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) in 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 aminophosphono-pentanoic 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 Ni²⁺. 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 Ca²⁺ 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 (Brodie and Bate, 1993).

By comparison, less is known about the modulation of inhibitory connections or their influence on the maturation of neuronal 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 Tsachioti, 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 activity. The animals were anesthetized with hypothermia such that the cardiac and respiratory cycles ceased and there was a complete absence of reflexive activity.

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were obtained under current-clamp conditions (PC-501A, Warner Instruments) throughout the experiments. Brain slices were fixed in 4% paraformaldehyde and processed for immunocytochemistry using the avidin-biotin-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.

Ablation

![Figure 1](https://example.com/figure1.png)

**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_{\text{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_{\text{REST}} and was -5 mV in amplitude. The IPSP reversed at -65 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_{\text{REST}} = -52 mV; ablation V_{\text{REST}} = -54 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.

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 ($V_{\text{IPSP}}$) was found to be 8 mV more depolarized in neurons from ablated animals compared with those from controls. 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 ($V_{\text{m}}$, 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 ($V_{\text{m}}$, arrow) produced a significant decrease in EPSP duration. See Table 3 for quantification and statistical analyses. Control $V_{\text{RST}}$ = -50; ablation $V_{\text{RST}}$ = -51 mV; SN-reared $V_{\text{RST}}$ = -49 mV.

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 ($R_{\text{INPUT}}$) (335 ± 19 Ω) 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

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**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 ± 2 (4) | 165 ± 17 (6)* | 103 ± 19 (9)* |
| Reduction of EPSP duration in AP-5 (msec) | 1.4 ± 2.9 (5) | 78 ± 26 (9)* | 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)* |

*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, $F = 5.06$, 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, $p = 0.012$.
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 Ni²⁺-sensitive rebound depolarizations, and the depolarization of EIPSP all support postsynaptic mechanisms.

The experimental manipulations that we used were intended to decrease glycineergic 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 complete 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 deafened 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 glycineergic 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 $R_{\text{input}}$ in experimental neurons may prolong EPSP duration, the profound effect of AP-5 indicates that $R_{\text{input}}$ 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 $[^{3}H]\text{muscimol}$, 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 occlusion 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 (Ramos 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 $\text{Cl}^{-}$ (Coombs et al., 1955; Lux, 1971; Linna and Baker, 1972; Gold and Martin, 1983; Bornmann et al., 1987), and that intracellular $\text{Cl}^{-}$ accumulation leads to a reduction of IPSP amplitude (Huguenard and Alger, 1986; Thompson and Gähwiler, 1989). In the present study, the mean $E_{\text{IPSP}}$ 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 $E_{\text{IPSP}}$ was attributable to a masking effect of depolarizing potentials. Rather, there appears to be a sizable decrement in the IPSP driving force, $E_{\text{M}} - E_{\text{Cl}^{-}}$.

In muscle cells, it has been found previously that membrane input resistance increases and $\text{Cl}^{-}$ conductance decreases after denervation, and that this is accompanied by $\text{Cl}^{-}$ accumulation (Albuquerque and Theleff, 1968; Westgaard, 1975; Camerino and Bryant, 1976; Lorkovic and Tomanek, 1977; Harris and Betz, 1987). Similar cellular mechanisms may account for both the increased $R_{\text{input}}$ and smaller IPSPs in LSO neurons after manipulations to decrease inhibitory transmission. Beyond its effect on driving force, higher cytoplasmic $\text{Cl}^{-}$ 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; Wolzson and Faber, 1989; Kuhse et al., 1990; Song and Huang, 1990; Vacllo et al., 1992). A transition in the expression of the $\alpha$ 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 [3H]glycine has also been demonstrated in the guinea pig LSO after unilateral cochlear ablation (Sunega et al., 1994). It is interesting that “silent” glycineergic synapses, which have recently been described in the goldfish brainstem, can be activated with use (Chapier 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 Ca2+ 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 (Kailla 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 Ca2+ 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 development 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 activity potentials as early as PND 9 (Kotak and Sanes, 1995).

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