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Synaptic Inhibition Influences the Temporal Coding Properties of Medial Superior Olivary Neurons: An *in vitro* Study

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The medial superior olive (MSO) functions as a coincidence detector for interaural time and phase differences by integrating excitatory synaptic inputs. Recent studies demonstrating glycinergic projections to MSO neurons suggest that coincidence detection results from the temporal integration of both EPSPs and IPSPs. We examined the impact of synaptic inhibition on the temporal coding properties of gerbil MSO neurons *in vitro* with intracellular recordings and electrical stimulation. For low-level bilateral electric stimulation, the EPSPs summated to produce an action potential in 73% of MSO neurons if they occurred within 50–500 μsec of one another. Synaptic inhibition became more prominent at higher stimulus amplitudes in 73% of MSO neurons, and could block an evoked action potential if the stimuli to each pathway were delivered within 250 μsec of one another. The glycine receptor antagonist strychnine influenced the response to simulated interaural time differences. In the presence of strychnine, interstimulus delays that originally resulted in full action potential suppression were sufficient to evoke an action potential.

For trains of stimuli, as stimulus intensity increased (spatial summation), or as stimulus repetition rate increased to 100–500 Hz (temporal summation), there was a decrease in the number of stimulus pulses that evoked an action potential. In the presence of strychnine, MSO neurons generated a greater percentage of action potentials to the stimulus trains. When stimulus trains were delivered bilaterally, MSO neurons fired a greater number of action potentials at specific interstimulus time differences, and were selectively inhibited at other time differences. We conclude that time-specific response characteristics of MSO neurons are governed not only by the coincidence of synaptic excitation, but also by the coincidence and temporal summation of synaptic inhibition. This should enable MSO neurons to respond selectively not only to interaural time differences, but also to sounds with complex time patterns.

[Key words: auditory pathways, temporal coding, medial superior olive, synaptic inhibition, gerbil, brain slice]

The medial superior olive (MSO) is thought to be the first mammalian auditory nucleus to process interaural time differences, largely due to the coincidence detection of excitatory inputs from each ear (Harrison and Irving, 1966; Warr, 1966; Masterton and Diamond, 1967; Goldberg and Brown, 1968, 1969; Watanabe et al., 1968; Clark, 1969; Guinan et al., 1972; Perkins, 1973; Caird and Klinke, 1983; Langford, 1984; Cant and Casseday, 1986; Yin and Chan, 1990).

At the beginning of this century Lord Raleigh published what has become known as the duplex theory of hearing (Raleigh, 1907). Today this theory is well established and states that mammals, including humans, primarily make use of two cues for azimuthal sound localization. These sources are interaural time or phase differences (ITDs or IPDs), used to localize low-frequency sounds, and interaural level differences (IIDs), used for localizing high-frequency sounds (Masterton and Diamond, 1967; Masterton and Imig, 1984). These two different physical parameters are thought to be processed in the mammalian brain via two different auditory pathways that are anatomically distinct at the level of the superior olivary complex (SOC).

The two principal nuclei of the SOC, the lateral superior olive (LSO) and the MSO, are the primary sites of binaural interaction in the ascending auditory system (Ramon y Cajal, 1909; Poljak, 1926). The LSO receives excitatory projections driven by the ipsilateral cochlea and inhibitory projections driven by the contralateral cochlea (Boudreau and Tsuchitani, 1968; Sanes and Rubel, 1988). The resulting interaction of excitation and inhibition enables LSO neurons to compute IIDs (Boudreau and Tsuchitani, 1968; Goldberg and Brown, 1969; Tsuchitani, 1977). In distinction, the MSO is thought to act as a coincidence detector by processing predominantly excitatory inputs. MSO bipolar principal cells are sensitive to ITDs and IPDs, due to the integration of the bilateral incoming excitation (Clark, 1969; Goldberg and Brown, 1969; Guinan et al., 1972; Caird and Klinke, 1983; Langford, 1984; Carr and Konishi, 1988; Yin and Chan, 1990). Although there are apparent difficulties recording from MSO neurons *in vivo* (Caird and Klinke, 1983; Yin and Chan, 1990), the single-neuron physiology and psychoacoustic studies are consistent with a coincidence detection theory (Colburn, 1973, 1977; Casseday and Neff, 1975; Creel et al., 1980; Jenkins and Masterton, 1982; Conlee et al., 1984, 1986; Colburn and Isabelle, 1992).

Recent anatomical and physiological results have identified significant inhibitory inputs to MSO neurons. First, there is evidence that collaterals of the projection from the medial trapezoid body (MNTB) innervate the MSO (Kiss and Majorossy, 1983; Spangler et al., 1985; Casseday et al., 1988; Cant, 1991;

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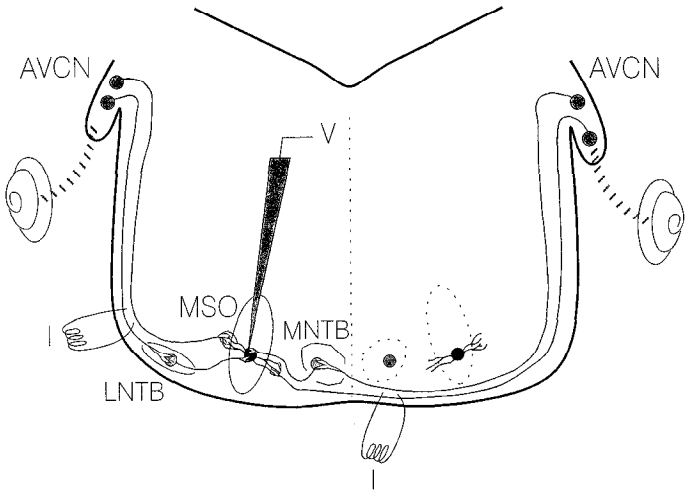

Figure 1. A schematic of the brain slice preparation. Intracellular recordings were obtained from MSO neurons while two afferent pathways were stimulated with current pulses. *AVCN,* anteroventral cochlear nucleus; *MSO,* medial superior olive; *LNTB,* lateral nucleus of the trapezoid body; *MNTB,* medial nucleus of the trapezoid body; *I,* stimulating electrodes; *V,* recording electrode.

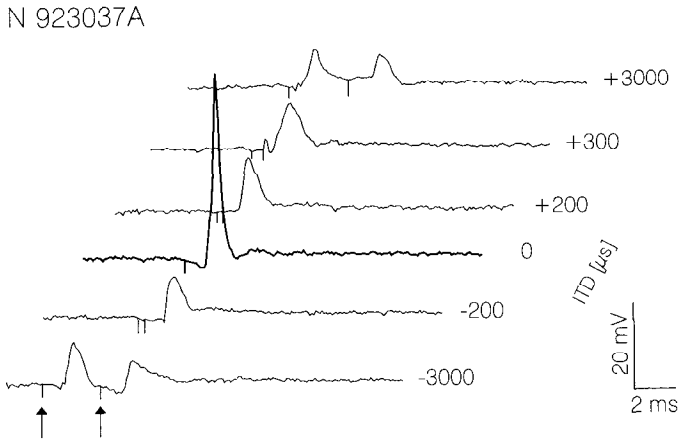

Figure 2. Convergence of EPSPs. Stimuli to each pathway were subthreshold, such that neither would evoke an AP alone. When the bilateral stimulus pulses were brought within ±200 μsec of one another, the EPSPs from both sides summated to evoke an AP. *Arrows* indicate the stimulus artifacts in the *bottom trace.* The stimulus artifacts have been clipped in this and the following figures.

Covey et al., 1991; Grothe et al., 1992; Kuwabara and Zook, 1992; B. Grothe, H. Schweizer, G. D. Pollak, G. Schuller, and C. Rosemann, unpublished observations). The MNTB is known to provide the glycinergic synaptic inhibition to LSO neurons that is evoked by contralateral stimulation, and its collaterals presumably provide glycinergic inhibition to the MSO as well (Moore and Caspary, 1983; Sanes et al., 1987; Sanes, 1990; Wu and Kelly, 1992b). Second, a projection from the lateral nucleus of the trapezoid body (LNTB) to MSO neurons has been identified in gerbils and bats (Cant and Hyson, 1992; Kuwabara and Zook, 1992). This LNTB-MSO projection is thought to be glycinergic as well (Peyret et al., 1987; Helfert et al., 1989; Saint-Marie et al., 1989). Third, electrophysiological recordings *in vivo* have provided some indirect evidence for synaptic inhibition. Some MSO neurons have nonmonotonic rate–level functions, and many MSO neurons exhibit a firing rate that falls below that elicited by monaural stimuli when binaurally presented pure tones are delivered at specific ITDs (out-of-phase suppression; Goldberg and Brown, 1969; Langford, 1984; Yin and Chan, 1990). More recently, an *in vitro* analysis of time difference coding in the chick has demonstrated inhibitory responses with simulated interaural time differences (Joseph and Hyson, 1993). Fourth, in the mustached bat's MSO glycinergic inhibition modulates the excitatory inputs to yield a phasic response pattern (Grothe et al., 1992).

A direct demonstration of synaptic inhibition in the MSO has now been generated in both gerbils and guinea pigs (Smith and Banks, 1992; Grothe and Sanes, 1993). Using the brain slice preparation, these intracellular analyses revealed synaptic inhibition from both the ipsilateral and contralateral pathways. Glycine was shown to be the neurotransmitter that mimicked synaptic inhibition in nearly all MSO neurons tested in the gerbil (Grothe and Sanes, 1993).

The question that has arisen from these data is whether inhibitory transmission influences bilateral time coding by MSO neurons. In particular, we wish to determine whether both excitatory and inhibitory synaptic integration have an impact on sensitivity to ITD. In the present study, we tested whether inhibitory transmission influenced the bilaterally evoked temporal coding properties of MSO neurons, using a gerbil (*Meriones unguiculatus*) brain slice preparation.

Materials and Methods

The methods used in our physiological experiments were previously described in some detail (Sanes, 1990). Briefly, gerbils aged 17–25 d postnatal were anesthetized with chloral hydrate (400–500 mg/kg, i.p.) and decapitated. The brain was rapidly dissected free in an oxygenated artificial cerebrospinal fluid (ACSF) at 15°C. The brainstem was sectioned at 350–400 μm on a vibratome, and the slices were then incubated in a holding chamber in oxygenated ACSF for 30–60 min.

For recordings the slices were placed in a custom chamber and perfused with oxygenated ACSF (8 ml/min, 31°C). Electric stimuli were delivered with two bipolar stimulation electrodes, positioned on the ipsilateral pathway lateral to the LSO and on the contralateral pathway medial to the MNTB (Fig. 1). Electrical stimuli were presented manually (Grass Instruments S11) or under computer control (RC Electronics, Everex 386). The stimuli consisted of 100 μsec pulses at 0–65 V.

Interaural time differences were simulated by successively shifting the latency of the electrical stimulus pulse to one side while keeping the other stimulus pulse constant in time. We refer to these interstimulus time differences as "ITDs" below. In a second set of experiments we presented stimulus trains (70 msec, 50–500 Hz) to one or both pathways. In this paradigm, relative time differences between the two pathways could also be introduced.

Intracellular recordings were obtained with glass electrodes filled with 2 M potassium citrate and having a resistance of 130–200 MΩ. The voltage signals were fed from an electrometer (Axon Instruments Axoprobe A-1) to an oscilloscope, and digitized with an A/D converter sampling at 10–20 kHz (ComputerBoards Inc.). The MSO cell body region was observed with a stereo microscope (Olympus) and electrodes were positioned under visual control. Recordings could rarely be obtained when the electrode was outside of the narrow cell body region. Analyses were performed off line. The mean resting potential for all analyzed neurons was −51.2 mV ($N = 148$).

In order to block synaptic inhibition mediated by glycine, its antagonist, strychnine, was prepared in relatively high concentrations (50 mM), and was added in small volumes (50–200 μl) to the superfusate in advance of the recording well. This allowed brief exposure periods, thus limiting the recovery period, which otherwise could be quite lengthy.

Results

Responses to simulated interaural time differences

The vast majority of MSO neurons (73 of 98 cells) responded with an action potential (AP) to bilaterally presented stimuli

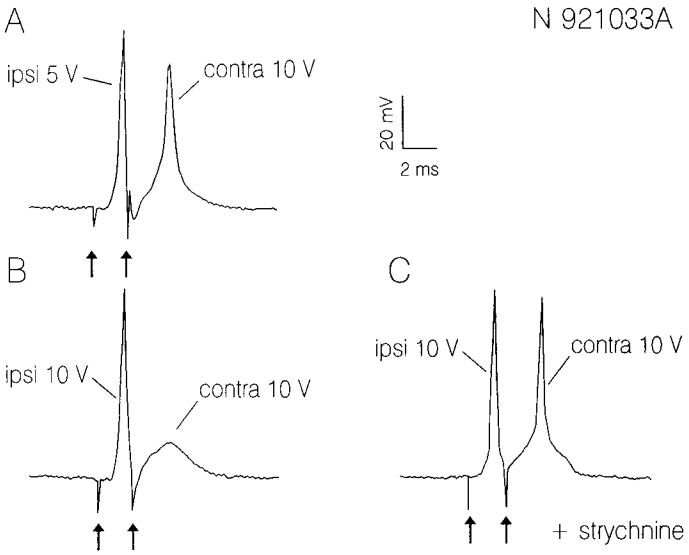

Figure 3. Strychnine reversed the stimulus-evoked blockade of an AP. *A,* The neuron in this example responded to both stimuli with an AP, if the amplitude of the leading (ipsilateral) stimulus was 5 V. *B,* An increase of the first stimulus from 5 V to 10 V led to a suppression of the AP to the second stimulus. *C,* In the presence of strychnine, the cell responded to both stimuli with an AP, independent of the stimulus amplitude of the leading stimulus. *Arrows* indicate stimulus artifacts (*A* and *B* adapted from Grothe and Sanes, 1993).

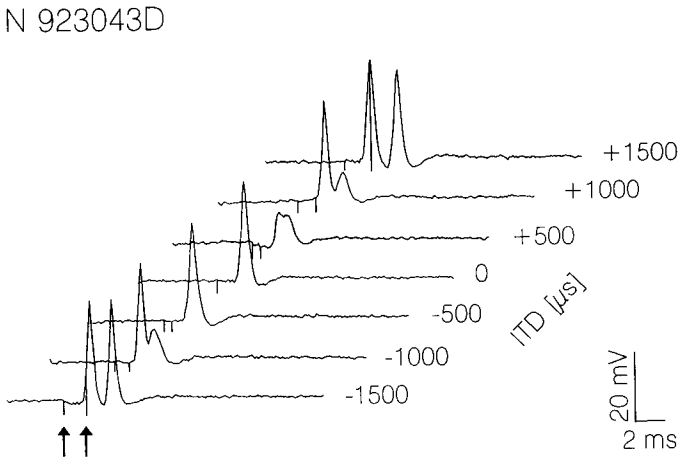

Figure 4. Response to varying interstimulus intervals (simulation of ITD shifts). If the interstimulus interval was greater than 1500 μsec, then the neuron responded with two APs. For shorter ITDs the neuron responded with one AP only. When the interstimulus interval was +500 μsec, discharge was completely suppressed. The stimulus amplitude was 10 V on both sides. *Arrows* indicate stimulus artifacts.

that were insufficient to evoke an AP if presented alone. When the relative timing of electrical stimuli to each pathway was varied (e.g., simulation of interaural time differences), it was found that EPSPs from both sides had to occur within a certain latency to one another in order to summate. This response was independent of the way a neuron responded to higher stimulus amplitudes. The mean duration of this interstimulus time difference (ITD) was 230 μsec, with a maximal period of 500 μsec. Figure 2 presents one example of this effect. When 5 V stimuli were delivered to both pathways, the cell responded with an AP if the stimuli occurred within 200 μsec of one another. In the presence of strychnine, the glycine antagonist, there was no change in the temporal characteristics of this response (not shown), although glycinergic inhibition was evident at higher stimulus amplitudes (see below).

In 47 of 65 cells tested (73%) the response pattern to bilateral stimuli was dependent on stimulus level. Figure 3 shows the response of one neuron at a fixed ITD with the leading stimulus close to threshold (Fig. 3*A*), and somewhat above threshold (Fig. 3*B*). When the first stimulus was near threshold, the neuron responded with an AP to each stimulus. When the intensity of the first stimulus was increased, the second stimulus was no longer able to evoke an AP (Fig. 3*B*). Thus, higher stimulus levels extended the apparent refractory period following the first stimulus-evoked AP. Strychnine abolished the extended refractory period observed at higher stimulus levels, demonstrating that synaptic inhibition was involved (Fig. 3*C*).

At the highest stimulus amplitudes, it was generally found that a distinct range of ITDs, between 50 and 300 μsec, resulted in the total suppression of APs. In general, the center point of this time range matched the point of coincidence of excitation observed at lower stimulus levels. Figure 4 presents an example of such a complete suppression. In this case, the range of ITDs that suppressed neuronal discharge was about 300 μsec. Within a larger range of ITDs (≈3000 μsec) there was a reduction of the response from two to one AP. In general, the interstimulus times that led to a reduction or full suppression of discharge occupied a broader range of time differences than the effective range for threshold stimuli that produced an AP due to a convergence of EPSPs (Fig. 2).

To determine whether the effects described above were due to the glycinergic inputs to the MSO, we used the same stimulus parameters in the presence of strychnine. In 9 of 11 cells, strychnine changed the response patterns elicited by the intermediate and the suprathreshold stimulus levels. As shown by the set of

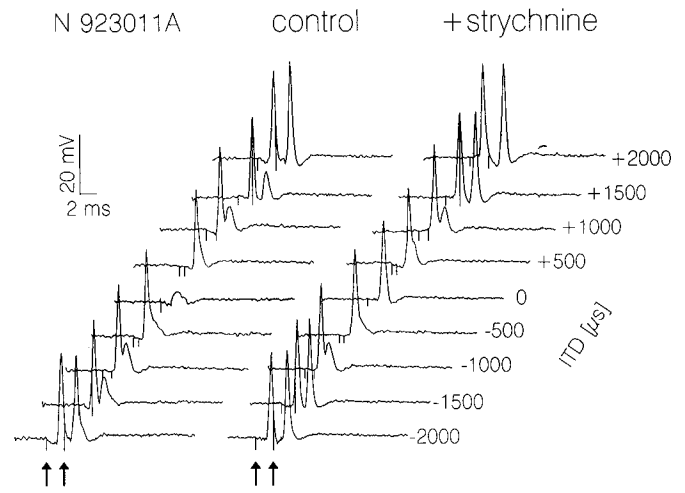

Figure 5. The suppression of discharge seen with bilateral stimuli was due to glycinergic transmission. A neuron that responded similarly to that shown in Figure 4 (*control*) displayed complete suppression of discharge at an ITD of 0 μsec. In the presence of strychnine (*+strychnine*), however, the neuron responded with one AP at an ITD of 0 μsec. In addition, strychnine extended the range of ITDs where the neuron responded with two APs. The stimulus amplitude was 10 V on both sides. *Arrows* indicate stimulus artifacts.

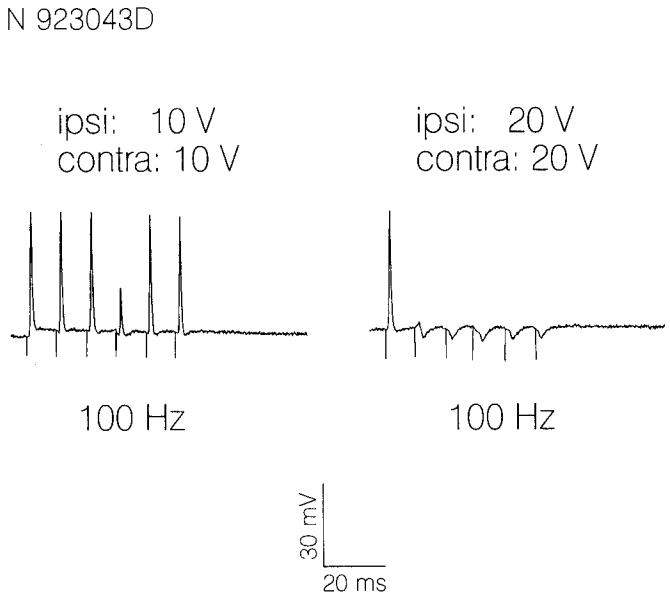

Figure 6. Temporal summation of synaptic inhibition in response to bilateral pulse trains. This neuron responded with five APs when the 100 Hz train was delivered bilaterally at 10 V. When stimulus amplitude was increased to 20 V, the neuron responded with only a single AP. In addition, prominent IPSPs were observed at higher stimulus levels.

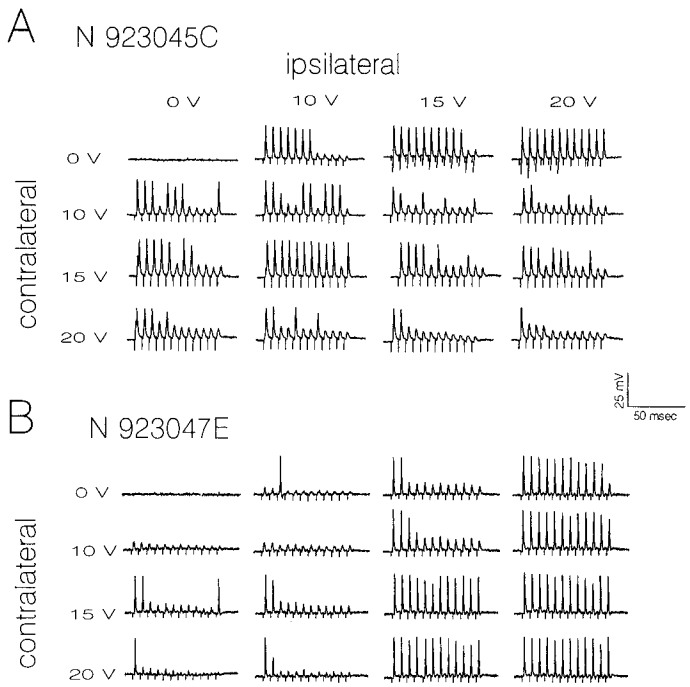

Figure 7. The relationship between stimulus amplitude and the response pattern evoked by stimulus trains. *A*, A neuron exhibiting strong contralateral inhibition and weak ipsilateral inhibition. Note that the neuron's response improved with increasing ipsilateral stimulus amplitude (*first row*), but not with increasing contralateral stimulus amplitude (*left column*). Bilateral stimulus presentation led to a summation of the inhibitory effects (*lower right trace*), and decreased the response (number of APs) even more than was possible with contralateral stimulation alone. *B*, A neuron exhibiting contralateral, but not ipsilateral, synaptic inhibition. In contrast to the traces shown in *A*, the response improved with increased bilateral stimulus amplitude. This was not the case for contralateral stimulation only.

control traces in Figure 5, bilateral stimuli led to a complete blockade of APs within a limited range surrounding 0 μsec. Moreover, there was a large time range of about ±1500 μsec (e.g., either the ipsilateral or the contralateral stimulus leading) during which only a single AP could be evoked. The addition of strychnine changed this pattern such that complete AP suppression did not occur at a time difference of 0 μsec, and two APs could be elicited at ±1500 μsec. In two neurons that exhibited no IPSPs when stimulated ipsilaterally and only weak inhibition when stimulated contralaterally, strychnine failed to change the response to ITD stimuli.

Response to bilateral pulse trains

In a second set of experiments we investigated the temporal summation of the synaptic inhibition in MSO neurons using short trains of stimuli delivered both unilaterally and bilaterally ($N = 46$). The number of APs that could be evoked in response to a stimulus train decreased as stimulus amplitude was raised (for an ITD of 0). The neuron shown in Figure 6 responded with five APs to concurrent bilateral stimuli of 10 V delivered at a repetition rate of 100 Hz. When the stimulus amplitudes were increased to 20 V, the response pattern of the cell changed dramatically, responding to only the first pulse of the stimulus train.

The differential influence of contralateral and ipsilateral inhibition is illustrated in Figure 7*A*. This neuron displayed significant contralateral inhibition and little ipsilateral inhibition (when stimulated). An increase of stimulus intensity of the ipsilateral pathway actually improved the cell's performance to the pulse trains (top row), when contralateral stimuli were absent or close to threshold. However, when higher stimulus level was delivered to the contralateral side the number of evoked APs was reduced. An increase of stimulus level to both sides simultaneously led to an even poorer response, possibly due to the summation of bilateral inhibition. Figure 7*B* presents the same test for a neuron that had a weak inhibitory influence from the contralateral side and no apparent inhibition from the ipsilateral side. In this neuron, the response was enhanced with increasing stimulus amplitude to the ipsilateral pathway, although increasing stimulus level to the contralateral side had only a slight effect. In this case the inhibition was not strong enough to overrule the coincidence of excitation that resulted in the best response for high stimulation levels at both sides.

Stimulus repetition rate also had a dramatic effect on the response pattern of MSO neurons. As shown in Figure 8, increasing repetition rate led to a reduction in the number of evoked APs. This neuron responded to a 70 msec train of pulses delivered at 100 Hz (identical to the 10 V/10 V panel in Fig. 6) with five APs. When the repetition rate was increased to 200 Hz, the neuron responded only to the first two pulses with an AP, and displayed only EPSPs to the next 10 pulses of the train. A further increase of the repetition rate to 300 Hz led to a suppression of all but the first AP, and elicited an obvious hyperpolarizing response.

In all cells tested there was a rate-dependent blockade of APs that usually occurred between 100 and 300 Hz. Bilateral presentation reduced the range for favorable repetition rates up to 100 Hz in some neurons, while in other neurons the coincidence of strong excitation improved the response to bilateral stimulation.

In order to demonstrate that the reduction of APs that occurred at different train rates was due to the glycinergic inhi-

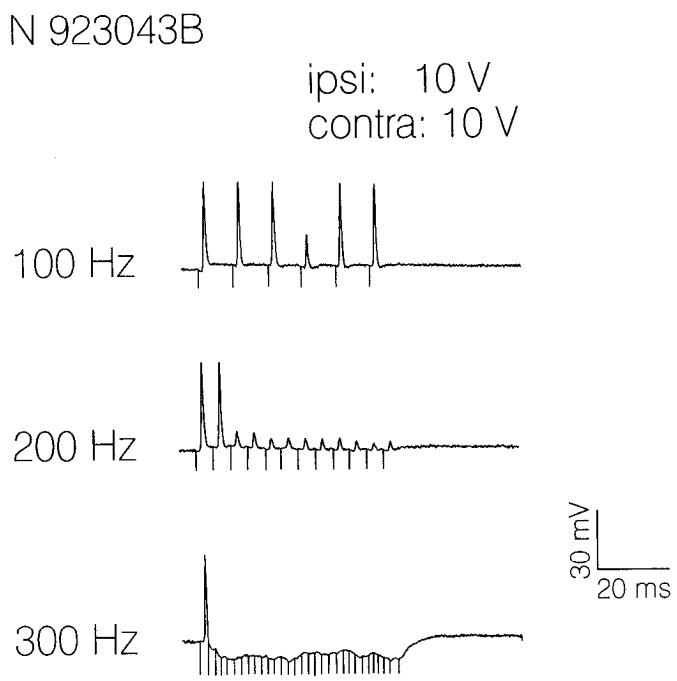

Figure 8. Temporal summation of synaptic inhibition was enhanced at higher stimulus frequency. This neuron responded with an AP to five of six stimuli to the 100 Hz train, but only to the first two pulses in the 200 Hz train. At 300 Hz, only a single AP was evoked, and there was a dramatic summation of the IPSPs that yielded a persistent hyperpolarization.

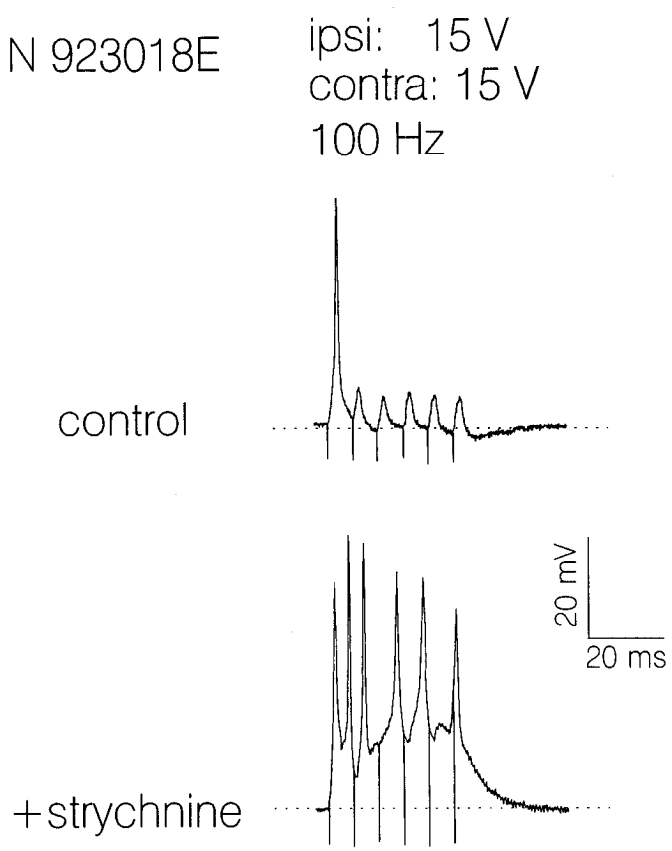

Figure 9. Glycinergic transmission limited the response to trains of stimuli. This neuron produced only a single AP to a bilateral 100 Hz pulse train, and exhibited a clear temporal summation of IPSPs as indicated by the hyperpolarization of about 10 msec following the last stimulus. In the presence of strychnine there was a prominent temporal summation of EPSPs, and the neuron responded with an AP to almost every stimulus in the train (note the two APs to the first pulse).

bition, we performed the same test in the presence of strychnine. In 10 of 11 cells tested, strychnine allowed the neuron to respond to more pulses per train than during the control period. As illustrated in Figure 9, a neuron responded with one AP for a bilateral stimulus train at 100 Hz. However, in the presence of strychnine, the cell was able to follow most pulses in the stimulus train. In addition, a hyperpolarization at the end of the control stimulus train that lasted for about 10 msec was abolished in the presence of strychnine.

Response to pulse trains with simulated ITDs

Favorable ITDs were also observed when bilateral trains of stimuli were employed. Figure 10 presents one example that is representative for the seven neurons tested with this paradigm. For certain ITDs the MSO neuron did not respond to the 100 Hz train with even a single AP (e.g., ipsilateral stimulus delayed by 750 μsec) due to the apparent bilateral summation of synaptic inhibition. However, with decreasing ITDs the neuron exhibited a maximal response of about 4 APs (e.g., ipsilateral stimulus delayed by 250 μsec). When the ipsilateral pulses were leading by 250 μsec, the neuron responded with only a single AP. For a 200 Hz train, the neuron discharged maximally once per train, and did not respond with a single AP when the contralateral train was leading by more than 300 μsec (data not shown).

Discussion

Glycinergic inhibition influences ITD coding

The main result of this study is that glycinergic inhibition has a profound impact on the way MSO neurons encode temporal stimulus patterns. Since this is consistent with anatomical and physiological data from previous studies (see below), we suggest that synaptic inhibition influences the sound-evoked temporal coding properties of MSO neurons. However, we discuss these effects below with the clear understanding that all conclusions are tentative until they are tested *in vivo*.

Inhibitory effects on the ITD function

As noted in our previous report (Grothe and Sanes, 1993), there is a consistent level dependency of the inhibitory events in the gerbil's MSO, as assessed *in vitro*. At low stimulus amplitudes inhibitory synaptic transmission does not appear to have a functional role. When bilateral stimuli were delivered below threshold for generating an AP (Fig. 2), the responses were fully consistent with the theory of excitatory coincidence detection (Jeffress, 1948). MSO cells integrated the bilateral EPSPs within a discrete range of interstimulus time differences.

The situation changed dramatically for higher stimulus amplitudes due to the recruitment of synaptic inhibition from the ipsilateral MNTB and/or LNTB. The recruitment of synaptic inhibition had two effects. First, it prolonged the apparent refractory period that followed the first of two presented pulses. Second, coincidence of bilaterally evoked inhibition was capable of suppressing all APs at specific interstimulus time differences. Thus, synaptic inhibition may significantly modify the "typical" MSO response characteristics.

Our results indicate a change of dominance from synaptic excitation to synaptic inhibition with higher stimulus levels. This transition directly influenced the response of an MSO cell,

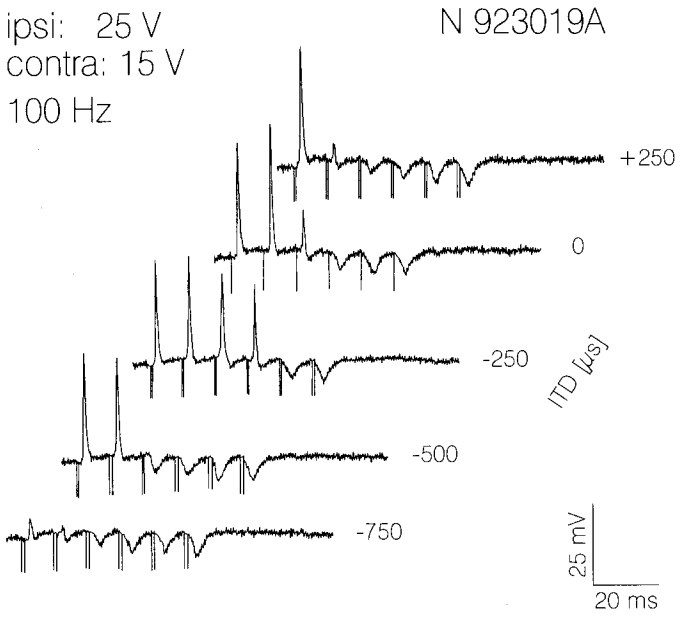

Figure 10. The response of an MSO neuron to bilateral stimulus trains presented with different interstimulus intervals. For certain ITDs the neuron did not respond to the 100 Hz train with even a single AP (*bottom trace*; −750 μsec). The neuron responded with two APs at 0 and −500 μsec, and it responded maximally with four APs at 250 μsec.

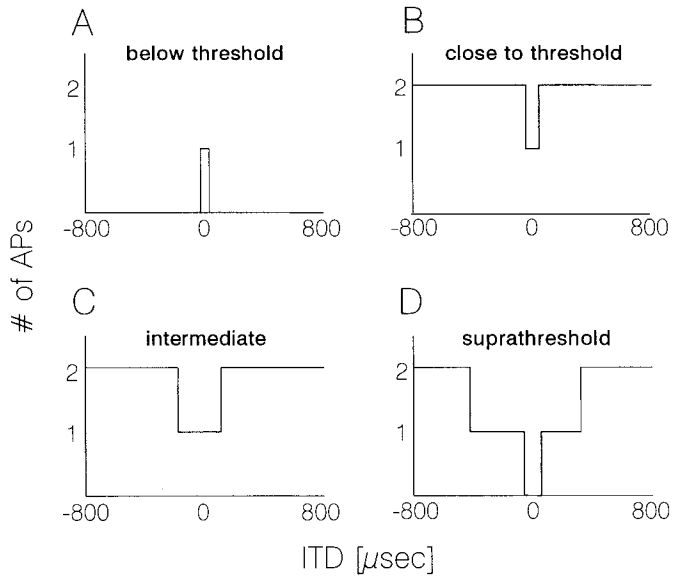

Figure 11. A schematic illustrating the dynamic range of the response patterns obtained in response to single bilateral pulses when amplitude and ITD are varied. *A*, Stimuli below threshold evoke an AP within a small range of ITDs where coincidence of the EPSPs produces the discharge. *B*, Stimuli above threshold evoke two APs at all ITDs except a small range around the 0 position, where only one spike occurs due to the absolute refractory period. *C*, As stimulus amplitude is increased, there appears a larger range of ITDs that elicit only a single AP, due to the recruitment of synaptic inhibition. *D*, When stimulus amplitude is maximal, the summation of bilateral inhibition is able to block all discharge at certain ITDs. Thus, synaptic inhibition serves to increase the dynamic range of the neuron's response.

especially when ITDs were varied. Figure 11 schematizes the sequence of response patterns as stimulus intensity was increased. At low stimulus amplitudes synaptic excitation was predominant (Fig. 11*A*), and increased stimulus levels led to the recruitment of synaptic inhibition (Fig. 11*B,C*). At the highest stimulus levels inhibition was dominant (Fig. 11*D*).

The inherent limitations of electrical stimulation prevent us from correlating the high-intensity stimulation with specific acoustic stimuli. It is possible that the recruitment of synaptic inhibition at high stimulus intensities reflects a higher threshold to electrical stimulation. For example, low-amplitude electrical stimuli may activate small-diameter fibers (e.g., fibers from spherical bushy cells that innervate MSO neurons), and high-amplitude electrical stimuli may activate larger-diameter fibers (e.g., fibers from globular bushy cells that innervate MNTB neurons). If this is the case, then the level-dependent recruitment of synaptic inhibition may be only an *in vitro* phenomenon. However, the nonmonotonic rate level functions exhibited by some neurons in the dog's MSO to monaural stimulation are consistent with the concept of level-dependent inhibition (Goldberg and Brown, 1969). Therefore, *in vivo* recordings will be required to resolve this issue.

From psychoacoustic experiments in humans it is known that ITD resolution partially depends upon stimulus level. Humans perform less precisely at low sound levels, but exhibit stability for azimuthal localization from 50 to 90 dB SPL. The resolution for ITDs at higher-intensity tones again decreased (Zwislocki and Feldman, 1956; Hershkowitz and Durlach, 1969), suggesting a level-dependent interaction in time difference processing.

A level dependency of periodic discharge curves (ITD functions) in electrophysiological experiments was first shown for single neurons in the inferior colliculus (IC) of the cat (Rose et al., 1966). It is useful to consider the difference between the right ear 50 dB/left ear 90 dB ITD function in Rose and coworkers' Figure 4*A* and the right ear 60 dB/left year 90 dB ITD function in their Figure 4*B*. In this case, the right ear has a monotonic rate–level function between 50 and 60 dB, yet the higher intensity leads to an enhancement of both the excitatory peak and the inhibitory trough. The absolute discharge rate for unfavorable ITDs dropped below the rate to monaural stimulation. Thus, the authors concluded that the changes in the periodic discharge curves are due to different interactions of excitation and inhibition at different stimulus levels. Although out-of-phase suppression has been reported for MSO neurons in the dog (Goldberg and Brown, 1969), the cat (Yin and Chan, 1990), and the chinchilla (Langford, 1984), there are no comparative results, to our knowledge, that have analyzed the level dependency of the ITD function in MSO neurons.

Taken together, there is evidence supporting the idea that synaptic inhibition influences ITD coding at all stations of the auditory system, including the MSO, and that coincidence of excitation as well as inhibition determines the response of MSO cells. The glycinergic inhibitory projection from the MNTB has been implicated in ITD coding by low-frequency LSO neurons (Joris and Yin, 1990; Finlayson and Caspary, 1991), and has also been implicated in temporal processing by LSO neurons using a brain slice preparation (Sanes, 1990). Therefore, it would be surprising if the MNTB projection to MSO neurons did not also provide a time-dependent input. At present, there is no *in vivo* data on the temporal properties of LNTB neurons.

The involvement of inhibition in processing ITDs is not an exclusively mammalian phenomenon, but is also true for birds. In the barn owl, inhibition was involved in sharpening ITD functions in the central and the external nuclei of the IC (Fujita and Konishi, 1991). This is similar to the sharpening of IID

functions in the IC of mammals (Caspary and Palombi, 1993; Park and Pollak, 1993; Pollak and Park, 1993). Recently, Joseph and Hyson (1993) have demonstrated *in vitro* that the discharge probability of neurons in the nucleus laminaris, an avian nucleus with functional and structural properties that are similar to the gerbil MSO, decreases to a value below that obtained with unilateral stimulation at certain simulated ITDs.

Nevertheless, it is possible that out-of-phase suppression is due to intrinsic properties of MSO neurons, and the convergence of excitatory potentials in the presence of significant spontaneous activity (Colburn et al., 1990; Han and Colburn, 1993). However, the results of Joseph and Hyson (1993) and our present results demonstrate that synaptic inhibition may affect ITD coding in the absence of spontaneous activity. In addition, our present results raise the possibility that synaptic inhibition influences the coding of ITDs at different stimulus levels. Unfortunately, the incorporation of synaptic inhibition into existing models will have to await *in vivo* analyses, similar to those that have been performed in the bat (Grothe et al., 1992).

Inhibition may play several possible roles in connection with ITD coding by MSO neurons. The first is to increase the dynamic range of the neurons' response to different ITDs if there are several repetitions of the acoustic event as is the case for low-frequency tones where phase-locking can occur. In comparison to the simulated ITD tests with single stimuli (Figs. 2–5), the dynamic range of the response was much greater for trains of stimuli (Fig. 10) since there were several possible responses (i.e., 1–4 APs). The synaptic basis for extending the dynamic range is the temporal summation of inhibitory potentials. Thus, the cell's response depended upon the specific combination of interstimulus interval and the stimulus structure (e.g., repetition rate). Joseph and Hyson (1993) have also noted that the ITD resolution is enhanced with several iterations of a time comparison (see their Fig. 10).

A second possible role for inhibition could be to constrain the summation of excitatory activity across the MSO within a certain range, independent of the stimulus level (M. Reed, personal communication). This would presumably be important for the stable performance of ITD detection over a large range of stimulus levels (Zwislocki and Feldman, 1956; Hershkowitz and Durlach, 1969). Inhibitory afferents may serve to limit the number of MSO neurons that are discharging maximally, thereby regulating the convergent excitation of MSO afferents at more central loci, such as the dorsal nucleus of the lateral lemniscus and the IC.

Asymmetric inhibitory inputs, as demonstrated for the MSO (Grothe and Sanes, 1993), could create different levels of activity in the MSO of each side. In most neurons the contralateral inhibition was stronger than that provided through ipsilateral stimulation. A difference in the strength and symmetry of the inhibitory afferents could contribute to the differences observed for ITD coding between species. For example, Yin and Chan (1990) remarked that the peaks of ITD curves in the MSO of the cat occurred when the contralateral side was leading, whereas MSO neurons in the chinchilla exhibited maximal discharges if the ipsilateral side was leading (Langford, 1984). The most prominent example of a different distribution of incoming projections to the MSO occurs in the mustached bat (Grothe, 1990; Covey et al., 1991; Grothe et al., 1992; Vater, 1993). The ipsilateral projections are severely reduced, resulting in predominantly monaural response characteristics.

It is difficult to judge the physiological and behavioral relevance of the sensitive ITD range measured *in vitro*, since there is no way to know the real "0" position that results in coincident EPSPs *in vivo*. The measurable delays changed with the position of the stimulus electrode and so did the ITD 0 point. The time range for effective excitatory coincidence for stimuli below threshold was on average much higher than 100 μsec. The gerbil's head has an interaural distance of about 33 mm and therefore creates ITDs up to about 100 μsec (Heffner and Heffner, 1988). Since we do not know the exact 0 position we can only speculate whether one of the limits for a change in the response patterns lies within the relevant ITD time range (±100 μsec), and whether the ITD resolution under the experimental conditions (31°C) is comparable with the response kinetics *in vivo*. Such a change would probably be equivalent to the steep portion of the periodic discharge curve seen in *in vivo* studies. It is interesting to note that when compared with *in vivo* functions, the simulated ITD ranges that influenced the MSO response *in vitro* were comparable to sound-evoked ITDs for MSO neurons in several mammals such as the kangaroo rat (Moushegian et al., 1975; Crow et al., 1978), the chinchilla (Langford, 1984), the molossid bats (Harnischfeger et al., 1985), the cat (Yin and Chan, 1990), and the dog (Goldberg and Brown, 1969).

However, it should be noted that ITD-sensitive neurons in higher auditory centers than the MSO exhibit similar ITD sensitivities (Rose et al., 1966; Stillman, 1971; Brugge and Merzenich, 1973; Yin and Kuwada, 1983; Yin et al., 1986; Batra et al., 1989; Kuwada et al., 1989; Kelly and Phillips, 1991), even though the exact position of the ITD function differs among mammals (Stillman, 1971; Crow et al., 1978) and may not match the behaviorally relevant range (Kelly and Phillips, 1991). Even LSO neurons that are traditionally thought to function exclusively as IID detectors also exhibited ITD and IPD sensitivity within a comparable range (Joris and Yin, 1990; Finlayson and Caspary, 1991). Additionally, there is evidence for similar time ranges and similar mechanisms for ITD coding of interaural delays of amplitude-modulated sounds. This seems to be true even if the characteristic frequency of a neuron is tuned well above the range limit to which mammalian neurons can phase-lock (Batra et al., 1989). Therefore, the significance of ITD coding by MSO neurons must be carefully examined within a species such as the gerbil, as the nucleus may contribute to nontraditional coding properties.

Inhibitory effects on temporal synaptic integration

There is strong evidence for temporal summation of inhibition, as illustrated by the responses of MSO neurons to repetitive pulses. This effect was also dependent upon the stimulus intensity, and was more prominent for contralateral stimulation. Blockade of the glycinergic transmission by bath application of strychnine enabled MSO neurons to follow higher pulse repetition rates. For the mustached bat it has been shown *in vivo* that the interaction of excitation and inhibition in the MSO leads to similar response characteristics for amplitude-modulated sounds. The bat MSO neurons exhibited strychnine-sensitive, low-pass filter characteristics with cutoff frequencies in the same range as those observed in the present study, generally between 100 and 300 Hz (Grothe, 1990). In contrast, neurons in the chick nucleus laminaris appear to follow more rapid stimulus rates with high fidelity (Joseph and Hyson, 1993, their Fig. 4*A*).

Since there is no temporal summation shown for the response of MNTB neurons (Wu and Kelly, 1992a) the summation of

inhibition clearly takes place within the MSO. Temporal summation has also been seen in the mustached bat's MSO (B. Grothe, unpublished observations). A similar phenomenon occurs in the LSO, where the duration of synaptic inhibition is longer lasting than that of excitation, and appears to summate during trains of bilateral stimuli such that later-occurring APs are suppressed (Sanes, 1990).

Taken together, the interaction of inhibitory and excitatory projections converging in the MSO provides a mechanism that is able to filter selectively not only ITDs but also sounds with complex time patterns. The results obtained with trains of stimuli and ITDs indicate that the response of MSO neurons could depend on the temporal fine structure of a stimulus and its spatial chronological position. In this case, the temporal summation of the synaptic inhibition appears to be the determining mechanism. Temporal integration is considered to be one of the essential mechanisms for processing complex sounds such as periodic modulations (Langner, 1988; van Stokkum, 1989). Therefore, the synaptic mechanisms that have been elucidated in the present study may have repercussions for acoustic processing that is independent of sound localization.

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