leave only a short discharge correlated with the offset of the stimulus.

These results demonstrate that MSO in the mustached bat receives excitatory input and glycinergic inhibitory input, both derived from the contralateral ear. Both inputs appear to originate from cells with sustained response patterns. The excitatory input is from spherical cells of the AVCN, which are known to respond in a primary-like pattern (23–26). The inhibitory input appears to be from cells of the MNTB, which also respond to pure tones in a primary-like pattern (refs. 27 and 28 and M.V., unpublished data). As in the LSO, the two inputs appear to share the same best frequency. The phasic discharge patterns of MSO units are due to the interaction of these two sustained inputs at the level of the MSO cell, and the relative timing of the two inputs creates the on or off response patterns seen in the MSO.

DISCUSSION

The anatomical and physiological data suggest that the MSO in echolocating bats has evolved to fulfill a function that is very different from the function it performs in mammals with low-frequency hearing. Although some neurons in the mustached bat MSO are binaural, the majority seem to be specialized for monaural analysis. In the bat, the evolutionary pressure has not been to develop a comparator for binaural time or phase differences, but rather to develop a processor for analyzing high-frequency echolocation sounds. Clearly, the MSO in the mustached bat fulfills some specialized function in echolocation, possibly encoding information about timing relationships between the emitted pulse and its echo (9, 18) or information about the rate of periodic amplitude modulations such as those that result from the beating of insect wings (10).

The results presented here cannot answer the question of whether the ancestral mammalian MSO was binaural and the ipsilateral excitatory input has become vestigial in the bat or whether it was monaural and the ipsilateral excitatory input has been elaborated in species with low-frequency hearing. However, the data do suggest that the inhibitory circuitry in the MSO may be common to all mammalian species. Certainly, the projection from the MNTB to the MSO is not unique to the bat; it is also present in nonecholocating mammals such as the cat (19, 20).

It is possible that an interaction between contralateral excitatory and inhibitory inputs, similar to that seen in the MSO of the mustached bat, might also occur in mammals with low-frequency hearing to sharpen selectivity for interaural phase difference. The mechanism originally proposed to explain the selectivity of low-frequency MSO neurons for an interaural time difference was the temporal coincidence of excitatory inputs from both ears, one being delayed with respect to the other (12, 29, 30). However, recent evidence from single-unit recording in the MSO of the cat indicates that simple coincidence of two excitatory inputs is insufficient to explain satisfactorily the selectivity of MSO neurons for interaural phase difference and shows that this selectivity requires inhibitory and excitatory inputs from both ears (13). Some binaurally excited phase-locking neurons in the cat have inhibitory regions that occur at certain interaural phase differences. In these inhibitory regions, the discharge rate is

less than that to monaural stimulation alone and sometimes less than the spontaneous rate.

The function of inhibitory inputs to the MSO is still not fully understood. However, since the inhibitory effects in both cat and bat MSOs are long lasting and dependent on the timing of inputs, it is possible that the inhibitory circuitry in the MSO is common to all mammals, and it is only because of the paucity of ipsilateral excitatory projections in the mustached bat that the resulting response properties are so different from those seen in MSO cells of other mammals. If so, this finding is an excellent example of how a specific neural network undergoes adaptation for different purposes during evolution; a difference in the strength of one connection has fundamentally altered the function of the MSO in the bat.

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