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Synaptic Pharmacology of the Superior Olivary Complex Studied in Mouse Brain Slice

Shu Hui Wu and Jack B. Kelly

Laboratory of Sensory Neuroscience, Psychology Department, Carleton University, Ottawa, Ontario K1S 5B6, Canada

The synaptic pharmacology of the lateral superior olive (LSO) and medial nucleus of the trapezoid body (MNTB) was examined in a brain slice preparation of the mouse superior olivary complex (SOC). Physiological responses in SOC were elicited by electrical stimulation of the trapezoid body ipsilateral or contralateral to the recording site, and bilateral interactions were investigated by combined ipsilateral and contralateral stimulation. Pharmacological effects were tested by bath application of amino acid agonists and antagonists. Neurons in MNTB were excited by contralateral stimulation and unaffected by ipsilateral stimulation. Excitatory amino acid (EAA) agonists—kainic acid (KA), quisqualic acid (QA), or L-glutamate—caused spontaneous firing at low concentrations and eliminated responses at higher concentrations in MNTB. The EAA agonist NMDA had relatively little effect at comparable concentrations. Stimulus-elicited responses were blocked by non-NMDA antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) and by the nonspecific EAA antagonist kynurenic acid, but were unaffected by the NMDA antagonist D,L-2-amino-5-phosphonovaleric acid (APV). LSO neurons were typically excited by ipsilateral stimulation and inhibited by contralateral stimulation. In LSO, KA, QA, and L-glutamate caused spontaneous firing at low concentrations and eliminated responses at higher concentrations, and NMDA had relatively little effect. Excitatory responses in the vast majority of LSO neurons were blocked by CNQX, DNQX, or kynurenic acid. Some responses were also blocked by APV. LSO neurons were affected by glycine, and contralateral inhibition in LSO was completely blocked by strychnine. NMDA also blocked inhibition in LSO. These results indicate that excitation of both MNTB and LSO neurons is mediated primarily by an EAA neurotransmitter through non-NMDA receptors and that contralateral inhibition of LSO cells is mediated through strychnine-dependent glycine receptors. NMDA receptors may play a role in binaural processing by modulating contralateral inhibitory input to LSO.

The superior olivary complex (SOC) is an important brainstem nucleus for binaural integration and sound localization (Masterton et al., 1967; Moore et al., 1974; Casseday and Neff, 1975; Jenkins and Masterton, 1982; Kavanagh and Kelly, 1986). Neurons in the SOC receive binaural input and are sensitive to small interaural time and intensity differences that serve as cues for sound source position (Masterton and Imig, 1984; Phillips and Brugge, 1985). In the mouse brainstem, the SOC is comprised of two main nuclei, the lateral superior olive (LSO) and the medial nucleus of the trapezoid body (MNTB). The MNTB receives its projection directly from the contralateral ventral cochlear nucleus and sends a projection to the LSO on the same side of the brain. The LSO receives a direct projection from the ipsilateral ventral cochlear nucleus. Thus, the cells in LSO are binaurally innervated through a monosynaptic ipsilateral and disynaptic contralateral pathway from the cochlear nucleus (Stotler, 1953; Harrison and Irving, 1966; Warr, 1966, 1972, 1982; Osen, 1969; Harrison and Feldman, 1970; Strominger and Strominger, 1971; Browner and Webster, 1975; Glendenning et al., 1985).

Physiological studies have shown that LSO neurons are excited by ipsilateral and inhibited by contralateral acoustic stimulation (Boudreau and Tsuchitani, 1968, 1970; Tsuchitani and Boudreau, 1969; Guinan et al., 1972a,b; Tsuchitani, 1977). Neurons in MNTB, on the other hand, are excited exclusively by contralateral acoustic stimulation (Goldberg and Brown, 1968; Guinan et al., 1972a,b; Caird and Klinke, 1983). Intracellular recordings, *in vivo* and *in vitro*, have shown that ipsilateral stimulation elicits EPSPs, and contralateral stimulation elicits IPSPs, in LSO. In contrast, MNTB neurons respond exclusively with EPSPs to contralateral stimulation (Finlayson and Caspary, 1989; Sanes, 1990; Wu and Kelly, 1991). Thus, binaural interaction is associated with the convergence of excitatory and inhibitory postsynaptic responses on cells located in LSO.

A better understanding of the mechanisms of binaural processing can be obtained from studies of synaptic transmission in SOC. Recently, studies have focused attention on the possible neurotransmitters in the superior olive. Histochemical and electrophysiological data suggest that MNTB neurons employ glycine as an inhibitory neurotransmitter in the circuit between MNTB and LSO (Zarbin et al., 1981; Moore and Caspary, 1983; Caspary et al., 1985; Schwartz, 1985; Sanes et al., 1987; Glendenning and Baker, 1988; Saint Marie et al., 1989). Our own previous study of the mouse brain slice has shown that glycine mediates contralateral IPSPs in LSO through neurons in MNTB (Wu and Kelly, 1991). Much less is known, however, about the excitatory neurotransmitters in SOC. The excitatory amino acids (EAAs), primarily L-glutamate and related compounds, are thought to be common neurotransmitters in the CNS (Watkins and Evans, 1981; Fonnum, 1984; Robinson and Coyle,

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Correspondence should be addressed to Jack B. Kelly, Department of Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

1987). They are well suited for the rapid synaptic transmission characteristic of the afferent auditory system. Receptors for EAAs have been subdivided on the basis of the depolarizing actions of selective agonists into two general types, NMDA and non-NMDA, which can be selectively blocked by specific antagonists (Watkins and Olverman, 1987; Monaghan et al., 1989). Iontophorenic studies of LSO neurons have suggested that an EAA mediates ipsilateral responses probably through non-NMDA receptors (Caspary and Faingold, 1989). Receptor binding studies, however, have provided evidence for both NMDA and non-NMDA receptors in LSO (Baker and Glendenning, 1991). So far, there have been no physiological studies of the potential excitatory neurotransmitters in MNTB.

In the present study we have examined the effects of EAA agonists and antagonists on responses in LSO and MNTB. Both NMDA and non-NMDA effects have been studied. We have also investigated the effects of glycine and strychnine on inhibition in LSO.

A preliminary report based on some of these observations has been published elsewhere (Wu and Kelly, 1992).

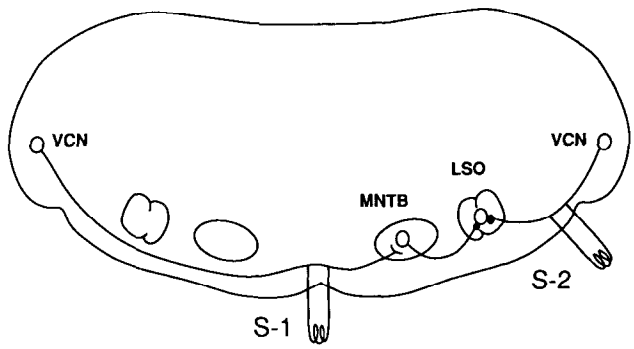

Figure 1. Schematic view of the superior olive mouse brain slice preparation. Bipolar tungsten electrodes were placed on the midline and lateral trapezoid body for contralateral and ipsilateral stimulation, respectively. Recording electrodes were inserted into the LSO or MNTB under visual control. Calyces of Held in MNTB are indicated by a *curved bar*, and terminal boutons in LSO are represented by *heavy black dots*. *S-1*, contralateral stimulating electrode; *S-2*, ipsilateral stimulating electrode; *VCN*, ventral cochlear nucleus.

Materials and Methods

The mouse brain slice preparation has been described in detail in a previous report (Wu and Kelly, 1991). In the present study 21–42-d-old mice of the inbred strain C 57 BL/6J were used. Brain slices about 400 μm thick were cut in the frontal plane through the trapezoid body and SOC with a Vibratome. Sectioning was performed with tissue immersed in 30°C oxygenated saline. For recording, the slice was placed in a chamber filled with warm oxygenated saline that flowed continuously around the submerged tissue at a rate of about 10–12 ml/min. The volume of the chamber was approximately 0.3 ml. The temperature of the saline was regulated by a servo-controlled heating element and maintained at 33–34°C. The normal saline in which the brain slice was immersed consisted of 124 mM NaCl, 5 mM KCl, 1.2 mM KH_2PO_4, 2.4 mM $CaCl_2$, 1.3 mM $MgSO_4$, 26 mM $NaHCO_3$, and 10 mM glucose in distilled water. The solution was saturated with 95% O_2 and 5% CO_2 and had a pH of 7.4.

Experimental drugs and test solutions were introduced into the chamber by redirecting the flow of liquid through a system of tubing and stopcocks from bottles located in an elevated, temperature-controlled water bath. The substitution of solutions was made without interruption of flow rate, temperature, or oxygen/carbon dioxide content. All pharmacological effects were determined by perfusion of brain tissue with an experimental solution usually followed by a return to normal saline. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 6,7-dinitroquinoxaline-2,3-dione (DNQX) (both from Research Biochemical Inc.), and kynurenic acid, D,L-2-amino-5-phosphonovaleric acid (APV), quisqualic acid (QA), kainic acid (KA), glutamate, NMDA, glycine, and strychnine (all from Sigma) were added to the normal saline solution to make the concentrations indicated in the text. The physiological effects of various neurochemicals usually took place within 1–4 min, and a comparable period of time was needed for recovery following normal saline wash. Negative responses were monitored for at least 5 min after bath application of chemicals.

Some experiments were conducted with pharmacological agents in a solution containing 0 Mg^{2+}. The 0 Mg^{2+} solution was prepared by omitting $MgSO_4$ from the normal saline. Also, occasionally, $MgCl_2$ was substituted for $CaCl_2$ to produce a 0 Ca^{2+} solution. These solutions are mentioned in the text where appropriate. If unspecified, the solution used as a vehicle for delivering pharmacological agents was normal saline.

The brain slice chamber was illuminated from below through a dark-field condenser that rendered the fibers of the trapezoid body and boundaries of the lateral superior olive visible with a dissecting microscope. Electrodes were positioned with a Burleigh piezoelectric (Inchworm) manipulator. Recordings were made from MNTB and LSO using 40–120 MΩ microelectrodes filled with 4 M potassium acetate. Both extracellular and intracellular recordings were obtained, but extracellular recordings were more frequently used because of their stability over long time periods during bath application of chemicals. An Axoprobe-1A microelectrode amplifier was employed for all recordings. Electrical potentials were stored with a Nicolet Benchtop Waveform Acquisition System 400 and plotted later for analysis.

Two bipolar tungsten electrodes were placed on the trapezoid body, one on the midline and one at a point just lateral to the LSO. Cells in the SOC were stimulated by a current pulse applied contralaterally through the midline electrode or ipsilaterally through the laterally placed electrode as indicated in Figure 1. Paired ipsilateral and contralateral current pulses were applied simultaneously. The duration of the stimulating pulse was 0.1 msec.

Results

Physiological properties

Physiological response properties were studied in 97 MNTB and 121 LSO neurons in 49 brain slice preparations. In MNTB, contralateral stimulation of the trapezoid body elicited extracellular spikes with very short latencies (0.4–0.6 msec), but ipsilateral stimulation was without effect. Neurons in LSO usually responded to ipsilateral stimulation with a relatively long-latency spike (0.5–2.0 msec), but sometimes also responded to contralateral stimulation. Twenty-three percent (28 of 121) of LSO neurons had excitatory responses to both ipsilateral and contralateral stimulation, and 7% (9 of 121) responded only to contralateral stimulation. The remaining 70% had excitatory responses to ipsilateral stimulation only. Regardless of whether contralateral responses are interpreted as a direct orthodromic influence or antidromic activation of axon collaterals (Wu and Kelly, 1991), their latencies were short and similar to those of ipsilateral responses. There were no differences in tne effects of EAA agonists or antagonists on the ipsilateral and contralateral responses in LSO. Occasionally, responses with very long latency (more than 2 msec) were evoked from MNTB or LSO. These responses were almost certainly multisynaptic, but the circuitry involved is unknown. These responses were not included in our final data analysis.

EAA agonists in MNTB

The effects of EAA agonists QA, KA, L-glutamate, and NMDA were tested on 26 cells in MNTB. QA and KA caused spontaneous firing at relatively low concentrations and eliminated both spontaneous and evoked responses at higher concentrations. The blocking effect was likely caused by depolarization of the cell membrane through activation of ion channels. Glutamate

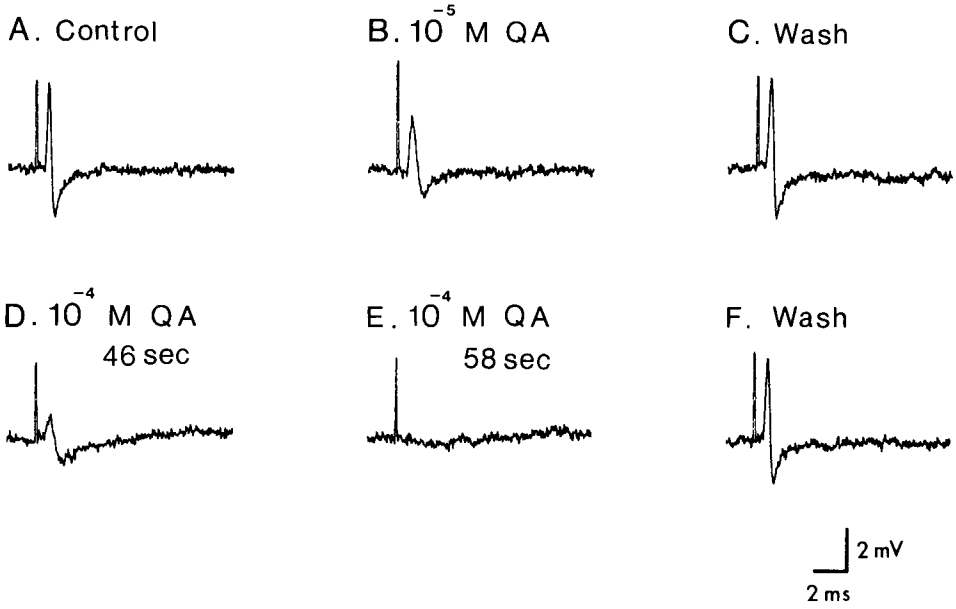

Figure 2. The effects of QA on a single MNTB neuron. *A*, A typical extracellular response of the cell in normal saline to stimulation of the midline trapezoid body. *B*, Application of 10 μM QA reduced the amplitude of the response relative to control. *C*, The response fully recovered after a wash with normal saline. *D*, Application of 100 μM QA for 46 sec further reduced the amplitude of the response to about one-third of control. *E*, Application of 100 μM QA for 58 sec completely abolished the response. *F*, The response fully recovered following normal saline wash.

had similar effects but the threshold concentration was variable from cell to cell. NMDA was ineffective at low concentrations but had some effect on the waveform and latency of action potentials at higher concentrations.

The detailed effects of QA are illustrated in Figure 2. Under control conditions, a current pulse delivered to the contralateral trapezoid body produced a single, short-latency action potential. Introduction of 10 μM QA reduced the amplitude of the action potential to about 65% of the control value. A 100 μM solution initially reduced response amplitude to about 30% and eventually abolished the response altogether. The response was fully restored after return to normal saline.

Figure 3 shows the effect of KA on the same neuron. A 10 μM concentration of KA reduced the amplitude of the response to about 70% of control. A 100 μM solution quickly abolished the response, which did not fully return to normal even after washing with saline for 1 hr. Following 100 μM KA, no further responses could be obtained from the SOC, suggesting that the entire slice had been permanently affected by KA. It seems likely that the lack of responsiveness was due to a widespread depolarization block of neurons in the slice and was possibly a reflection of the well-known excitotoxicity of KA.

Figure 4 shows the effect of NMDA on an MNTB neuron. The contralaterally elicited response was completely unaffected by a 100 μM concentration of NMDA. Similar results were obtained from 12 other MNTB neurons with concentrations of NMDA between 50 and 100 μM. Because NMDA receptors are known to be blocked by Mg^{2+}, 4 of the 12 cells were tested in a 0 Mg^{2+} saline solution. Even under these conditions, 100 μM NMDA had no effect on evoked responses in MNTB. Occasionally, NMDA at these concentrations increased spontaneous dischages. With a 1 mM concentration of NMDA there was some reduction in response amplitude in one MNTB neuron. For this particular neuron, the same effect was obtained with NMDA in either a normal or 0 Mg^{2+} saline solution.

The effect of L-glutamate was tested in five neurons with concentrations ranging from 0.1 to 5.0 mM. Although the nature of the effect was similar to that produced by QA and KA, the critical concentration was quite variable from cell to cell. At relatively low concentrations, glutamate caused spontaneous fir-

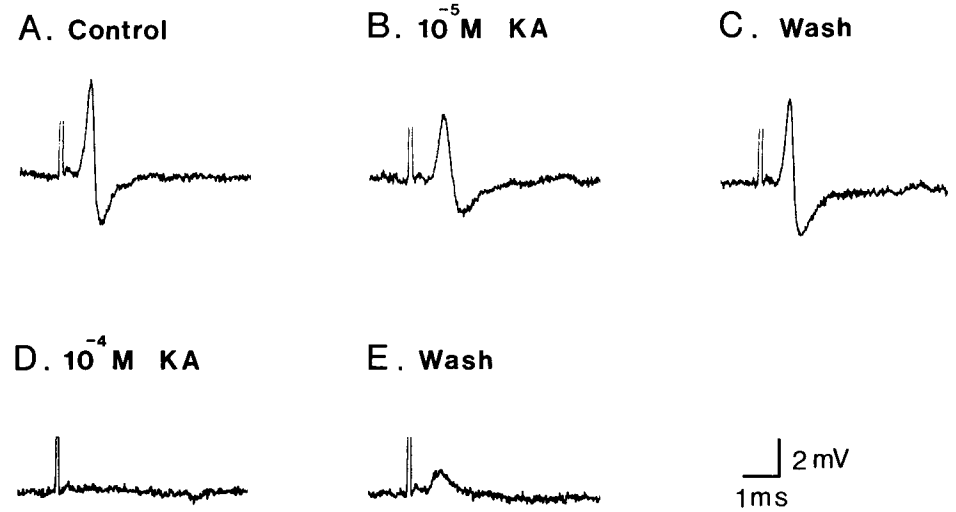

Figure 3. The effects of KA on an MNTB cell. *A*, The extracellular response of the MNTB cell in normal saline to stimulation of the midline trapezoid body. *B*, Slight reduction in amplitude following application of 10 μM KA. *C*, Return to normal saline. *D*, Complete elimination of response following 100 μM KA. *E*, Partial return of response after saline wash. This response never fully returned to normal even after prolonged wash in saline solution.

ing and reduced spike amplitude. At higher concentrations, the response was completely eliminated. The lowest effective concentration was 0.5 mM, but responses of some MNTB neurons were not completely blocked even at a concentration of 5 mM.

EAA antagonists in MNTB

Sixty-six MNTB neurons were tested with either the non-NMDA antagonists CNQX and DNQX, an NMDA antagonist, APV, or a nonspecific EAA antagonist, kynurenic acid. In general, both CNQX and DNQX at very low concentrations completely blocked contralaterally elicited spikes. Kynurenic acid was also a very effective blocker. APV had no blocking effect even at relatively high concentrations. A summary of the effects of all antagonists is provided in Table 1.

Responses in MNTB typically took the form of a single short-latency spike elicited by contralateral stimulation. Occasionally, the spike was accompanied by an earlier potential, the amplitude of which depended upon recording conditions. It is likely that the presence or size of the early potential was related to the position of the electrode tip relative to axon terminals and cell bodies in MNTB. An example of a neuron exhibiting this response pattern is shown in Figure 5. Initially during the course of recording from this cell, the early potential was quite large, larger in fact than the subsequent spike. The latency of the first potential was only about 0.2 msec, whereas the latency of the spike was closer to 1.0 msec. Over the next few minutes, the amplitude of the second potential became larger and the recording became more stable. Finally, the response settled into a constant waveform with the earlier potential still present but at reduced amplitude relative to the now more prominent spike. The form and latency of this response suggested that the early component was a presynaptic event comparable to the "prepotential" reported in *in vivo* studies of MNTB neurons.

The effects of EAA antagonists on a neuron with early and late response components are shown in Figure 6. Application of 5 μM CNQX completely blocked the contralaterally evoked spike in this MNTB neuron, but had no effect on the early potential. In contrast, 100 μM APV did not affect either response component. Perfusion with a saline solution lacking Ca^{2+} totally

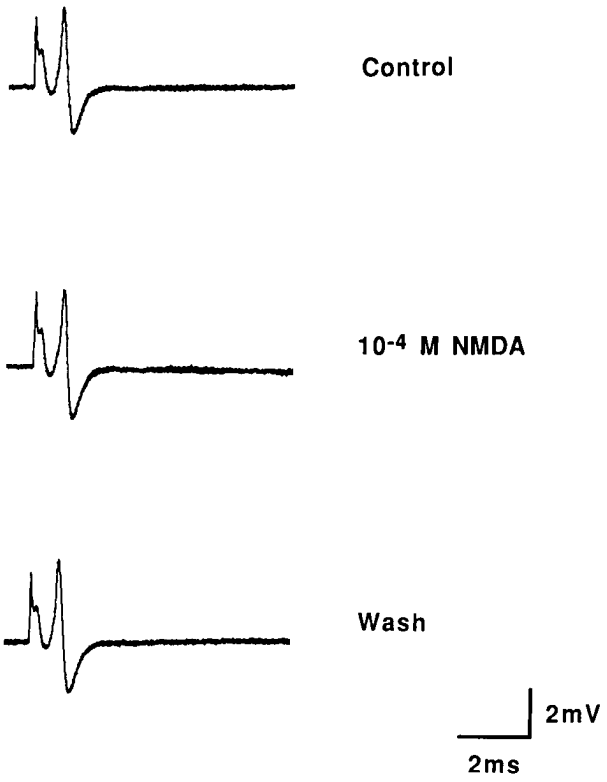

Figure 4. The absence of effect of NMDA on evoked response from an MNTB cell. The *top trace* shows the extracellular response of an MNTB cell to stimulation of the midline trapezoid body. The *middle trace* demonstrates the complete lack of effect after application of 100 μM NMDA for 4 min. The *bottom trace* shows that the response was not changed after washing with normal saline. Each trace is based on the average of 10 individual responses to ipsilateral stimulation.

abolished the spike but had no effect on the earlier potential. These data demonstrate that the early response component derives from a presynaptic source whereas the longer-latency spike is a postsynaptic event mediated by non-NMDA receptor mechanisms.

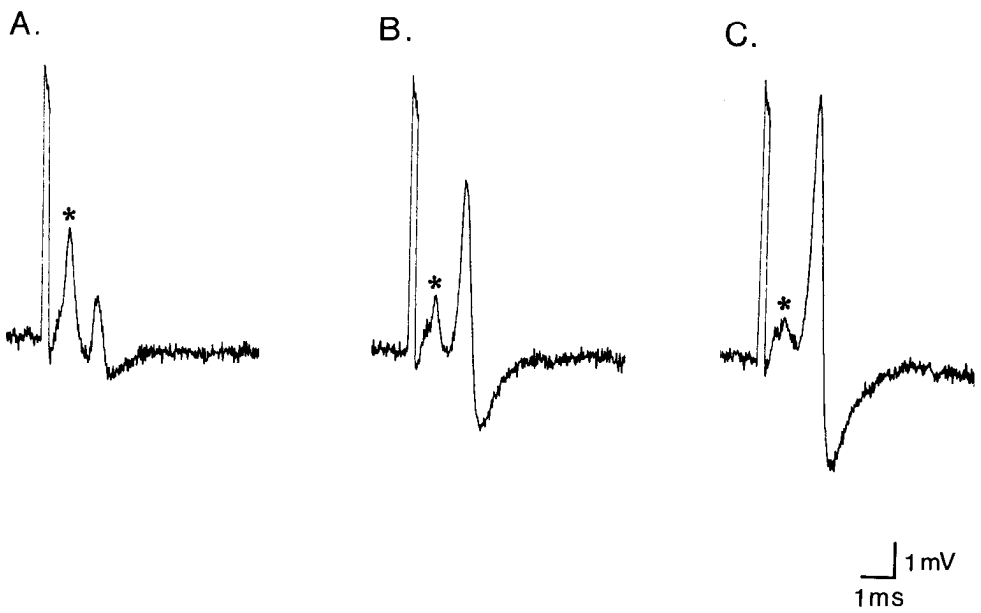

Figure 5. Pre- and postsynaptic responses in MNTB. *A–C* illustrate a sequence of extracellular responses recorded at different times from a single MNTB cell to stimulation of the midline trapezoid body. The response consisted of short- and long-latency potentials that were identified as pre- and postsynaptic events. The presynaptic potential is indicated by an *asterisk* and is followed immediately by the postsynaptic response. Initially the prepotential was larger than the postsynaptic response (*A*). Rapidly, the prepotential became smaller until the postsynaptic response, a typical biphasic action potential, dominated the recording (*B* and *C*). Pre- and postsynaptic components could be identified in many, but not all, of the responses recorded from MNTB.

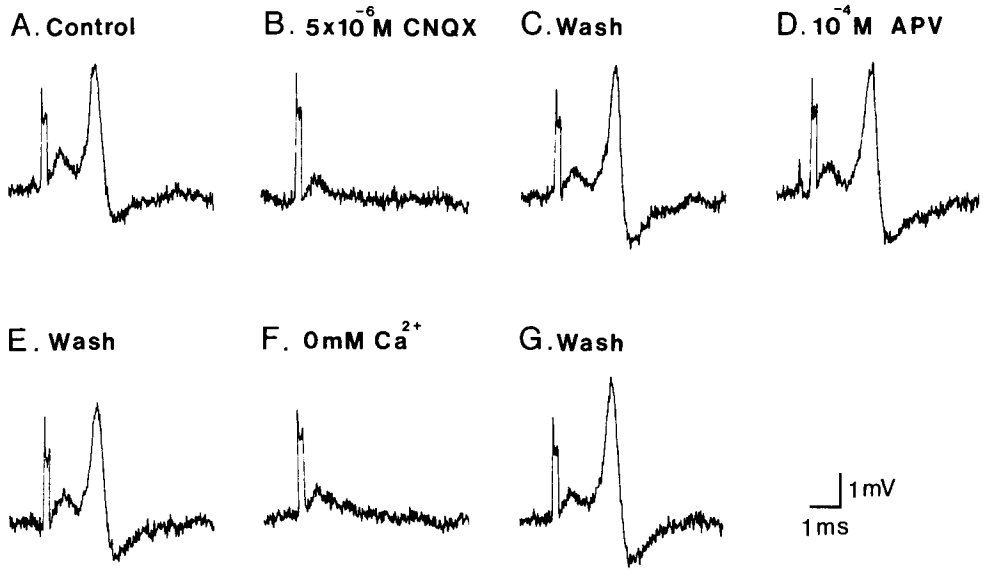

Figure 6. The effects of CNQX and APV on the response of an MNTB cell with clearly identified pre- and postsynaptic components. *A*, The extracellular response in normal saline consisted of a prepotential followed by a postsynaptic spike. *B*, Bath application of 5 μM CNQX completely blocked the postsynaptic response, but did not affect the prepotential. *C*, The postsynaptic response was fully restored following return to normal saline. *D*, Application of 100 μM APV had no effect on either pre- or postsynaptic responses. *E*, Both pre- and postsynaptic responses were unchanged after washing with normal saline. *F*, The postsynaptic response was eliminated but the prepotential was unaffected by saline solution containing 0 Ca^{2+}. *G*, The postsynaptic response fully recovered following return to normal saline.

Figure 7 shows the effects of 5 μM CNQX and 100 μM APV on the contralateral response of an MNTB neuron without a presynaptic potential. After 83 sec of perfusion with 5 μM CNQX, the latency of the response was increased and the amplitude of the response was reduced slightly. Within the next 8 sec, the response was completely blocked. After washing with normal saline, the response quickly returned to normal. A solution of 100 μM APV had no effect on the response of this neuron.

The NMDA antagonist APV was completely without effect on contralaterally elicited responses in MNTB with solutions prepared in normal saline. However, since NMDA receptors are known to be selectively blocked in a voltage-dependent manner by Mg^{2+}, we were concerned that tests in normal saline may have reduced the probability of revealing NMDA responses. Therefore, we conducted a parallel series of tests in which the brain slice was immersed in 0 Mg^{2+} saline. The results were identical to those obtained with the 1.3 mM Mg^{2+} solution. Regardless of Mg^{2+} content, solutions containing 5–10 μM CNQX always completely blocked contralaterally elicited spikes in MNTB (with 20 cells tested in normal saline and 10 cells tested in 0 Mg^{2+}). A 1.0 μM CNQX solution had the effect of increasing response latency in some cases. In contrast, APV in 50–100 μM solutions never had an effect (with 12 cells tested in normal saline and 10 cells tested in 0 Mg^{2+}).

Some of the cells in MNTB exhibited very stable responses that allowed testing with several pharmacological substances in sequence (as illustrated in Figs. 6, 7). Sixteen MNTB cells were tested with both CNQX at 5 μM and APV at 50–100 μM. Regardless of whether testing was conducted in normal saline or 0 Mg^{2+} solution, the responses of all 16 of these neurons were completely blocked by CNQX and totally unaffected by APV.

Eight MNTB neurons were tested with 1–20 μM DNQX, a non-NMDA receptor antagonist. The effects were similar to those seen with CNQX. Response latencies were increased shortly after application of DNQX, and continued administration at concentrations of 10–20 μM blocked the responses completely in six of the eight cells. In one cell, both 5 and 10 μM DNQX produced an increase in response latency without completely eliminating the spike, and in another 10 μM DNQX failed to produce an effect. Both cells were lost before they could be tested at higher concentrations. In all cells blocked by DNQX the responses were completely restored by washing the slice with normal saline.

Kynurenic acid, a nonspecific EAA antagonist, clearly blocked

Table 1. Antagonism of excitatory responses in LSO and MNTB: number of cells examined

	Normal saline						0 Mg^{2+} Saline					
	LSO			MNTB			LSO			MNTB		
Antagonist	Total block	No block	Partial block[a]	Total block	No block	Partial block[a]	Total block	No block	Partial block[a]	Total block	No block	Partial block[a]
CNQX (5–10 μM)	16	3	0	20	0	0	15	2	0	10	0	0
DNQX (10–20 μM)	4	0	0	6	2	0	–	–	–	–	–	–
APV (50–100 μM)	1	12	1	0	12	0	2	18	1	0	10	0
Kynurenic acid (100–200 μM)	3	0	0	4	2	0	–	–	–	–	–	–

[a] Partial block denotes a reduced probability of response and/or an increase in response latency.

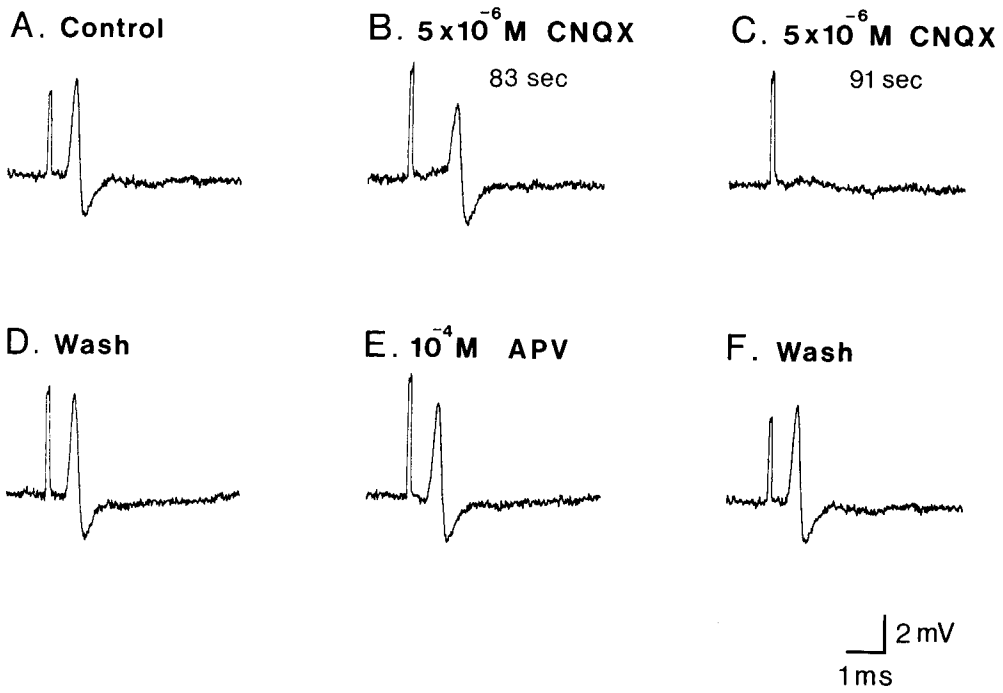

Figure 7. The effects of CNQX and APV on a typical MNTB cell. *A*, The extracellular response of an MNTB neuron to stimulation of the trapezoid body at midline revealed a postsynaptic spike without a prepotential. Spike latency was about 0.5 msec. *B*, Application of 5 μM CNQX for 83 sec caused the latency of the response to increase to about 1 msec. The amplitude of the response was also slightly reduced. *C*, Application of CNQX for 91 sec completely blocked the response. *D*, The response was fully restored by a wash with normal saline. *E*, Application of 100 μM APV had no effect on the response. *F*, The response was unaltered after wash with normal saline.

contralateral excitation in four out of six MNTB neurons tested with concentrations between 100 μM and 1 mM. The nature of the effect of kynurenic acid was similar to that of CNQX and DNQX, that is, an increased latency followed by the complete elimination of the contralaterally elicited spike. Two neurons were not blocked by kynurenic acid. The first was tested with only a 100 μM solution, which failed to produce an effect, but was lost before further testing could be conducted. The second was tested with a 200 μM concentration, which increased response latency but failed to produce a complete block. The cell was not tested with higher concentrations.

EAA agonists in LSO

The effects of EAA agonists were tested in 14 LSO neurons. Both non-NMDA agonists, QA and KA, and the nonspecific EAA agonist, L-glutamate, caused spontaneous firing at relatively low concentrations. At higher concentrations, evoked action potentials were reduced in amplitude and eventually eliminated altogether. The effective concentrations were 10–100 μM for QA, 10–100 μM for KA, and 0.1–5.0 mM for glutamate. Among the three EAA agonists, KA had the most potent effect, resulting in an irreversible cessation of neural activity at high concentrations. The effective concentration of glutamate varied substantially from cell to cell. The effects of NMDA were tested in 12 neurons, 4 with 0 Mg^{2+} solution and 8 with normal saline. NMDA (100 μM) had little immediate effect on spontaneous or evoked responses in LSO, but did cause spontaneous firing, reduction of spike amplitude, or elimination of spikes in some cells with continued application. Specifically, spontaneous activity was increased in several cells, spike amplitude was reduced in two cells, and the evoked response was eliminated in one cell after 5 min of exposure to NMDA.

EAA antagonists in LSO

The effects of CNQX, DNQX, and kynurenic acid on LSO neurons were similar to those reported for MNTB neurons. CNQX, at 5–10 μM concentrations, completely blocked ipsilateral responses in 16 of 19 neurons tested in normal saline and 15 of 17 tested in 0 Mg^{2+} saline. Two of the five neurons whose responses were unaffected by 5–10 μM CNQX were affected at higher concentrations. Solutions of 20 μM and 40 μM CNQX abolished excitatory responses in these neurons. Kynurenic acid at 100–200 μM blocked ipsilateral responses in all three LSO neurons tested.

The NMDA antagonist APV (50–100 μM) had no effect on ipsilateral responses of most LSO neurons tested. Negative results were obtained in 12 of 14 neurons tested with normal saline and 18 of 21 neurons tested with 0 mM Mg^{2+} saline. However, unlike MNTB neurons, a small percentage of LSO neurons (5 of 35 cells) were influenced by APV. Figure 8 shows an example of an LSO neuron whose response was completely eliminated by both 100 μM APV (in normal saline) and 5 μM CNQX (in 0 Mg^{2+}). A solution of 100 μM APV increased response latencies in an additional neuron tested in normal saline, and 100 μM APV in 0 Mg^{2+} saline blocked responses in another two neurons. One other LSO neuron was partially blocked by 100 μM APV in 0 Mg^{2+}.

Twenty-two LSO cells were tested with both CNQX at 5 μM and APV at 50–100 μM. Sixteen of these LSO cells were blocked by CNQX but were unaffected by APV. Of the remaining six cells, two were blocked by 100 μM APV as well as by CNQX at 5 μM and 40 μM, respectively. In two other cells, 100 μM APV produced increased response latencies. These two cells were also blocked by 5 μM CNQX in 0 Mg^{2+}. Two cells were unaffected by either 100 μM APV or 5 μM CNQX. Thus, all neurons that were blocked by APV were also blocked by CNQX at concentrations between 5 and 40 μM.

Agonist and antagonist of inhibition in LSO

In our previous study of intracellular potentials in the mouse brain slice, we found that contralateral IPSPs in LSO were blocked by strychnine, a well-known glycine antagonist, suggesting that glycine plays a major role in mediating contralateral inhibition in SOC (Wu and Kelly, 1991). To investigate this possibility

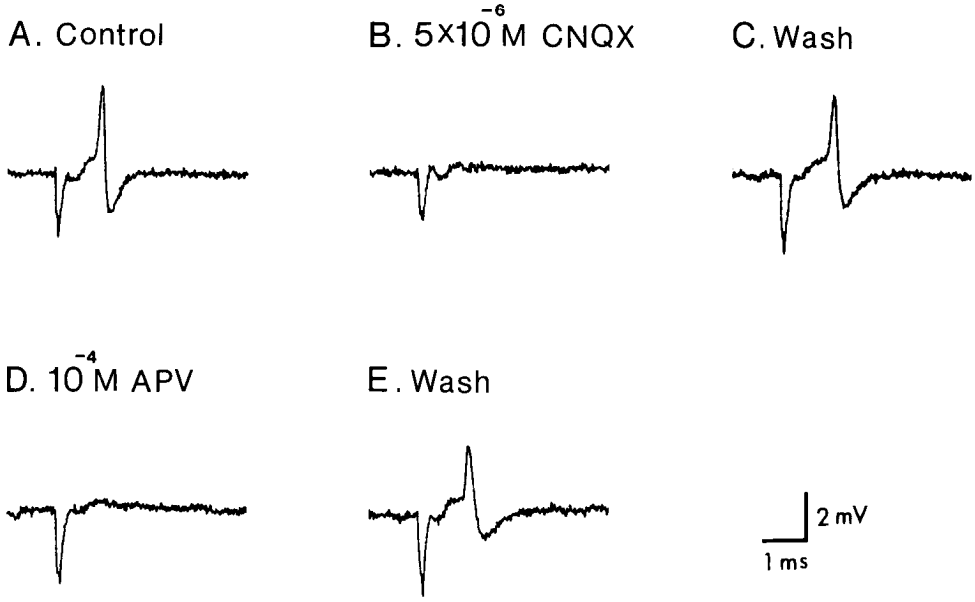

Figure 8. The effects of CNQX and APV on a single LSO neuron. *A*, The extracellular response of the LSO neuron in normal saline was characterized by a single biphasic spike elicited by electrical stimulation lateral to the SOC. *B*, The response was completely blocked by an application of 5 μM CNQX in 0 Mg^{2+} saline. *C*, The response was restored by wash with normal saline. *D*, Application of 100 μM APV blocked the response in this neuron. *E*, The response recovered following wash with normal saline. Blocking effects by APV were unusual among LSO neurons. Most LSO neurons were unaffected by APV.

further, in the present study we examined the effects of glycine itself and its antagonist, strychnine, on inhibition in LSO. Since most of the data were derived from extracellular recordings in which IPSPs could not be observed directly, the presence of inhibition was inferred from the effects of combined stimulation of the ipsilateral and contralateral trapezoid body. Many LSO neurons that were excited by ipsilateral stimulation were completely suppressed by simultaneous contralateral stimulation. The suppression of ipsilaterally elicited spikes in LSO was taken as evidence of contralateral inhibition.

The effect of glycine on the ipsilaterally produced action potential of an LSO neuron is illustrated in Figure 9. Four minutes after application of 5 mM glycine, the amplitude of the evoked spike was reduced to about 40% of the control value. Continuous perfusion with glycine for another half minute completely abolished the response. The response recovered completely after washing with normal saline. Similar results were obtained in four additional LSO neurons.

The effect of strychnine on contralateral inhibition was examined in six LSO neurons. Inhibition was blocked by a 0.5 μM solution in every cell tested. A typical example of the effect of 0.5 μM strychnine is shown in Figure 10. In this neuron, ipsilateral stimulation alone generated a single spike and simultaneous contralateral stimulation produced complete suppression of the response. Application of strychnine completely restored the ipsilateral spike. Recovery from the effect of strychnine on this and other LSO cells was prolonged. As in the case of neurons in the cochlear nucleus, at least 30 min were needed to reverse the effects of strychnine by washing in normal saline (Wu and Oertel, 1986). Because of the extended time period involved, full recovery was not documented for most neurons tested with strychnine.

Effect of NMDA on contralateral inhibition in LSO

Although 100 μM NMDA had relatively little effect on excitatory responses in MNTB or LSO, the same concentration completely blocked contralateral inhibition of LSO neurons. This effect was apparently independent of any action of NMDA on excitatory synapses in SOC. The time course over which NMDA affected excitation and inhibition was different. Usually the suppression of LSO neurons was completely reversed by NMDA within 1–2 min, whereas the effect of NMDA on excitatory responses became apparent only after at least 5 min of continuous perfusion. NMDA consistently and completely blocked contralateral inhibition in every cell tested (12 cells), but at the same concentration had only inconsistent effects on excitation. Examples of the effect of NMDA on contralateral inhibition are shown in Figures 11–13.

The LSO neuron shown in Figure 11 responded to ipsilateral stimulation with a single extracellular spike that was totally suppressed by simultaneous contralateral stimulation. A 100 μM solution of NMDA in 0 Mg^{2+} resulted in the complete restoration of the spike. The spike could not be inhibited in the presence of NMDA even when the intensity of contralateral stimulation was increased. After washing the slice with normal saline, the ipsilateral response was blocked again by contralateral stimulation. An identical effect on extracellular responses was obtained for four other LSO neurons tested with 100 μM NMDA. One of these cells was tested with NMDA in normal saline, and three were tested with NMDA in a 0 Mg^{2+} solution. In one cell (tested in 0 Mg^{2+}), a 50 μM solution of NMDA produced the same result as a 100 μM solution.

Intracellular data were obtained from seven additional neurons in LSO. Three neurons were tested with 100 μM, and four were tested with 50 μM NMDA in normal saline. The selective effect of NMDA on contralateral inhibitory potentials in LSO is illustrated in Figure 12. An intracellular recording was made from an LSO neuron that responded with an EPSP to ipsilateral stimulation and an IPSP to contralateral stimulation. The ipsilateral stimulus intensity was set to a subthreshold value so that the EPSP was not accompanied by an action potential. The latency of the IPSP was somewhat longer and that of the EPSP somewhat shorter than usually found among LSO neurons (Wu and Kelly, 1991), but otherwise the response was typical. Introduction of a 100 μM solution of NMDA completely blocked the IPSP, but had no effect on the EPSP. After washing with normal saline the IPSP was fully restored.

A second example is shown in Figure 13. For this neuron,

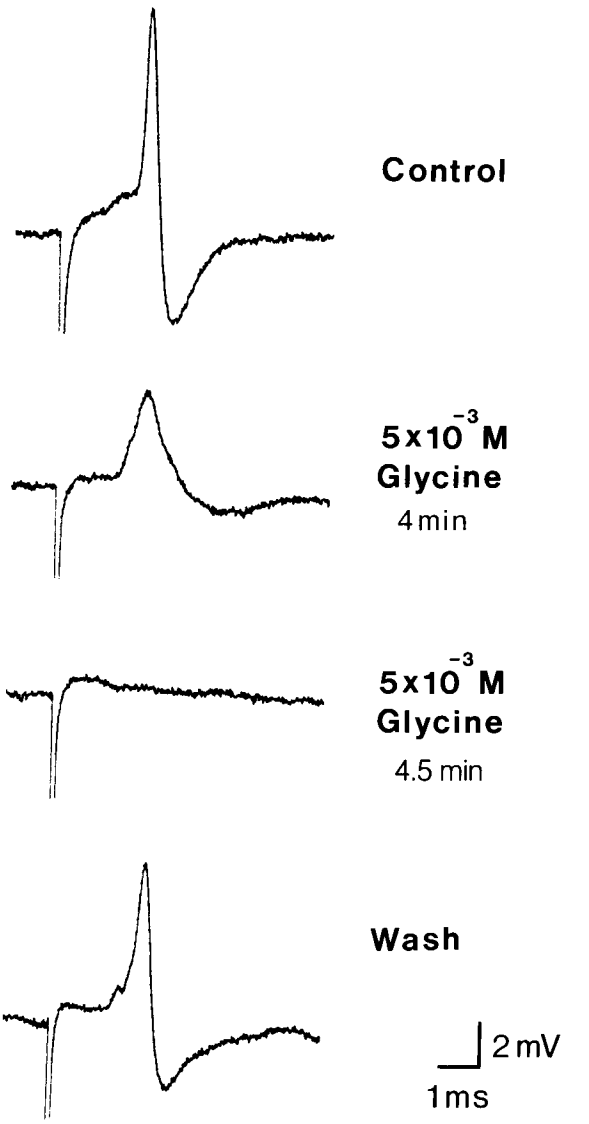

Figure 9. The effect of glycine on an LSO neuron. The *top trace* illustrates the extracellular spike elicited by stimulation of the ipsilateral trapezoid body. The *second trace* shows a reduction in amplitude of the spike after application of 5 mM glycine for 4 min. The *third trace* shows complete elimination of the spike after continued application of 5 mM glycine for 4.5 min. The *bottom trace* shows restoration of the response following normal saline wash.

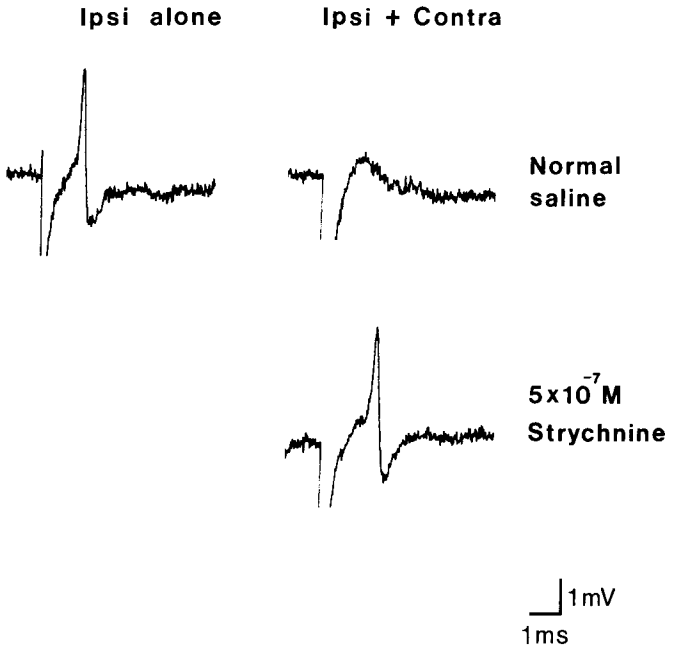

Figure 10. The effect of strychnine on contralateral inhibition in an LSO cell. The *upper left trace* illustrates the extracellular response of an LSO cell to stimulation of the ipsilateral trapezoid body. The *upper right trace* shows that the response evoked by ipsilateral stimulation was blocked by simultaneous stimulation of the contralateral trapezoid body. The *bottom trace* demonstrates the complete restoration of the response following application of 0.5 μM strychnine. Strychnine eliminated the suppression produced by contralateral stimulation in every cell tested.

the latencies of the ipsilateral EPSP and the contralateral IPSP were similar. Ipsilateral stimulation was capable of eliciting an action potential, but for critical tests the intensity was set below threshold so that the EPSP was not accompanied by a spike. Application of 100 μM NMDA rapidly blocked the IPSP but had no obvious effect on the EPSP. This result further demonstrates the selective effect of NMDA on inhibitory potentials in LSO. A similar selectivity was found in each of the seven neurons tested in this manner.

Discussion

Overview of results

The present results indicate that glutamate, or a closely related EAA, serves as a neurotransmitter mediating excitatory responses in both LSO and MNTB. Glutamate and the glutamate agonists KA and QA activate neurons in both LSO and MNTB and, at sufficiently high concentrations, eliminate neural responses altogether, probably by opening ion channels and depolarizing the cell membrane. Furthermore, the EAA antagonists CNQX, DNQX, and kynurenic acid block excitatory responses in both LSO and MNTB. Since both CNQX and DNQX at low concentrations are thought to have their action specifically on non-NMDA receptor types, it seems likely that excitatory responses in LSO and MNTB are mediated largely by non-NMDA receptors. The glutamate agonist NMDA has relatively little effect on neural responses in either MNTB or LSO. The NMDA anatagonist APV fails to block responses in MNTB and blocks only a small proportion of excitatory responses in LSO. The lack of action of APV contrasts sharply with the highly consistent effect of the non-NMDA antagonists CNQX and DNQX.

Our data support the conclusion that contralateral inhibition in LSO is mediated by glycine (Zarbin et al., 1981; Moore and Caspary, 1983; Wenthold and Martin, 1985; Sanes et al., 1987; Wenthold et al., 1987; Wu and Kelly, 1991). In the mouse brain slice, LSO neurons are affected by glycine. Furthermore, ipsilateral excitatory responses that are normally blocked by simultaneous contralateral stimulation of the trapezoid body are fully restored by the glycine antagonist strychnine. These results add weight to accumulating evidence that contralateral inhibition in LSO is mediated by strychnine-sensitive glycine receptors.

Finally, NMDA blocks contralateral inhibition in LSO, an effect that cannot be explained by the influence of NMDA on excitatory responses. The disinhibition cannot be attributed to a depolarization block of cells in MNTB because depolarization

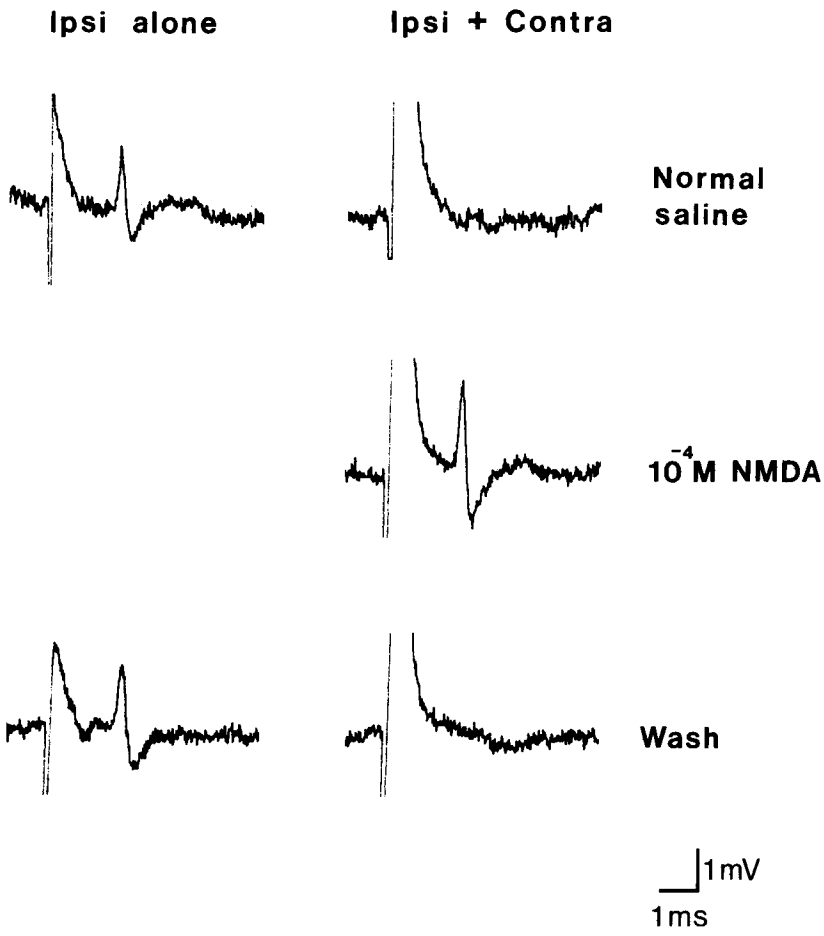

Figure 11. The effect of NMDA on contralateral inhibition in LSO. The *upper left trace* illustrates the extracellular response of an LSO cell to ipsilateral stimulation of the trapezoid body. The *upper right trace* shows the complete suppression of the response by simultaneous stimulation of the contralateral trapezoid body. Note the increased stimulus artifact associated with combined ipsilateral and contralateral stimulation. The *middle trace* demonstrates release of the response from contralateral suppression following application of 100 μM NMDA in 0 Mg^{2+} saline for 1.5 min. The inhibition was blocked over the full range of contralateral stimulus intensities. The shape and amplitude of the ipsilateral response were unchanged. The *bottom left trace* shows the effect of ipsilateral stimulation alone, and the *bottom right trace* shows complete restoration of the contralateral suppression effect following wash in normal saline.

block does not occur at these concentrations. It cannot be attributed to increased ipsilateral excitation in LSO because elimination of contralateral inhibition occurs more rapidly and more consistently than any effect of NMDA on excitation in LSO. Furthermore, intracellular data demonstrate that NMDA can eliminate contralateral IPSPs in LSO without affecting ipsilateral EPSPs. Thus, it seems likely that the effect of NMDA on SOC neurons is specific to inhibition in LSO. These results suggest that the NMDA receptor plays a role in binaural processing by modulating contralateral inhibition, possibly through

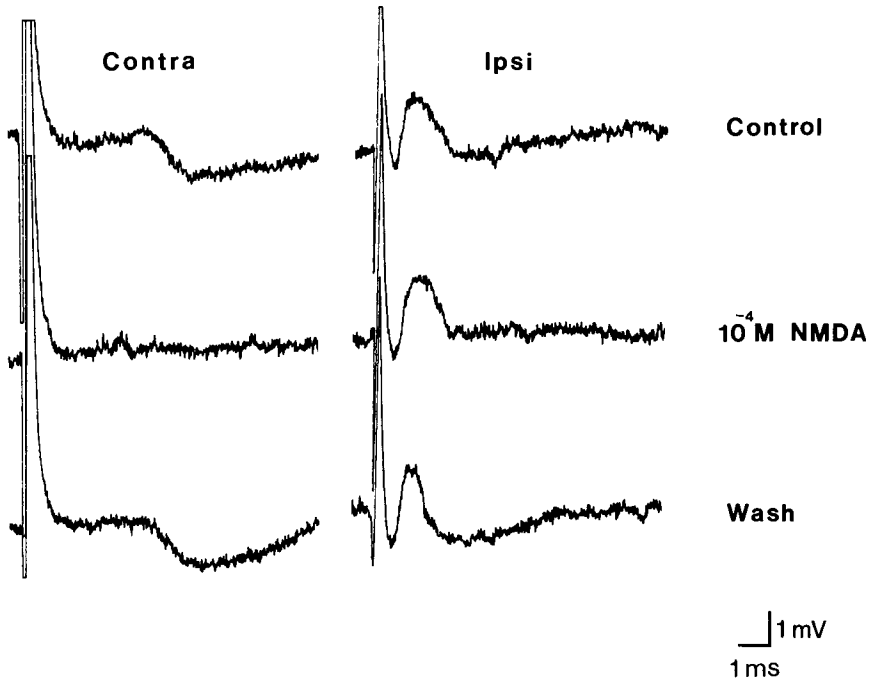

Figure 12. The effect of NMDA on contralateral inhibition in LSO revealed by intracellular recordings from an LSO neuron. The *upper traces* (*left* and *right*, respectively) illustrate the IPSP produced by contralateral stimulation and the EPSP produced by ipsilateral stimulation of the trapezoid body under normal saline conditions. The *middle traces* show the complete elimination of the IPSP and the total lack of effect on the EPSP following application of 100 μM NMDA. A complete blocking effect was evident after 1 min, but the waveforms illustrated here were obtained after 3 min in NMDA. The *bottom traces* show responses following normal saline wash. Full recovery of the IPSP is apparent. The membrane potential for these recordings was between -50 and -55 mV.

interaction with glycine receptors on LSO neurons. The NMDA receptor could provide a mechanism for regulating binaural balance in LSO, the maintenance of which is important for the normal processing of cues involved in sound localization.

EAA mediates excitation in MNTB through non-NMDA receptors

The principal cells in MNTB are innervated by bushy/globular cells located in the contralateral anteroventral cochlear nucleus (AVCN). These cells send large-diameter axons through the trapezoid body to form highly specialized synaptic terminals, the calyces of Held, on neurons in MNTB (Harrison and Irving, 1966; Lenn and Reese, 1966; Morest, 1968; Warr, 1972, 1982; Elverland, 1978). Very little is known, however, regarding the identity of the neurotransmitter at this synapse. Immunocytochemical studies have shown an intense glutamate-like immunoreactivity in large-diameter fibers that cross the midline ventrally in the trapezoid body and in axon terminals located on principal cells in MNTB. These glutamate-like positive fibers and endings likely originate from bushy/globular cells in AVCN (Ottersen and Storm-Mathisen, 1984). Recent immunocytochemical studies using antibodies to glutaraldehyde conjugated with aspartate or glutamate demonstrate aspartate-like or glutamate-like immunoreactivity among neurons in the ventral cochlear nucleus, providing further evidence that MNTB afferents may utilize one or another of the EAAs as a neurotransmitter (Aoki et al., 1987; Madl et al., 1987). Also, Caspary and Finlayson (1991) have provided preliminary physiological evidence that MNTB neurons are sensitive to agonists and antagonists of EAA. These data are consistent with our results showing that MNTB neurons are strongly affected by EAA agonists and antagonists, particularly those acting upon non-NMDA receptor types.

The suggestion made here that excitation in MNTB is mediated by non-NMDA receptors is consistent with the rapid and efficient conduction of sensory information provided by MNTB synapses. Both the morphology (Lenn and Reese, 1966; Morest, 1968; Nakajima, 1971; Jean-Baptiste and Morest, 1975) and extracellular physiology (Goldberg and Brown, 1968; Guinan et al., 1972a,b) of MNTB neurons indicate that the MNTB synapse is specialized for rapid conduction. Also, intracellular recordings from MNTB cells in mouse brain slice reveal nonlinear current–voltage relations and other membrane characteristics that promote a secure and rapid relay between AVCN and LSO (Wu and Kelly, 1991). Studies of other neural systems suggest that fast excitatory monosynaptic events, such as those seen in MNTB, are mediated by non-NMDA receptors, whereas NMDA receptors are involved with slower synaptic processes (Fagg and Foster, 1983; Fagg et al., 1986; Jessell et al., 1986; Langdon and Freeman, 1986; Brodin et al., 1987; MacDermott and Dale, 1987; Massey and Miller, 1987).

The selective block of contralateral evoked responses in MNTB by the specific non-NMDA antagonists CNQX and DNQX and the corresponding lack of effect by the specific NMDA antagonist APV provide evidence that non-NMDA EAA receptors mediate excitatory synaptic transmission in MNTB (Davies et al., 1981; Honore et al., 1988). Tests with non-NMDA antagonists under conditions of 0 Mg^{2+} confirm the lack of NMDA effects in MNTB. In other neural systems, Mg^{2+} blocks NMDA receptor activity in a voltage-dependent manner (Nowak et al., 1984; Coan and Collingridge, 1985). If any part of the contralateral excitatory response in the MNTB had been mediated by

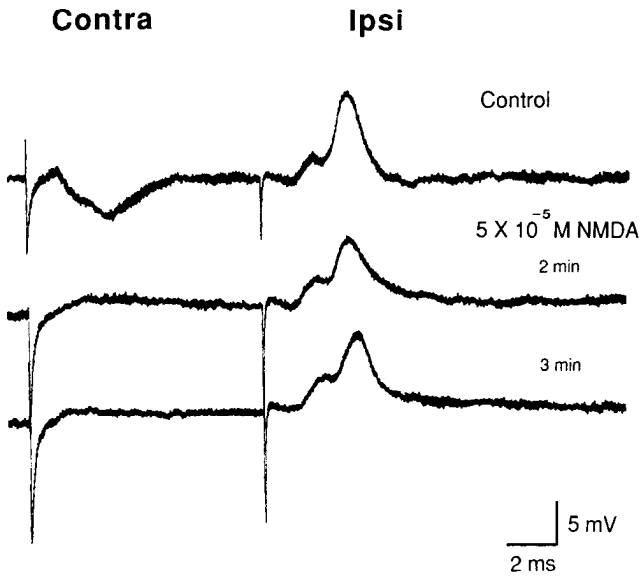

Figure 13. The effect of NMDA on intracellular potentials recorded from a neuron in LSO. Under control conditions (normal saline), contralateral stimulation elicited an IPSP and ipsilateral stimulation elicited an EPSP, as shown in *left* and *right traces*, respectively. Application of 100 μM NMDA rapidly eliminated the IPSP but had no systematic effect on the EPSP. The IPSP remained blocked and the EPSP remained stable for the duration of the recording. The membrane potential for this cell was −51 mV.

NMDA receptors, it should have become apparent in the absence of Mg^{2+}. Under 0 Mg^{2+} conditions, however, there was no evidence of any neural response following application of CNQX. The lack of effect of APV in 0 Mg^{2+} solution further supports the idea that NMDA receptors are not involved in synaptic transmission between contralateral AVCN and MNTB.

The positive effects of the non-NMDA agonists KA and QA, together with the relative lack of effect of NMDA itself, support the conclusion that non-NMDA receptors mediate excitation in MNTB. The fact that non-NMDA agonists caused increased neural activity at low concentrations and reduced and ultimately eliminated responses at higher concentrations is compatible with what is known about the action of EAAs. At threshold concentrations, EAAs typically cause neuronal membrane depolarization and lead to the production of action potentials (Curtis et al., 1960). At higher concentrations, activation of EAA receptors can lead to the cessation of firing by opening nondesensitizing ion channels to produce a complete depolarization block (Barker and Nicoll, 1973; Hosli et al., 1976; Hablitz and Langmoen, 1982). The apparently irreversible effects of KA on MNTB responses may be related to its well-established role as a neurotoxin (Olney, 1978; Rothman, 1985).

In vivo recordings from MNTB have shown that tone-evoked responses are often preceeded by a potential that appears to reflect presynaptic activity associated with the calyces of Held (Li and Guinan, 1971; Guinan et al., 1972a,b). The present *in vitro* study of mouse brain slice has shown that some cells in MNTB have a similar prepotential in response to electrical stimulation of the trapezoid body. This response is not eliminated by a 0 Ca^{2+} saline solution, which is known to block postsynaptic activity, nor is it affected by CNQX or DNQX, both of which exert a powerful blocking effect on action potentials in MNTB neurons. These findings provide direct pharmacological evidence that the prepotentials in MNTB are of presynaptic origin,

probably generated from the calyces of Held, and that the effects of CNQX and DNQX are specific to the postsynaptic component of the response.

EAA mediates ipsilateral excitation in LSO through non-NMDA receptors

The neurotransmitter mediating ipsilateral excitation in LSO is probably an EAA. Immunocytochemical studies have revealed aspartate- and glutamate-like immunoreactivity in the ventral cochlear nucleus, indicating that the neurotransmitter between cochlear nucleus and LSO is an EAA (Madl et al., 1986; Aoki et al., 1987). Receptor binding has provided evidence for the presence of both non-NMDA and NMDA receptor sites on LSO neurons (Baker and Glendenning, 1991). The most pronounced labeling is seen with the non-NMDA antagonist CNQX, but some labeling can also be detected for the NMDA antagonist CGS 19755. Thus, cytochemical evidence suggests that ipsilateral excitation in LSO is mediated by an EAA neurotransmitter acting on non-NMDA receptors with the possible additional involvement of NMDA receptors.

Physiological recordings from LSO neurons *in vivo* provide further evidence that non-NMDA receptors are involved in ipsilateral excitation. Caspary and Faingold (1989) found that iontophoretic application of the non-NMDA agonist QA enhanced both spontaneous and tone-evoked activity in LSO, whereas NMDA was relatively ineffective. Furthermore, the non-NMDA antagonist DNQX reduced ipsilateral tone-evoked and QA-evoked activity. The nonselective EAA antagonist kynurenic acid also effectively blocked ipsilateral excitation. These physiological data indicate that EAA receptors are present in LSO and that an EAA neurotransmitter mediates ipsilateral excitation through non-NMDA receptors (Caspary and Faingold, 1989; Finlayson and Caspary, 1989).

Our results confirm the importance of non-NMDA receptors in LSO. In the mouse brain slice, bath application of the non-NMDA agonists QA and KA as well as of the general agonist L-glutamate excited LSO neurons at low dosages and eliminated excitatory responses at higher concentrations. NMDA had relatively little effect. Furthermore, most ipsilateral excitatory responses in LSO were blocked by the non-NMDA antagonists CNQX or DNQX at very low concentrations, but were unaffected by the NMDA antagonist APV at much higher concentrations. These findings indicate that EAA receptors are present in LSO and that ipsilateral excitation is mediated by glutamate or a glutamate-like neurotransmitter acting primarily on non-NMDA receptor sites.

In the mouse brain slice preparation, the activity of a small percentage of LSO neurons was affected by APV. In most of these cases, ipsilateral excitatory responses were completely blocked by *both* CNQX and APV, suggesting the possibility that NMDA and non-NMDA receptors are present on the same neurons in LSO. In our sample, no cells were blocked exclusively by APV, although some neurons that were sensitive to APV were relatively insensitive to CNQX. Immunocytochemical receptor binding studies have demonstrated the presence of NMDA, as well as non-NMDA, receptors in LSO, but the functional significance of the receptors is not known and the cellular distribution within the nucleus is unclear (Baker and Glendenning, 1991). Whether both non-NMDA and NMDA receptors play a role in mediating ipsilateral excitation in some LSO neurons is uncertain. Several lines of physiological, morphological, and biochemical evidence suggest that multiple neuronal classes exist in LSO (Helfert and Schwartz, 1986, 1987; Irvine, 1986; Saint Marie et al., 1989), but the relation between cell type and the distribution of NMDA and non-NMDA receptors is not known.

Glycine mediates contralateral inhibition in LSO

Both anatomical and physiological data point to glycine as the most likely neurotransmitter mediating contralateral inhibition in LSO. It is now well established that the neural connections from MNTB to LSO serve an inhibitory function (Tsuchitani and Boudreau, 1966, 1969; Rasmussen, 1967; Boudreau and Tsuchitani, 1968; Morest, 1968; Guinan et al., 1972a,b; Borg, 1973; Browner and Webster, 1975; Tsuchitani, 1977; Elverland, 1978; Caird and Klinke, 1983; Glendenning et al., 1985; Irvine, 1986). Recent immunocytochemical studies have shown that MNTB neurons are glycinergic. Both the MNTB principal cells and axons coursing from MNTB to LSO exhibit an intense immunoreactivity to antibodies for glycine. Immunoreactivity is also seen within the LSO as puncta, usually interpreted as terminals of MNTB neurons, surrounding the LSO principal cells (Campistron et al., 1986; Peyret et al., 1987; Wenthold et al., 1987; Aoki et al., 1988; Saint Marie et al., 1989). These bouton endings also show an affinity for ^{3}H-glycine (Schwartz, 1985). Glycine receptors can be identified on LSO principal cells using either a monoclonal antibody or ^{3}H-strychnine as a ligand (Zarbin et al., 1981; Frostholm and Rotter, 1985; Wenthold et al., 1985; Altschuler et al., 1986; Baker et al., 1986; Sanes and Wooten, 1987; Sanes et al., 1987).

Electrophysiological studies support the concept that glycine is an inhibitory neurotransmitter in the LSO. Iontophoretic application of the glycine antagonist strychnine blocks contralateral inhibition recorded from LSO neurons during binaural stimulation, and the application of glycine mimics contralateral inhibition during *in vivo* recordings (Moore and Caspary, 1983; Caspary et al., 1985; Caspary and Finlayson, 1991). Intracellular recordings from LSO neurons in anesthetized chinchilla have shown that IPSPs evoked by contralateral acoustical stimulation are likely associated with increased chloride conductance, a property that is characteristic of glycinergic IPSPs in other systems (Finlayson and Caspary, 1989). In the auditory brain slice preparation, strychnine completely blocks contralaterally evoked IPSPs in LSO, a result that provides further support for the role of glycine as an inhibitory transmitter (Wu and Kelly, 1991). The present finding that very low concentrations of strychnine completely blocked contralateral suppression of extracellular responses in LSO is consistent with our previous intracellular results.

In the present study, the application of glycine itself resulted in a reduced amplitude and ultimately the complete elimination of shock-elicited extracellular spikes, probably due to the prolonged opening of glycine-mediated Cl^- channels (Miller et al., 1981; Barker et al., 1982; Wu and Oertel, 1986; Martin, 1990). These data demonstrate that LSO neurons are sensitive to glycine, and support the suggestion that contralateral inhibition is mediated by glycinergic neurons in MNTB acting on strychnine-sensitive glycine receptors in LSO.

Possible role of NMDA in modulation of binaural responses in LSO

Although NMDA had relatively little effect on excitation in either LSO or MNTB, it did have a powerful blocking effect on contralateral inhibition in LSO. The inhibition of neural re-

sponses produced by contralateral stimulation of the trapezoid body was completely eliminated within 1–2 min after application of 100 μM NMDA in every case tested. The effect was clearly not due to a general disruption of neural activity in the LSO because the extracellular spikes previously suppressed by stimulation of the trapezoid body were completely restored by NMDA. The amplitude and waveform of the spikes appeared normal and unaltered. Furthermore, intracellular recordings from LSO neurons showed that NMDA selectively blocked contralateral IPSPs without affecting ipsilateral EPSPs in the same cell. The disinhibition produced by NMDA cannot be attributed to an enhancement of excitation in LSO or a reduction of excitatory events in MNTB. Although 100 μM NMDA did cause an increase in spontaneous firing in some LSO cells, and altered spike waveforms in others, these effects were apparent only after prolonged application of the drug and cannot in any way account for the rapid and complete blocking action of NMDA on contralateral inhibiton. A 100 μM solution of NMDA never produced depolarization block or reduced excitability of cells in MNTB. Therefore, the effect of NMDA seems specifically related to inhibitory processes in LSO.

The mechanism by which NMDA blocks inhibition in LSO is unknown. There are no published data indicating that NMDA can directly modulate strychnine-dependent glycine receptors. On the other hand, there is no evidence that would rule out this possibility. There are reports of interactions between transmitter substances at other receptor sites that may involve similar processes. For example, glycine greatly enhances NMDA-induced responses in a variety of neurons through strychnine-insensitive glycine receptors at the NMDA receptor site (Johnson and Asher, 1987; Fletcher et al., 1990). Also, Stelzer and Wong (1989) report that $GABA_A$ responses in hippocampal pyramidal cells are enhanced by glutamate, a result that suggests the possibility of interaction through a common postsynaptic mechanism. Immunocytochemical studies have recently shown that glutamate and glycine are colocalized in MNTB cells, raising the possibility that both transmitters are involved in regulating contralateral inhibition in LSO (Helfert et al., 1989; Glendenning et al., 1991). It is also possible that NMDA modulates binaural responses through presynaptic inhibition of glycinergic terminals in LSO.

The modulating influence of NMDA on binaural processing is probably due to factors other than those brought into play by electrical stimulation of the trapezoid body. Since APV has little or no effect on responses elicited by current pulses applied to the trapezoid body, it is likely that NMDA receptors are normally activated by some other mechanism. Neurons other than the primary sensory afferents may be involved. Experiments with local application of NMDA, through either iontophoretic or pressure injection, would be useful to determine the critical circuits for producing NMDA blockade of contralateral inhibition in LSO.

It is not known whether other EAA agonists affect glycineric inhibition in LSO or whether the effect is restricted to NMDA. In our preparation it is impossible to test the effect of QA, KA, or glutamate on contralateral inhibition, because these agents also have profound effects on excitation in MNTB and LSO. Further experiments using other techniques are needed to clarify the possible mechanism of action of NMDA on glycine receptors. However, regardless of the mechanism involved, these data suggest that NMDA receptors play an important role in regulating binaural responses in LSO through modulation of the contralateral inhibitory projection from MNTB.

References

Altschuler RA, Betz H, Parakkal MH, Reeks KA, Wenthold RJ (1986) Identification of glycinergic synapses in the cochlear nucleus through immunocytochemical localization of the postsynaptic receptor. Brain Res 369:316–320.

Aoki E, Semba R, Kato K, Kashiwamata S (1987) Purification of specific antibody against aspartate and immunocytochemical localization of aspartergic neurons in the rat brain. Neuroscience 21:755–765.

Aoki E, Semba R, Keino R, Kato K, Kashiwamata S (1988) Glycine-like immunoreactivity in the rat auditory pathway. Brain Res 442: 63–71.

Baker BN, Glendenning KK (1991) Excitatory amino acid receptors in the auditory brainstem of the cat. Assoc Res Otolaryngol Abstr 14:10.

Baker BN, Glendenning KK, Hodges P (1986) Acoustic chiasm: distribution of glycine and GABA receptors in brainstem auditory nuclei of cat. Assoc Res Otolaryngol Abstr 9:7.

Barker JL, Nicoll RA (1973) The pharmacology and ionic dependency of amino acid responses in the frog spinal cord. J Physiol (Lond) 228: 259–277.

Barker JL, McBurney RN, MacDonald JF (1982) Fluctuation analysis of neutral amino acid responses in cultured mouse spinal neurons. J Physiol (Lond) 322:365–387.

Borg E (1973) A neuroanatomical study of the brainstem auditory system of the rabbit. 1. Ascending connections. Acta Morphol Neerl Scand 11:31–48.

Boudreau JC, Tsuchitani C (1968) Binaural interaction in the cat superior olive S segment. J Neurophysiol 31:442–454.

Boudreau JC, Tsuchitani C (1970) Cat superior olive S-segment cell discharge to tonal stimulation. In: Contributions to sensory physiology, Vol 4 (Neff WD, ed), pp 143–213. New York: Academic.

Brodin L, Christenson J, Grillner S (1987) Single sensory neurons activate excitatory amino acid receptors in the lamprey spinal cord. Neurosci Lett 75:75–79.

Browner RH, Webster DB (1975) Projections of the trapezoid body and the superior olivary complex of the kangaroo rat (*Dipodomys merriami*). Brain Behav Evol 11:322–354.

Caird D, Klinke R (1983) Processing of binaural stimuli by cat superior olivary complex neurons. Exp Brain Res 52:385–399.

Campistron G, Buijs RM, Geffard M (1986) Glycine neurons in the brain and spinal cord. Antibody production and immunocytochemical localization. Brain Res 376:400–405.

Caspary DM, Faingold CL (1989) Non-*N*-methyl-D-aspartate receptors may mediate ipsilateral excitation at lateral superior olivary synapses. Brain Res 503:83–90.

Caspary DM, Finlayson PG (1991) Superior olivary complex: functional neuropharmacology of the principal cell types. In: Neurobiology of hearing, Vol 2 (Altshuler RA, Hoffman DW, Bobbin RP, Clopton B, eds), pp 141–161. New York: Raven.

Caspary DM, Rybak LP, Faingold CL (1985) The effects of inhibitory and excitatory amino acid neurotransmitters on the response properties of brainstem auditory neurons. In: Auditory biochemistry (Drescher DG, ed), pp 198–226. Springfield, IL: Thomas.

Casseday JH, Neff WD (1975) Auditory localization: role of auditory pathways in brainstem of the cat. J Neurophysiol 38:842–858.

Coan EJ, Collingridge GL (1985) Magnesium ions block an *N*-methyl-D-aspartate receptor-mediated component of synaptic transmission in rat hippocampus. Neurosci Lett 53:21–26.

Curtis DR, Phillis JW, Watkins JC (1960) The chemical excitation of spinal neurones by certain acidic amino acids. J Physiol (Lond) 150: 656–682.

Davies J, Francis AA, Jones AW, Watkins JC (1981) 2-Amino-5-phosphonovalerate (2-APV), a potent and selective antagonist of amino acid-induced and synaptic excitation. Neurosci Lett 21:77–81.

Elverland HH (1978) Ascending and intrinsic projections of the superior olivary complex in the cat. Exp Brain Res 32:117–134.

Fagg GE, Foster AC (1983) Amino acid neurotransmitters and their pathways in the mammalian central nervous system. Neuroscience 9:701–719.

Fagg GE, Foster AC, Ganong AH (1986) Excitatory amino acid synaptic mechanisms, neurological function. Trends Pharmacol Sci 7: 357–363.

Finlayson PG, Caspary DM (1989) Synaptic potentials of chinchilla lateral superior olivary neurons. Hearing Res 38:221–228.

Fletcher EJ, Beart PM, Lodge D (1990) Involvement of glycine in excitatory neurotransmission. In: Glycine neurotransmission (Otterson OP, Storm-Mathisen J, eds), pp 193–217. New York: Wiley.
Fonnum F (1984) Glutamate: a neurotransmitter in mammalian brain. J Neurochem 42:1–11.
Frostholm A, Rotter A (1985) Glycine receptor distribution in mouse CNS: autoradiographic localization of [^{3}H] strychnine binding sites. Brain Res Bull 15:473–486.
Glendenning KK, Baker BN (1988) Neuroanatomical distribution of receptors for three potential inhibitory neurotransmitters in the brainstem auditory nuclei of the cat. J Comp Neurol 275:261–285.
Glendenning KK, Hutson KA, Nudo RJ, Masterton RB (1985) Acoustic chiasm II: anatomical basis of binaurality in lateral superior olive of cat. J Comp Neurol 232:261–285.
Glendenning KK, Masterton RB, Baker BN, Wenthold RJ (1991) Acoustic chiasm III: nature, distribution and sources of afferents to the lateral superior olive in cat. J Comp Neurol 310:377–400.
Goldberg JM, Brown PB (1968) Functional organization of the dog superior olivary complex: an anatomical and electrophysiological study. J Neurophysiol 31:639–656.
Guinan JJ Jr, Guinan SS, Norris BE (1972a) Single auditory units in the superior olivary complex. I. Responses to sounds and classifications based on physiological properties. Int J Neurosci 4:101–120.
Guinan JJ Jr, Norris BE, Guinan SS (1972b) Single auditory units in the superior olivary complex. II. Locations of unit categories and tonotopic organization. Int J Neurosci 4:147–166.
Hablitz J, Langmoen I (1982) Excitation of hippocampal cells by glutamate in the guinea pig and rat. J Physiol (Lond) 325:317–331.
Harrison JM, Feldman ML (1970) Anatomical aspects of the cochlear nucleus and superior olivary complex. In: Contributions to sensory physiology, Vol 4 (Neff WD, ed), pp 95–142. New York: Academic.
Harrison JM, Irving R (1966) Ascending connections of the anterior ventral cochlear nucleus in the rat. J Comp Neurol 126:51–64.
Helfert RH, Schwartz IR (1986) Morphological evidence for the existence of multiple neuronal classes in the cat lateral superior olivary nucleus. J Comp Neurol 244:533–549.
Helfert RH, Schwartz IR (1987) Morphological features of five neuronal classes in the gerbil lateral superior olive. Am J Anat 179:55–69.
Helfert RH, Juiz JM, Bledsoe SC Jr, Bonneau JM, Wenthold RJ, Altschuler RA (1989) Two classes of glutamate-immunoreactive synaptic terminals in the guinea pig superior olivary complex. Soc Neurosci Abstr 15:941.
Honore T, Davies SN, Drejer J, Fletcher EJ, Jacobsen P, Lodge D, Nielsen FE (1988) Quinoxalinediones: potent competitive non-NMDA glutamate receptor antagonists. Science 241:701–703.
Hosli L, Andres PF, Hosli E (1976) Ionic mechanisms associated with the depolarization by glutamate and aspartate on human and rat spinal neurones in tissue culture. Pfluegers Arch 363:43–48.
Irvine DRF (1986) The auditory brainstem: a review of the structure and function of auditory brainstem processing mechanisms. In: Progress in sensory physiology, Vol 7 (Ottoson D, ed). Berlin: Springer.
Jean-Baptiste M, Morest DK (1975) Transneuronal changes of synaptic endings and nuclear chromatin in the trapezoid body following cochlear ablations in cats. J Comp Neurol 162:111–134.
Jenkins WM, Masterton RB (1982) Sound localization: effects of unilateral lesions in central auditory system. J Neurophysiol 47:987–1016.
Jessell TM, Yoshioka K, Jahr CE (1986) Amino acid receptor-mediated transmission at primary synapses in rat spinal cord. J Exp Biol 124:239–258.
Johnson JW, Asher P (1987) Glycine potentiates the NMDA response in cultured mouse brain neurons. Nature 325:529–531.
Kavanagh GL, Kelly JB (1986) The effects of kainic acid lesions of the superior olivary nucleus on sound localization by the ferret. Soc Neurosci Abstr 12:1246.
Langdon RB, Freeman JA (1986) Antagonists of glutaminergic neurotransmission block retinotectal transmission in goldfish. Brain Res 398:169–174.
Lenn NJ, Reese TS (1966) The fine structure of nerve endings in the nucleus of the trapezoid body and the ventral cochlear nucleus. Am J Anat 118:375–389.
Li RYS, Guinan JJ (1971) Antidromic and orthodromic stimulation of neurons receiving calyces of Held. In: Quarterly Progress Report of the Research Laboratory of Electronics, Vol 100, pp 227–234. Cambridge, MA: MIT.
MacDermott AB, Dale N (1987) Receptors, ion channels, and synaptic potentials underlying the integrative actions of excitatory amino acids. Trends Neurosci 10:34–42.
Madl JE, Larson AA, Beitz AJ (1986) Monoclonal antibody specific for carbodiimide-fixed glutamate: immunocytochemical localization in the rat CNS. J Histochem Cytochem 34:317–326.
Madl JE, Beitz AJ, Johnson RL, Larson AA (1987) Monoclonal antibodies specific for fixative-modified aspartate: immunocytochemical localization in the rat CNS. J Neurosci 7:2639–2650.
Martin AR (1990) Glycine- and GABA-activated chloride conductances in lamprey neurons. In: Glycine neurotransmission (Otterson OP, Storm-Mathisen J, eds), pp 171–191. New York: Wiley.
Massey SC, Miller RF (1987) Excitatory amino acid receptors of rod- and cone-driven horizontal cells in the rabbit retina. J Neurophysiol 57:645–659.
Masterton RB, Imig TJ (1984) Neural mechanisms for sound localization. Annu Rev Physiol 46:275–287.
Masterton RB, Jane JA, Diamond IT (1967) Role of brainstem auditory structures in sound localization. I. Trapezoid body, superior olive, and lateral lamniscus. J Neurophysiol 30:341–359.
Miller RF, Frumkes TE, Slaughter M, Dacheux RF (1981) Physiological and pharmacological basis of GABA and glycine action on neurons of mudpuppy retina. I. Receptors, horizontal cells, bipolars, and G-cells. J Neurophysiol 45:743–763.
Monaghan DT, Bridges RJ, Cotman CW (1989) The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. Annu Rev Pharmacol Toxicol 29:365–402.
Moore CN, Cassaday JH, Neff WD (1974) Sound localization: role of the commissural pathways of the auditory system of the cat. Brain Res 82:13–26.
Moore MJ, Caspary DM (1983) Strychnine blocks binaural inhibition in lateral superior olivary neurons. J Neurosci 3:237–242.
Morest DK (1968) The collateral system of the medial nucleus of the trapezoid body of the cat, its neuronal architecture and relation to the olivo-cochlear bundle. Brain Res 9:288–311.
Nakajima Y (1971) Fine structure of the medial nucleus of the trapezoid body of the bat with special reference to two types of synaptic endings. J Cell Biol 50:121–134.
Nowak L, Bregestovski P, Ascher P, Herbet A, Prochiantz A (1984) Magnesium gates glutamate-activated channels in mouse central neurones. Nature 307:462–465.
Olney JW (1978) Neurotoxicity of excitatory amino acids. In: Kainic acid as a tool in neurobiology (McGeer EG, Olney JW, McGeer PL, eds), pp 95–121. New York: Raven.
Osen KK (1969) Cytoarchitecture of the cochlear nucleus in the cat. J Comp Neurol 136:453–484.
Ottersen OP, Storm-Mathisen J (1984) Glutamate- and GABA-containing neurons in the mouse and rat brain, as demonstrated with a new immunocytochemical technique. J Comp Neurol 229:374–392.
Peyret D, Campistron G, Geffard M, Aran J-M (1987) Glycine immunoreactivity in the brainstem auditory and vestibular nuclei of the guinea pig. Acta Otolaryngol (Stockh) 104:71–76.
Phillips D, Brugge JF (1985) Progress in neurophysiology of sound localization. Annu Rev Psychol 36:245–274.
Rasmussen GL (1967) Efferent connections of the cochlear nucleus. In: Sensorineural hearing processes and disorders (Graham AB, ed), pp 61–75. Boston: Little Brown.
Robinson MB, Coyle JT (1987) Glutamate and related acidic excitatory neurotransmitters: from basic science to clinical application. Fed Am Soc Exp Biol 1:446–455.
Rothman SM (1985) The neurotoxicity of excitatory amino acids is produced by passive chloride influx. J Neurosci 5:1483–1489.
Saint Marie RL, Ostapoff E-M, Morest DK, Wenthold RJ (1989) A glycine-immunoreactive projection of the cat lateral superior olive: possible role in midbrain ear dominance. J Comp Neurol 279:382–396.
Sanes DH (1990) An *in vitro* analysis of sound localization mechanisms in the gerbil lateral superior olive. J Neurosci 10:3494–3506.
Sanes DH, Wooten GF (1987) Development of glycine receptor distribution in the lateral superior olive of the gerbil. J Neurosci 7:3803–3811.
Sanes DH, Geary WA, Wooten GF, Rubel EW (1987) Quantitative distribution of the glycine receptor in the auditory brainstem of the gerbil. J Neurosci 7:3793–3802.
Schwartz IR (1985) Autoradiographic studies of amino acid labeling

of neural elements in the brainstem. In: Auditory biochemistry (Drescher DG, ed), pp 258–277. Springfied, IL: Thomas.

Stelzer A, Wong RKS (1989) $GABA_A$ responses in hippocampal neurons are potentiated by glutamate. Nature 337:170–173.

Stotler WA (1953) An experimental study of the cells and connections of the superior olivary complex of the cat. J Comp Neurol 98:401–431.

Strominger NL, Strominger AI (1971) Ascending brain stem projections of the anteroventral cochlear nucleus in the rhesus monkey. J Comp Neurol 143:217–242.

Tsuchitani C (1977) Functional organization of lateral cell groups of cat superior olivary complex. J Neurophysiol 40:296–318.

Tsuchitani C, Boudreau JC (1966) Single unit analysis of cat superior olive S segment with tonal stimuli. J Neurophysiol 29:684–697.

Tsuchitani C, Boudreau JC (1969) Stimulus level of dichotically presented tones and cat superior olive S-segment cell discharge. J Acoust Soc Am 46:978–988.

Warr WB (1966) Fiber degeneration following lesions in the anterior ventral cochlear nucleus of the cat. Exp Neurol 14:453–474.

Warr WB (1972) Fiber degeneration following lesions in the multipolar and globular cell areas in the ventral cochlear nucleus of the cat. Brain Res 40:247–270.

Warr WB (1982) Parallel ascending pathways from the cochlear nucleus: neuroanatomical evidence of functional specialization. In: Contributions to sensory physiology, Vol 7 (Neff WD, ed), pp 1–38. New York: Academic.

Watkins JC, Evans RH (1981) Excitatory amino acid transmitters. Annu Rev Pharmacol Toxicol 21:165–204.

Watkins JC, Olverman HJ (1987) Agonists and antagonists for excitatory amino acid receptors. Trends Neurosci 10:265–272.

Wenthold RJ, Martin MR (1985) Neurotransmitters of the auditory nerve and central auditory system. In: Hearing science (Berlin C, ed), pp 341–369. San Diego: College-Hill.

Wenthold RJ, Betz H, Reeks KA, Parakkal MH, Altschuler RA (1985) Localization of glycinergic synapses in the cochlear nucleus and superior olivary complex with monoclonal antibodies specific for glycine receptor. Soc Neurosci Abstr 11:1048.

Wenthold RJ, Huie D, Altschuler RA, Reeks KA (1987) Glycine immunoreactivity localized in the cochlear nucleus and superior olivary complex. Neuroscience 22:897–912.

Wu SH, Kelly JB (1991) Physiological properties of neurons in the mouse superior olive: membrane characteristics and postsynaptic reponses studied *in vitro*. J Neurophysiol 65:230–246.

Wu SH, Kelly JB (1992) NMDA, non-NMDA and glycine receptors mediate binaural interaction in the lateral superior olive: physiological evidence from mouse brain slice. Neurosci Lett 134:257–260.

Wu SH, Oertel D (1986) Inhibitory circuitry in the ventral cochlear nucleus is probably mediated by glycine. J Neurosci 6:2691–2706.

Zarbin MA, Wamsley JK, Kuhar MJ (1981) Glycine receptor: light microscopic autoradiographic localization with [^{3}H]-strychnine. J Neurosci 1:532–547.