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Developmental Influence of Glycinergic Transmission: Regulation of NMDA Receptor-Mediated EPSPs

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The influence of excitatory transmission on postsynaptic structure is well established in developing animals, but little is known about the role of synaptic inhibition. We addressed this issue in developing gerbils with two manipulations designed to decrease glycinergic transmission in an auditory nucleus, the lateral superior olive (LSO), before the onset of sound-evoked activity. First, contralateral cochlear ablation functionally denervated the glycinergic pathway from the medial nucleus of the trapezoid body (MNTB) to the LSO, while leaving the excitatory pathway intact. Second, continuous release of a glycine receptor antagonist, strychnine (SN), was used to decrease transmission.

The strength of excitatory and inhibitory synapses was examined with whole-cell recordings from LSO neurons in a brain-slice preparation. The percentage of LSO neurons exhibiting MNTB-evoked IPSPs was reduced in both ablated and SN-treated animals. In those neurons displaying IPSPs, the amplitude was significantly reduced. This decrease was accompanied by an 8 mV depolarization in the IPSP equilibrium potential. In contrast, the ipsilaterally evoked EPSPs were of unusually long duration in experimental animals. These long-duration EPSPs were significantly shortened by hyperpolarizing the neuron to −90 mV or exposing them to aminophosphonopentanoic acid (AP-5), an NMDA receptor antagonist. Membrane hyperpolarization and AP-5 had little effect in control neurons. In addition, LSO neurons from ablated or SN-treated animals displayed broad rebound depolarizations after membrane hyperpolarization, and these were abolished in the presence of Ni^{2+}. Because both cochlear ablation and SN-rearing were initiated before the onset of sound-evoked activity, the results suggest that *spontaneous* glycinergic transmission influences the development of postsynaptic properties, including the IPSP reversal potential, NMDA receptor function, and a Ca^{2+} conductance.

Key words: inhibition; glycine; synaptic plasticity; development; calcium; auditory pathways

Spontaneous and evoked excitatory synaptic activity plays an essential role in the genesis and strength of excitatory synapses. A chronic reduction in neural transmission during development is commonly associated with a decrease in synaptic efficacy (Wiesel and Hubel, 1965; Sherman and Spear, 1982; Dubin et al., 1986). The activity at one set of excitatory synapses may also affect the strength of other excitatory connections (Constantine-Paton et al., 1990; Shatz, 1990). For example, heterosynaptic suppression has been demonstrated at the neuromuscular junction *in vitro* (Lo and Poo, 1991). Synaptic activity can also regulate the appearance of postsynaptic receptors and ion channels. For example, blockade of acetylcholine receptors prevents the loss of extrajunctional receptors at the vertebrate neuromuscular junction (Burden, 1977), and decreased presynaptic activity reduces glutamate receptor expression at the insect neuromuscular junction (Broadie and Bate, 1993).

By comparison, less is known about the modulation of inhibitory connections or their influence on the maturation of nervous system properties. There is experimental evidence suggesting that GABAergic transmission influences neuron morphology (Wolff et al., 1978; Hansen et al., 1987; Meier et al., 1991) and the formation of synapses (Wolff et al., 1979; Van Huizen et al., 1987). In the gerbil lateral superior olive (LSO), glycinergic transmission influences the maturation of both pre- and postsynaptic neuronal morphology (Sanes and Chokshi, 1992; Sanes et al., 1992; Sanes and Takacs, 1993). Therefore, we wished to investigate whether inhibitory synaptic transmission contributed to the maturation of neuronal function.

The LSO is a brainstem auditory nucleus with a favorable arrangement of afferents, allowing for the selective manipulation of inhibitory synapses. Excitatory afferents from the cochlear nucleus to the LSO are activated primarily by sound at the ipsilateral ear, whereas the contralateral ear activates a group of inhibitory afferents arising in the medial nucleus of the trapezoid body (MNTB) (Rasmussen, 1946; Boudreau and Tsuchitani, 1970; Browner and Webster, 1975; Warr, 1982; Caird and Klinke, 1983; Moore and Caspary, 1983; Glendenning et al., 1985; Harnischfeger et al., 1985; Spangler et al., 1985; Cant and Casseday, 1986; Wenthold et al., 1987; Sanes and Rubel, 1988; Zook and DiCaprio, 1988; Sanes, 1990; Wenthold et al., 1990). When the ear contralateral to the LSO is ablated during development, the inhibitory MNTB afferents are functionally denervated, thus limiting glycinergic transmission within the LSO (Moore, 1992; Sanes et al., 1992). Both excitatory and inhibitory synaptic transmission are prominent in LSO neurons from birth, as assessed *in vitro* (Sanes, 1993). Moreover, spontaneous action potentials have been recorded in the auditory brainstem before the onset of sound-evoked activity (Woolf and Ryan, 1985; Kotak and Sanes, 1995). In the present report, we investigate whether a chronic decrease of synaptic inhibition perturbs the maturation of these synaptic properties in the LSO.

MATERIALS AND METHODS

Cochlear ablations. Gerbil (*Meriones unguiculatus*) pups, aged 7 d postnatal, were anesthetized with hypothermia such that the cardiac and respiratory cycles ceased and there was a complete absence of reflexive

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Table 1. Comparison of afferent-evoked maximum EPSPs and IPSPs, and membrane properties of LSO neurons in control and manipulated animals

	Control	Ablated	Strychnine
IPSP amplitude (mV)	8.7 ± 0.4 (21)	4.8 ± 0.6 (7)*	---- (1)
EPSP duration (msec)	68 ± 7 (31)	252 ± 18 (29)*	253 ± 18 (10)*
EPSP amplitude (mV)	11.2 ± 0.4 (31)	15.8 ± 0.6 (29)*	13.6 ± 1.2 (10)*
Resting potential (mV)	−52.0 ± 0.3 (31)	−52.9 ± 0.5 (29)	−49.8 ± 0.4 (10)*
Input resistance (MΩ)	326 ± 15 (31)	666 ± 41 (29)*	705 ± 57 (10)*

Mean ± SEM. *n* values in parentheses.

* Significantly different from control values:

IPSP amplitude: Student's *t* test, control versus ablated: $t = 4.72$, df = 26, $p < 0.001$.

EPSP duration: ANOVA, $F = 59$, df = 2, $p < 0.0001$; Student's *t* test, control versus ablated: $t = 6.54$, df = 58, $p < 0.0001$; control versus strychnine: $t = 6.54$, df =39, $p < 0.0001$.

EPSP amplitude: ANOVA, $F = 17$, df = 2, $p < 0.0001$; Student's *t* test, control versus ablated: $t = 5.38$, df = 58, $p < 0.0001$; control versus strychnine: $t = 1.99$, df = 39, $p < 0.04$.

Resting potential: ANOVA, $F = 6.2$, df = 2, $p = 0.003$; Student's *t* test, control versus ablated: $p > 0.05$; control versus strychnine: $t = 1.99$, df = 39, $p < 0.05$.

Input Resistance: ANOVA, $F = 37.9$ df = 2, $p < 0.0001$; Student's *t* test, control versus ablated: $t = 5.98$, df = 57, $p < 0.0001$; control versus strychnine: $t = 5.98$, df = 38, $p < 0.0001$.

withdrawal to noxious stimulation on the integument. The right cochlea was exposed and extirpated with a fine forceps, as described previously (Sanes et al., 1992). The cavity was packed with gel foam and the wound was closed. The pups were revived slowly on a heating pad and returned to the litter when locomotor activity resumed.

Strychnine implants. Gerbil pups, aged 3–4 d postnatal, were anesthetized by hypothermia, and 0.05 mg strychnine (SN) timed-release pellets (Innovative Research of America, Toledo, OH) were implanted subcutaneously at a posterior middorsal location. The wound was closed, and the pups were revived on a heating blanket before being returned to the litter. This manipulation permits a continuous release of SN for ~21 d. The dosage used was previously determined as the maximum sublethal dose by using intraperitoneal administration and by evaluating 0.01–0.1 mg SN implants (Sanes and Chokshi, 1992).

Brain-slice preparation. Control and manipulated gerbils at postnatal days (PND) 8–14 were used to produce 300 μm transverse slices through the auditory brainstem (Sanes, 1990, 1993). The slices selected for recording contained the LSO, the inhibitory afferent pathway from the medial nucleus of the trapezoid body (MNTB), and the excitatory afferent pathway from the ipsilateral cochlear nucleus. The artificial CSF (ACSF) contained (in mM): 123 NaCl, 4 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 28 $NaHCO_3$, 15 glucose, 2.4 $CaCl_2$, and 0.4 L-ascorbic acid. Slices were generated in cold oxygenated ACSF and transferred to an incubation chamber for 2 hr. The recording chamber was superfused with oxygenated ACSF (8 ml/min) at room temperature. An ACSF with reduced calcium, and added Ni^{2+} (400 μM $CaCl_2$, 2 mM $NiCl_2$), was used to block calcium currents during current injections. To block NMDA receptors, slices were superfused with ACSF containing 25 μM aminophosphonopentanoic acid (AP-5). In some experiments, AMPA receptors also were blocked by the addition of 10 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). Slices were exposed to antagonists for 2–3 min before determining their effect on synaptic potentials.

To examine whether acute blockade of glycinergic inhibition could perturb synaptic physiology, some slices were superfused with ACSF containing 2 μM SN for 5–30 min during an LSO neuron recording. A second set of slices were preincubated in ACSF containing 2 μM SN for 3–7 hr before recording.

Electrophysiology. Whole-cell patch-clamp recordings were obtained in a manner similar to that described by Marty and Neher (1985). Recording electrodes were fabricated from 1.5 mm outer diameter borosylicate glass microcapillaries (4–6 MΩ). The composition of the internal pipette solution was (in mM): 130 K-gluconate, 0.6 EGTA, 10 HEPES, 2 $MgCl_2$, 5 KCl, 2 ATP, 0.3 GTP, pH 7.2. To mark the location of recorded neurons, 0.2% biocytin was added to the pipette solution. Recordings were obtained under current-clamp conditions (PC-501A, Warner Instruments, Hamden, CT), and the access resistance was compensated throughout the experiments. Brain slices were fixed in 4% paraformaldehyde, and the biocytin was visualized with an avidin–biotin–horseradish peroxidase amplification procedure (Vector Laboratories, Burlingame, CA). The borders of LSO were easily recognized in the 300 μm sections because of the dense array of fibers that surround the nucleus. Therefore, all recorded neurons were verified to be located within the LSO borders.

Custom-designed software running on a PC/AT computer was used for programmed stimulus delivery, data acquisition, and analysis (Sanes, 1993). Bipolar stimulating electrodes were placed on the MNTB, medial to the LSO (i.e., inhibitory afferents), and on the cochlear nucleus pathway lateral to the LSO (i.e., excitatory afferents). A set of EPSPs and IPSPs, including potentials of maximum amplitude, was collected by applying 100 μsec current pulses at ≤0.5 Hz in 0.5 V increments until the action potential threshold was attained.

RESULTS

The data were obtained from whole-cell patch-clamp recordings in 136 neurons from 125 brain slices. Because both denervation of the inhibitory afferents to the LSO (i.e., unilateral cochlear ablation) and pharmacological blockade of glycine receptors (i.e., SN-rearing) produced comparable results, these manipulations are treated jointly below and referred to as manipulations to decrease inhibitory transmission.

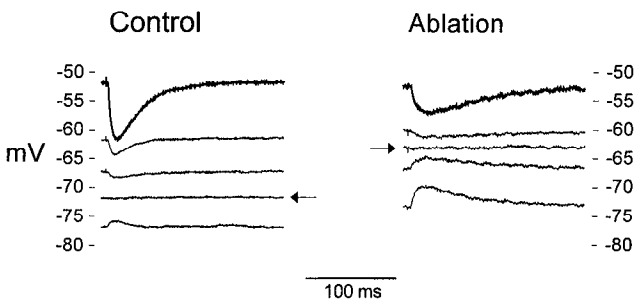

Figure 1. MNTB-evoked IPSPs from LSO neurons in control and ablated animals. *Left*, A series of MNTB-evoked IPSPs obtained at different holding potentials is shown for a neuron from a 9 d control neuron. The *top IPSP* was obtained at the resting membrane potential (V_{REST}) and was 10 mV in amplitude. The IPSP reversed at −72 mV (*arrow*). *Right*, A series of MNTB-evoked IPSPs obtained at different holding potentials is shown for a neuron from a 9 d ablated animal. The *top IPSP* was obtained at V_{REST} and was ~5 mV in amplitude. The IPSP reversed at −63 mV (*arrow*). Both recordings were obtained in the presence of 10 μM CNQX and 25 μM AP-5. See Table 2 for quantification and statistical analyses. Control V_{REST} = −52 mV; ablation V_{REST} = −54 mV.

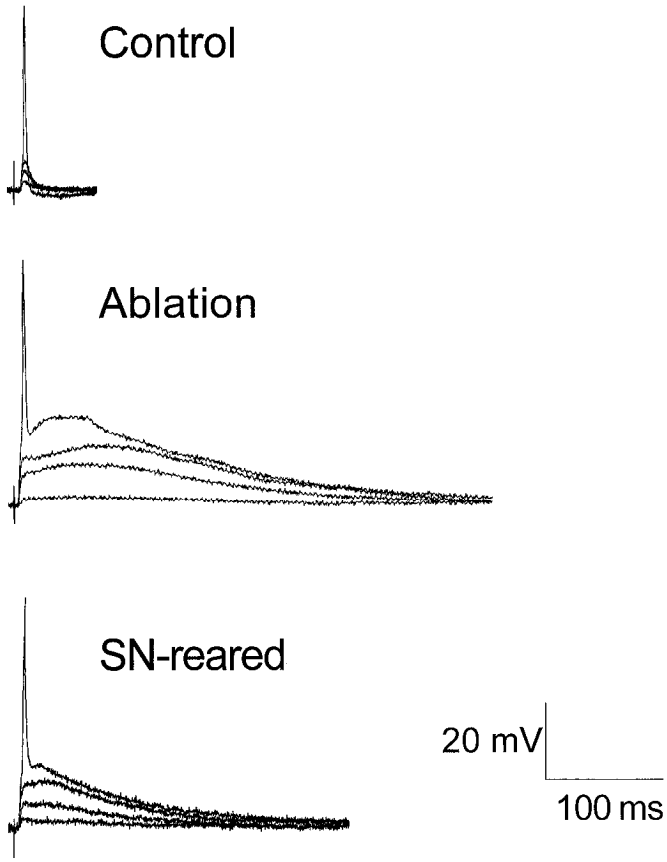

Figure 2. EPSPs are larger after decreased inhibitory transmission. *Top*, A family of EPSPs and an action potential for a 10 d neuron from a control animal. *Middle*, A family of EPSPs and an action potential for a 10 d neuron from an ablated animal. *Bottom*, A family of EPSPs and an action potential for a 10 d neuron from an SN-reared animal. Note the dramatic increase in EPSP duration and amplitude in the two manipulated neurons. EPSPs were evoked by stimulating the excitatory pathway from the cochlear nucleus to the LSO. See Table 1 for quantification and statistical analyses. Control V_{REST} = −54; Ablation V_{REST} = −55 mV; SN-reared V_{REST} = −50 mV.

Manipulations to decrease inhibitory transmission: effect on inhibitory synapses

MNTB-evoked IPSPs were examined in brain slices from normal and manipulated animals. Of those neurons that displayed an MNTB-evoked IPSP, the maximum IPSP amplitude decreased from 8.7 mV in control neurons to 4.8 mV in neurons from ablated animals (Table 1). However, it was also found that both manipulations led to a decrease in neurons exhibiting IPSPs. Whereas 21 of 31 (68%) control neurons displayed MNTB-evoked IPSPs, only 7 of 29 (24%) neurons from ablated animals and 1 of 10 (10%) neurons from SN-reared animals displayed IPSPs.

Table 2. Comparison of maximum IPSP amplitude and E_{IPSP} of LSO neurons superfused with 10 μM CNQX and 25 μM AP-5 in controls and manipulated animals

	Control	Ablated
IPSP amplitude (mV)	9.0 ± 1.3 (11)	5.3 ± 1.3 (14)*
E_{IPSP} (mV)	−72.1 ± 1.5 (10)	−63.9 ± 1.6 (9)*
Resting potential (mV)	−51.8 ± 0.7 (11)	−51.3 ± 1 (14)

Mean ± SEM. *n* values in parentheses.

* Significantly different from control values:

IPSP amplitude: Shapiro–Wilk W test for normality, ablated: $W = 0.85$, $p = 0.02$; median test, chi square = 8.64, df = 1, $p < 0.01$.

E_{IPSP}: Student's *t* test, $t = 3.67$, df = 17, $p < 0.002$.

Resting potential: Student's *t* test, $p > 0.05$.

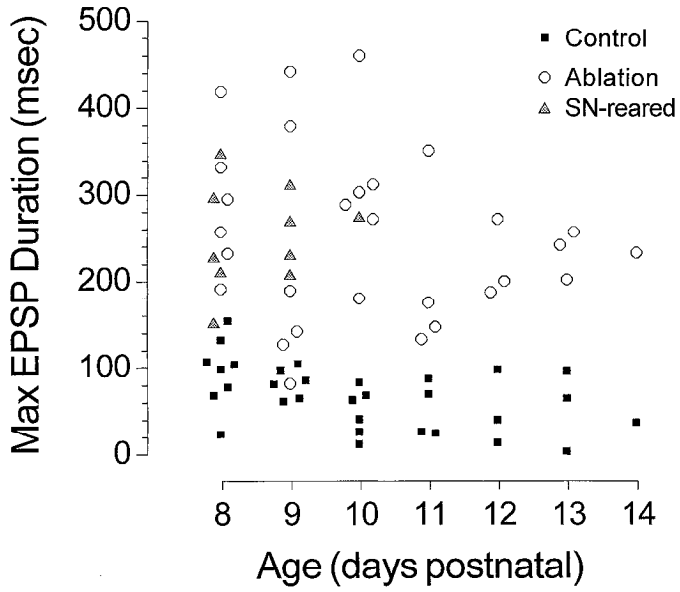

Figure 3. The response to decreased inhibitory transmission was rapid. The distribution of maximum EPSP durations for neurons in control (*filled squares*), ablated (*open circles*), and SN-reared (*gray triangles*) animals. Note the significant extension of EPSP durations in manipulated neurons from PND 8 onward. This indicates that the effect was elicited within 24 hr.

To examine the basis for this decreased inhibitory transmission, a second set of recordings was performed in the presence of two glutamate receptor antagonists, 10 μM CNQX and 25 μM AP-5, and these recordings were restricted to PND 8–11. These recordings confirmed that MNTB-evoked IPSPs were 41% smaller in neurons from ablated animals compared with those from controls (Fig. 1, Table 2). Furthermore, the IPSP reversal potential (E_{IPSP}) was found to be 8 mV more depolarized in neurons from ablated animals (Fig. 1, Table 2). In this second data set, 5 of 14 neurons (35%) from ablated animals did not exhibit MNTB-evoked inhibitory potentials, whereas synaptic inhibition was absent from only 1 of 11 (9%) control neurons. There was no difference in the resting membrane potential for neurons from control and ablated animals (Table 2).

Manipulations to decrease inhibitory transmission: effect on excitatory synapses

The anatomically discrete excitatory pathway allowed us to test whether manipulations to decrease inhibitory transmission also influenced the development of excitatory synapses. EPSPs from the LSO neurons of both ablated and SN-reared animals differed significantly from those obtained in control neurons. After a period of decreased inhibitory transmission, the initial synaptically evoked depolarization was typically followed by a second, long-lasting phase (Fig. 2). The maximum duration of these EPSPs was more than threefold longer compared with age-matched controls, and there was a commensurate increase of EPSP amplitude (Table 1). As shown in Figure 3, the effects of decreased inhibitory transmission were apparent at PND 8, within 24 hr of the surgical manipulation. Neurons from SN-reared animals also exhibited the

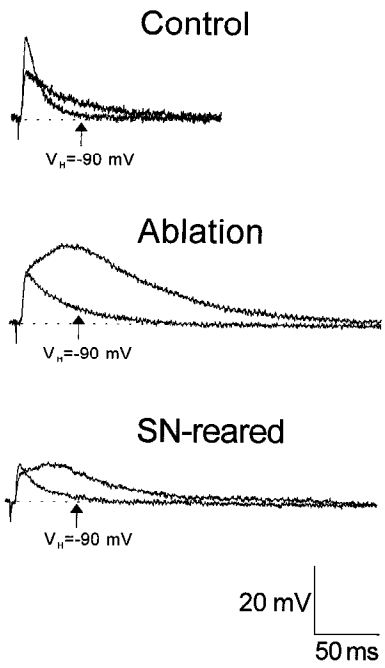

Figure 4. EPSPs were shortened at a hyperpolarizing membrane potential. *Top*, In an 11 d neuron from a control animal, holding the cell at −90 mV (V_H, *arrow*) decreased the EPSP duration only marginally. *Middle*, *bottom*, In neurons from an 8 d ablated animal and a 9 d SN-reared animal, holding the cell at −90 mV (V_H, *arrow*) produced a significant decrease in EPSP duration. See Table 3 for quantification and statistical analyses. Control V_{REST} = −50; ablation V_{REST} = −51 mV; SN-reared V_{REST} = −49 mV.

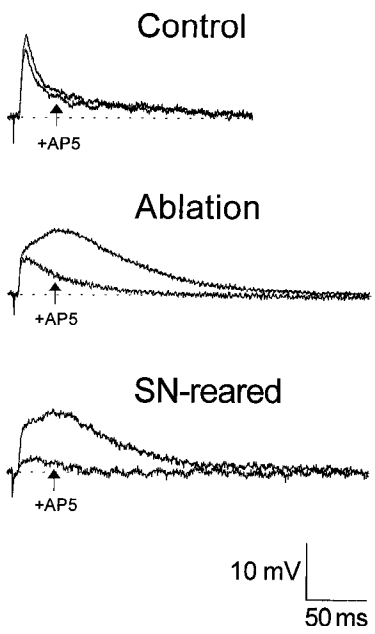

Figure 5. EPSPs were shortened in the presence of the NMDA receptor antagonist AP-5. *Top*, In a neuron from an 8 d control animal, superfusion of 25 μM AP-5 (*+AP5*, *arrow*) produces only a slight decrease in EPSP amplitude and duration. *Middle*, *bottom*, In neurons from 8 d ablated and a 9 d SN-reared animals, superfusion of AP-5 (*+AP5*, *arrow*) produced a significant decrease in EPSP duration. See Table 3 for quantification and statistical analyses. Control V_{REST} = −57; ablation V_{REST} = −51 mV; SN-reared V_{REST} = −48 mV.

effect on the first day of recording, PND 8, although this was 3–4 d after the exposure period began.

To test whether acute removal of glycinergic transmission would produce a loss of synaptic inhibition, slices were incubated in 2 μM SN for 5 min to 7 hr. In five LSO neurons, ipsilaterally evoked EPSPs were monitored immediately before and after 5–30 min exposure to SN. Whereas SN did eliminate contralaterally evoked IPSPs, there was no change in the ipsilaterally evoked EPSP duration (before, 93 ± 16 msec; after, 95 ± 16 msec) or amplitude (before, 10.9 ± 0.6 mV; after, 10.6 ± 0.6 mV). For five neurons, the brain slice was incubated in SN for 3–7 hr before obtaining the recording. Whereas contralaterally evoked IPSPs were absent, there was no difference in the ipsilaterally evoked EPSP duration (74 ± 14 msec), EPSP amplitude (10.4 ± 0.6 mV), or the input resistance (R_{INPUT}) (335 ± 19 MΩ) compared with control neurons (see Table 1).

To examine the basis of these enhanced EPSPs, two experimental strategies were used. First, we tested whether the large EPSPs were voltage-dependent by hyperpolarizing control and manipulated neurons to −90 mV. After hyperpolarization, the EPSP duration was shortened by 103–133 msec in manipulated neurons compared with a reduction of only 8.2 msec in controls (Fig. 4, Table 3). Second, we tested whether NMDA receptors contributed to the large EPSPs by bathing the preparation in 25 μM AP-5, an NMDA receptor antagonist. In the presence of AP-5, the EPSP duration was shortened by 78–125 msec in manipulated neurons compared with only 1.4 msec in controls (Fig. 5, Table 3). AP-5 had a comparable effect on EPSP amplitude.

Manipulations to decrease inhibitory transmission: membrane properties

Decreased inhibitory transmission also resulted in a doubling of the membrane input resistance in LSO neurons. However, there

Table 3. Reduction of afferent-evoked EPSPs in the presence of AP-5, and at a holding potential of −90 mV

	Control	Ablated	Strychnine
Reduction of EPSP duration at −90 (msec)	8 ± 21 (4)	165 ± 17 (6)*	103 ± 19 (9)*
Reduction of EPSP duration in AP-5 (msec)	1.4 ± 2.9 (5)	78 ± 26 (8)*	125 ± 31 (4)*
Reduction of EPSP amplitude in AP-5 (mV)	0.8 ± 0.3 (5)	3.6 ± 0.5 (9)*	4.2 ± 0.9 (4)*

Mean ± SEM. *n* values in parentheses

* Significantly different from control values:

EPSP amplitude in AP-5: ANOVA, F = 6.87, df = 2, p = 0.008; control versus ablated: t test, t = 3.365, df = 12, p = 0.006; control versus strychnine: t test, t = 3.806, df = 7, p = 0.007.

EPSP duration in AP-5: ANOVA, F = 5.006, df = 2, p = 0.023; control versus ablated: t test, t = 2.260, df = 11, p = 0.045; control versus strychnine: t test, t = 4.537, df = 7, p = 0.003.

EPSP duration at −90 mV: ANOVA, F = 7.219, df = 2, p = 0.006; control versus ablated: t test, t = 3.985, df = 8, p = 0.004; control versus strychnine: t test, t = 3.021, df = 11, p = 0.012.

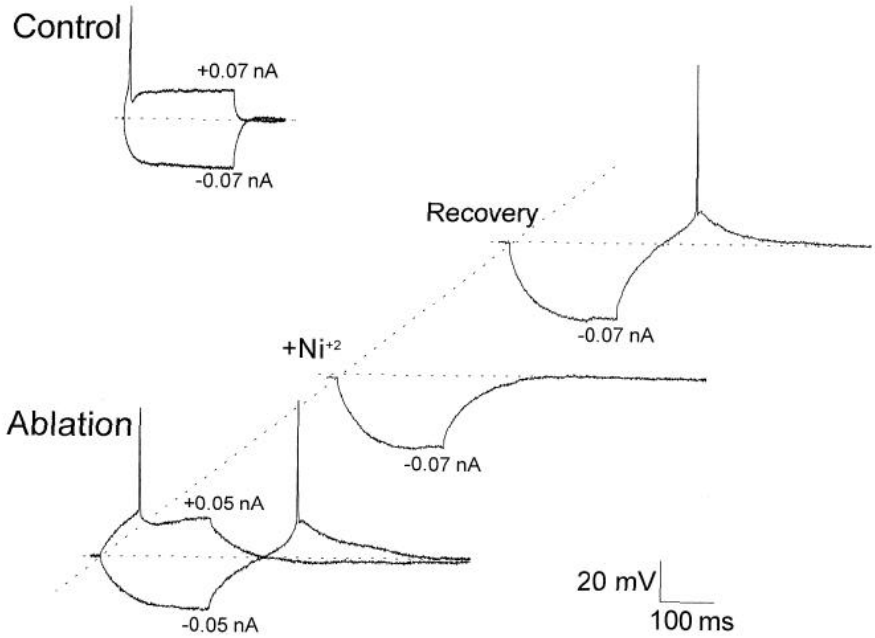

Figure 6. Evidence for recruitment of a calcium conductance after decreased inhibitory transmission. *Top*, A hyperpolarizing current pulse did not produce a rebound depolarization in a neuron from a 10 d control animal. *Bottom*, A hyperpolarizing current pulse did produce a rebound depolarization and action potential in a neuron from a 10 d ablated animal. This response was abolished in low Ca^{2+} (400 μM), high Ni^{2+} (2 mM) ACSF, and it recovered (*Recovery*) when Ni^{2+} was removed. Similar results were obtained in SN-reared gerbils. Control V_{REST} = −53 mV; ablation V_{REST} = −55 mV.

was not an associated alteration in the resting membrane potential of neurons from ablated animals, although there was a slight but significant reduction after SN-rearing (Table 1). A second characteristic of manipulated neurons was that hyperpolarizing current pulses (−0.05 nA, 200 msec) elicited a broad rebound depolarization and action potential in 24 of 29 (83%) neurons tested (Fig. 6). These responses were completely and reversibly eliminated by superfusion with 2 mM Ni^{2+}. In control neurons, hyperpolarizing current pulses elicited rebound depolarizations in only 3 of 31 (10%) neurons tested.

DISCUSSION

The major finding of this study is that inhibitory synaptic transmission in the LSO has a profound influence on the development of functional properties. Decreasing synaptic inhibition for as little as 24 hr leads to an enormous increase of EPSP amplitude and duration (Fig. 2, Table 1). Somewhat more expected, there is a decline in the number of neurons exhibiting contralaterally evoked IPSPs and IPSP amplitude after a short period of disuse (Fig. 1, Table 2). Although long-duration postsynaptic potentials are characteristic of younger animals, the extended EPSP durations after unilateral ablation or SN-rearing are more than threefold larger than age-matched controls and more than twice as long as those obtained from 2 d postnatal gerbils (Sanes, 1993). Therefore, inhibitory dysfunction led to long-term alterations of synaptic efficacy in the LSO. As summarized in Figure 7, the enhancement in functional NMDA receptors, the presence of Ni^{2+}-sensitive rebound depolarizations, and the depolarization of E_{IPSP} all support postsynaptic mechanisms.

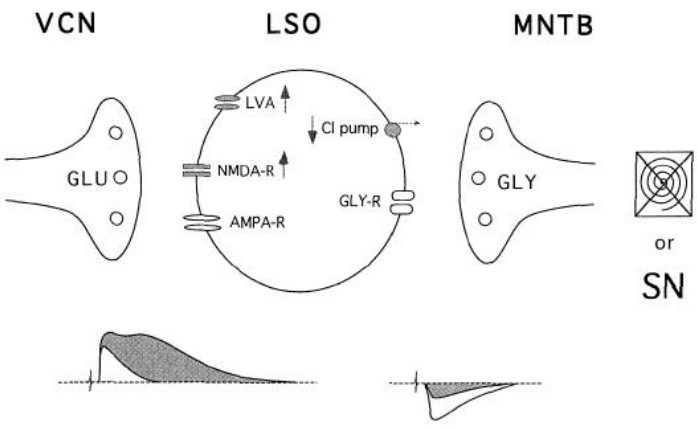

Figure 7. Summary of postsynaptic changes resulting from decreased inhibitory transmission (denoted by *gray symbols* with *arrows*). An *LSO* neuron is shown to be innervated by a glutamate (*GLU*)-containing terminal from the *VCN*, and a glycine (*GLY*)-containing terminal from the *MNTB*. The two manipulations, elimination of one cochlea (*x*) or SN-rearing (*SN*), were designed to decrease glycinergic transmission in the LSO. The manipulations result in enhanced excitatory potentials (*gray EPSP*), largely attributable to addition of functional NMDA receptors (*NMDA-R*), although low voltage-activated Ca^{2+} channels (*LVA*) also may contribute. These manipulations also result in decreased inhibitory potentials (*gray IPSP*).

The experimental manipulations that we used were intended to decrease glycinergic transmission in the LSO. Although contralateral cochlear ablation does denervate MNTB neurons, we have made the assumption that the MNTB afferents are spontaneously active before the onset of sound-evoked activity. There are several lines of evidence that support this assumption. First, spontaneous action potentials have been recorded in the gerbil cochlear nucleus and auditory midbrain as early as PND 9, 2 d after the age when cochleae were removed (Woolf and Ryan, 1985; Kotak and Sanes, 1995). It is not yet clear whether spontaneous discharge in the neonatal gerbil auditory brainstem arises from cochlear activity. However, it has been found that cochlea removal or inactivation with tetrodotoxin (TTX) in the embryonic chick produces a compete cessation of spontaneous activity in the cochlear nucleus (Lippe, 1994). Second, inactivation of the cochlea with TTX leads to a rapid reduction in soma size and protein synthesis in the gerbil cochlear nucleus at PND 14, just after the onset of hearing (Sie and Rubel, 1992). Furthermore, cochlear removal at PND 7 also leads to a rapid decrease of soma size in the cochlear nucleus (Hashisaki and Rubel, 1989). Soma size and protein synthesis have been shown previously to be sensitive indicators of synaptic transmission in the chick auditory brainstem (Born and Rubel, 1988; Hyson and Rubel, 1989). Third, synaptic transmission in the auditory brainstem is possible from birth, as demonstrated in the brain-slice preparation (Wu and Oertel, 1987; Sanes, 1993; Kandler

and Friauf, 1995). Although it has yet to be directly demonstrated that these synapses are active at PND 7, it should be noted that spontaneous release of neurotransmitters has been detected from the onset of synaptic contact (Young and Poo, 1983).

It is also important to recognize that sprouting of afferent projections occurs in the gerbil superior olive after cochlear removal (Sanes and Takacs, 1993; Kitzes et al., 1995; Russell and Moore, 1995). For example, MNTB arbors within the LSO are known to retain an expanded arborization when deafferented by contralateral cochlear ablation (Sanes and Takacs, 1993). In the present study, decreased inhibitory transmission provides the most plausible explanation for the electrophysiological alterations that result from cochlear ablation because: (1) the alterations were elicited within 24 hr; (2) an independent manipulation, SN-rearing, produced nearly identical findings; and (3) SN-rearing or ablation produces nearly identical effects on LSO dendritic morphology (Sanes and Chokshi, 1992; Sanes et al., 1992). Finally, we have recently observed dendritic hypertrophy in SN-treated organotypic cultures of the LSO in which the cochlear nucleus afferents were not present (Sanes and Hafidi, 1993) (D. Sanes and A. Hafidi, unpublished data), further suggesting that there is a unique influence of spontaneous glycinergic transmission.

Enhanced excitatory potentials

In control LSO neurons, the NMDA receptors account for only a minor component of the total EPSP amplitude (Fig. 5, Table 3), yet they become the principal EPSP component after manipulations to decrease inhibitory transmission. This was clearly illustrated by the substantial reduction in EPSP duration during membrane hyperpolarization or AP-5 application (Figs. 4, 5; Table 3). As schematized in Figure 7, it is also possible that a voltage-gated calcium conductance participates in the synaptically evoked depolarization (Fig. 6). Although the increased R_{INPUT} in experimental neurons may prolong EPSP duration, the profound effect of AP-5 indicates that R_{INPUT} is not the primary basis of our observation.

A developmental reduction of NMDA receptors has been reported previously in the avian cochlear nucleus (Zhou and Parks, 1992), and our results are consistent with a mechanism by which spontaneous inhibitory transmission contributes to this process. In fact, unilateral cochlear extirpation in the chick prevents the normal developmental reduction in NMDA receptors in the cochlear nucleus (Zhou and Parks, 1993), and this is accompanied by a transient reduction in the binding of [^{3}H]muscimol, a $GABA_A$ receptor agonist (Code and Churchill, 1991). In the present study, the extended EPSPs from manipulated LSO neurons (Fig. 2) also resemble an immature state (Sanes, 1993), although normal developmental alterations in glutamate receptor expression remain unexplored.

There are precedents for activity-dependent modulation of NMDA receptor expression. After visual deprivation, the normal decrease in NMDA receptors in the kitten cortex is delayed (Carmignoto and Vicini, 1992; Fox et al., 1992). It remains to be determined whether inhibitory synaptic function is involved in this transition. However, it should be noted that monocular enucleation or visual deprivation has been shown to decrease GABA immunoreactivity and $GABA_A$ receptor levels in the visual cortex of adult monkeys (Hendry and Jones, 1986; Hendry et al., 1990; Hendry, 1991). In addition, manipulations of GABAergic transmission in the visual cortex suggest that they influence the development of synaptic connections. For example, chronic treatment of visual cortex with a $GABA_A$ receptor antagonist (bicuculline) largely prevents a response to monocular deprivation (Ramoa et al., 1988), whereas treatment with agonist (muscimol) leads to the expansion of afferents from the deprived eye (Hata and Stryker, 1994). Similarly, the maturation of functional properties in rat neocortical cultures is perturbed by chronic exposure to picrotoxin, the $GABA_A$ receptor antagonist (Corner and Ramakers, 1992).

The onset of enhanced NMDA receptor sensitivity at excitatory synapses is rapid, occurring within 24 hr of cochlear ablation (Fig. 3). Unfortunately, the time course does not allow us to discriminate among the possible cellular mechanisms: transcription, translation, posttranslational modification, or receptor clustering. Given the prominent role played by NMDA receptors at developing neural circuits (Constantine-Paton et al., 1990; Schnupp et al., 1995), our findings imply that excitatory connections in the LSO are highly modifiable after manipulations to decrease inhibitory transmission. It is also of interest that NMDA receptor antagonists can increase glycine receptor expression in mouse spinal cord cultures (Hoch et al., 1992). Taken together with our results (i.e., glycine receptor antagonist-enhanced expression of functional NMDA receptors), we speculate that the appropriate balance of excitatory and inhibitory receptor molecules relies on parallel regulatory mechanisms.

Reduced IPSPs

The decline in IPSP amplitude after cochlear removal suggested that decreased inhibitory transmission could perturb the glycine-gated chloride current in experimental animals. It has been demonstrated previously that the glycine receptor-coupled channel is highly selective for Cl^- (Coombs et al., 1955; Lux, 1971; Llinás and Baker, 1972; Gold and Martin, 1983; Bormann et al., 1987), and that intracellular Cl^- accumulation leads to a reduction of IPSP amplitude (Huguenard and Alger, 1986; Thompson and Gähwiler, 1989). In the present study, the mean E_{IPSP} of manipulated neurons was depolarized by 8 mV compared with control neurons, whereas resting membrane potential was unchanged (Fig. 1, Table 2). Because these recordings were performed in the presence of glutamate receptor blockers AP-5 and CNQX, it is unlikely that the change in E_{IPSP} was attributable to a masking effect of depolarizing potentials. Rather, there appears to be a sizable decrement in the IPSP driving force, $E_M - E_{Cl}$.

In muscle cells, it has been found previously that membrane input resistance increases and Cl^- conductance decreases after denervation, and that this is accompanied by Cl^- accumulation (Albuquerque and Thesleff, 1968; Westgaard, 1975; Camerino and Bryant, 1976; Lorkovic and Tomanek, 1977; Harris and Betz, 1987). Similar cellular mechanisms may account for both the increased R_{INPUT} and smaller IPSPs in LSO neurons after manipulations to decrease inhibitory transmission. Beyond its effect on driving force, higher cytoplasmic Cl^- levels also may reduce the conductance state of single glycine-gated channels (Gold and Martin, 1982, 1983; McNiven and Martin, 1993). Our results leave open the possibility that glycine receptors are modified posttranslationally or that the expression of one or more subunits is altered in manipulated LSO neurons (Becker et al., 1988; Wolszon and Faber, 1989; Kuhse et al., 1990; Song and Huang, 1990; Vaello et al., 1992). A transition in the expression of the α glycine receptor subunit has recently been described in the postnatal rat LSO (Friauf et al., 1994).

Most LSO neurons from ablated and SN-treated animals did not display any IPSPs, suggesting that there are additional grounds for the observed reduction of inhibition. For example, it is possible that disuse of the inhibitory pathway leads to decreased transmitter re-

lease from presynaptic terminals. Glycine immunoreactivity in deep dorsal cochlear nucleus neurons decreases over an 8 hr period *in vitro*, but can be restored by afferent stimulation (Wickesberg et al., 1994). Decreased uptake of [^{14}C]glycine has also been demonstrated in the guinea pig LSO after unilateral cochlear ablation (Suneja et al., 1994). It is interesting that "silent" glycinergic synapses, which have recently been described in the goldfish brainstem, can be activated with use (Charpier et al., 1995).

The manipulations used in the present study, extirpation of one cochlea or SN implantation, have been shown previously to produce hypertrophy of LSO dendrites (Sanes and Chokshi, 1992; Sanes et al., 1992) and to influence MNTB terminal arborizations within the LSO (Sanes and Takacs, 1993). These anatomical changes and the functional alterations described in the present study are both a consequence of altered inhibitory transmission. It is interesting to consider the mechanism of action. In one scenario, inhibitory synapses ordinarily limit postsynaptic depolarization, but their dysfunction leads to a net depolarization of LSO neurons. Membrane depolarization, often accompanied by increased Ca^{2+} conductance, is known to enhance neuron survival and differentiation *in vitro* (Lasher and Zagon, 1972; Chalazonitis and Fischbach, 1980; Nishi and Berg, 1981; Walicke and Patterson, 1981; Gallo et al., 1987). Alternatively, because GABA and glycine-activated Cl^- channels are also permeable to bicarbonate, it is possible that decreased inhibitory transmission perturbs the cytoplasm or extracellular pH (Kaila and Voipio, 1987; Chen and Chesler, 1992), thereby influencing postsynaptic metabolism. The dramatic increase in NMDA receptor-mediated EPSPs could lead to further increases in intracellular free Ca^{2+} and promote LSO dendritic outgrowth (Kater and Mills, 1991).

Taken together, our observations indicate that glycinergic transmission plays an important regulatory role during the normal developmental of synaptic function. In particular, a short-term disruption of glycinergic transmission led to weaker inhibitory connections and stronger excitatory connections on LSO neurons. Our analyses suggest that these changes were largely postsynaptic, including a reduction in the chloride equilibrium potential and an increase in functional NMDA receptors. Finally, because inhibitory transmission was disrupted before the onset of sound-evoked activity, the influence of glycinergic transmission must be because of spontaneous activity. In fact, we have recently shown that auditory midbrain neurons display spontaneous action potentials as early as PND 9 (Kotak and Sanes, 1995).

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