Glycinergic Transmission Regulates Dendrite Size in Organotypic Culture

Dan H. Sanes [1,2,*] and Aziz Hafidi [1,3]

[1]Center for Neural Science and [2]Department of Biology, 6 Washington Place, New York University, New York, New York 10003 and [3]Laboratoire de Neurobiologie, Universite Blaise Pascal, BP145, 63177, Aubiere, France

SUMMARY

We previously demonstrated that inhibitory synaptic transmission influences dendrite development *in vivo*. We now report an analogous finding in an organotypic culture of a glycinergic projection nucleus, the medial nucleus of the trapezoid body (MNTB), and its postsynaptic target, the lateral superior olive (LSO) of gerbils. Cultures were generated at 6–7 days postnatal and grown in serum containing medium with or without the glycine receptor antagonist, strychnine (SN), at 2 μM. LSO neurons were then labeled with biocytin, and the dendritic arbors were analyzed morphometrically. Compared to neurons from age-matched *in vivo* tissue, the neurons cultured in control media were somewhat atrophic, including decreases in dendritic branching and length. Incubation in strychnine led to a dramatic increase in dendritic branching and total dendritic length. Control neurons averaged 6.3 branches, compared to 18 branches/neuron in SN-treated cultures. There was a similar increase in primary dendrites and total dendritic length. The physical elimination of MNTB cells did not mimic SN treatment, presumably because glycinergic LSO neurons generated intrinsic connections. In fact, the LSO soma area was significantly greater following MNTB removal, suggesting that these afferents provide a second signal to postsynaptic neurons. These results suggest that spontaneous glycinergic transmission regulates the growth of postsynaptic processes.

Keywords: **lateral superior olive, glycine receptors, strychnine, inhibition, trophic, gerbil, development, dendrites.**

INTRODUCTION

Dendritic arbors generally decrease in size when their excitatory synapses are blocked or eliminated (Levi–Montalcini, 1949; Valverde, 1968; Rakic, 1972; Rakic and Sidman, 1973; Benes et al., 1977; Berry et al., 1980; Vaughn et al., 1988; Parks, 1981; Harris and Woolsey, 1981; Feng and Rogowski, 1980; Trune, 1982; Deitch and Rubel, 1984; Vogel and Prittie, 1995; Kalb, 1994). However, our previous *in vivo* studies suggest that inhibitory transmission can also regulate the growth of neuronal processes. Two manipulations were employed to depress glycinergic synapses in the lateral superior olive (LSO) during development: (1) surgical extirpation of the contralateral cochlea effectively denervated the glycinergic afferents from the medical nucleus of the trapezoid body (MNTB) to the LSO (Sanes et al., 1992a; Aponte et al., 1996), and (2) strychnine (SN)-containing continuous release pellets decreased the level of glycinergic transmission throughout the nervous system (Sanes and Chokshi, 1992). These two manipulations produced nearly identical results. LSO dendrites were hypertrophic compared to control animals. However, both manipulations unintentionally produced systemic effects that restricted interpretation. For example, cochlear extirpation is known to induce sprouting in the superior olivary complex

Received May 13, 1996; accepted July 22, 1996
Journal of Neurobiology, Vol. 31, No. 4, pp. 503–511 (1996)

CCC 0022-3034/96/040503-09
* To whom correspondence should be addressed (e-mail: sanes@cns.nyu.edu).

(Kitzes et al., 1995), and SN treatment undoubtedly blocks glycinergic synapses throughout the central auditory system. Therefore, we tested whether glycinergic synapses influence dendrite growth in organotypic explants of the gerbil superior olivary complex.

The LSO has a favorable afferent projection pattern for studies on inhibitory development. The glutamatergic afferents from the ipsilateral cochlear nucleus are physically separate from the glycinergic afferents projection from the MNTB (Moore and Caspary, 1983; Glendenning et al., 1985; Spangler et al., 1985; Cant and Casseday, 1986; Wenthold et al., 1987; Zook and DiCaprio, 1988; Sanes, 1990; Wu and Kelly, 1992). Both excitatory and inhibitory synaptic transmission are prominent at birth, as assessed in the brain slice preparation (Sanes, 1993). Glycine receptor expression is also quite prominent at birth, as assessed by densitometric measures of ^{3}H-SN autoradiographs (Sanes and Wooten, 1987). Moreover, we recently showed that there is measurable spontaneous activity in gerbil auditory brain stem neurons during the second postnatal week (Kotak and Sanes, 1995). Therefore, it is likely that spontaneous inhibitory postsynaptic potentials (IPSP) are present before the onset of LSO neural response to sound stimulation (Sanes and Rubel, 1988).

The morphology of the LSO principal neuron dendrites was shown to vary along the LSO tonotopic axis in adult gerbils, with high frequency neurons having a more restricted field (Sanes et al., 1990). The mature dendritic pattern emerges during the third postnatal week as a result of regressive events, such as a reduction in dendritic branching, particularly in the high frequency projection region of the LSO (Sanes et al., 1992b). During this same period of time, glycinergic MNTB arborizations become spatially restricted within the LSO and there is a striking alteration in the distribution of glycine receptor density (Sanes and Wooten, 1987; Sanes and Takacs, 1993). Therefore, glycinergic connections within the LSO are initially quite pronounced and undergo developmental alterations that parallel dendritic maturation. The present study introduces an organotypic culture system that allows us to directly test whether spontaneous glycinergic synaptic transmission is able to regulate neuron shape.

MATERIALS AND METHODS

Organotypic Cultures

Gerbils (*Meriones unguiculatus,* 6–7 days postnatal) were used to produce static interface explant cultures (Stoppini et al., 1991). All of the explant procedures were performed under a laminar flow hood (Baker), and the equipment was ultraviolet irradiated for at least 4 h prior to the generation of cultures. The microdissection tools and glassware were sterilized in ethanol and flamed or were presterilized disposable plastic. Gerbil pups were deeply anesthetized with hypothermia (Sanes et al., 1992a), the brain was rapidly removed, and the brain stem was vibratome sectioned at 275–300 μm in minimum essential medium at 4°C.

Slices were examined under a stereomicroscope to insure that the full LSO (i.e., medial and lateral limbs) was present at both surfaces. The superior olive was then dissected away from the brain stem section by cutting lateral to the LSO along the facial nerve medial to the MNTB along the midline and dorsal to the LSO (Fig. 1). In some cultures, the MNTB was also removed to produce an LSO preparation. The explants were then passed through fresh media and transferred onto a filter insert (Millipore) within the well of a tissue culture plate. Each well contained 350 μL of sterilized medium, and the explant was held to the filter by surface tension. The culture medium contained 25% horse serum (GIBCO), 50% minimum essential medium (GIBCO), 25% Earle's balanced salt solution (GIBCO), 0.5 g/100 mL glucose, and 1 mL/100 mL of 200 m*M* L-glutamine (GIBCO); each component was filtered with a 0.22-μm filter unit (Falcon). Neither the minimum essential medium nor the Earle's balanced salt contained glycine. Culture medium was supplemented with 10 μM veratridine, the sodium channel agonist, to enhance synaptic activity. Experimental tissue was grown in the same media, with the addition of 2 μM of SN.

The multiwell plate was placed in a humidified incubator (Forma) with 5% CO_2 at 37°C. The explant culture was provided fresh medium every 24 h and eventually thinned to approximately 100 μm. On the fourth day *in vitro,* the explants were exposed to antimitotic agents for 24 h to suppress glial cell proliferation (e.g., 5-fluoro-2′-deoxyuridine, 10 μM; uridine, 10 μM; cytosine B-D-arabinofuranoside, 10 μM).

Labeling Cultured Neurons

To label LSO neurons or MNTB axonal projections, small crystals of biocytin (Sigma) were applied to the section at 6–13 days *in vitro* (DIV) and the tracer was allowed to transport for approximately 12 h (Kenan-Vaknin et al., 1992). The tissue was fixed for 24 h in a solution of 4% paraformaldehyde in 0.1 *M* phosphate buffer (PBS), rinsed in PBS, and the endogenous peroxidase was quenched in 1% H_2O_2. The tissue was then preincubated in 0.5% Triton X-100 for 1 h and then incubated in avidin-biotin-horseradish peroxidase complex (Vector Labs) for 2 h (Horikawa and Armstrong, 1988). The tissue was washed in 0.1 *M* Tris buffer solution, incubated in diaminobenzidine (50 mg/100 mL Tris buffer) for 15 min, and then reacted in a similar solution with H_2O_2.

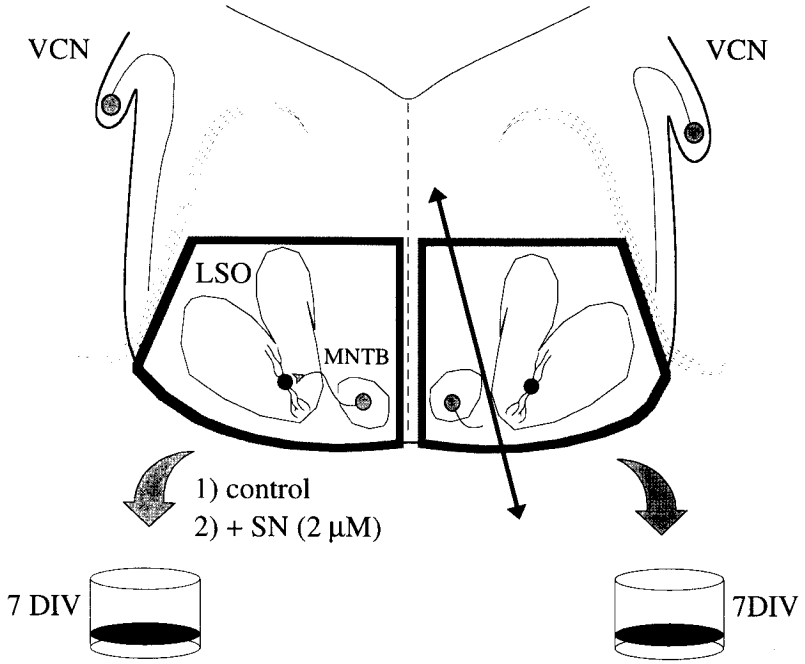

Figure 1 Schematic of the organotypic preparations. Sections through the MNTB and LSO were trimmed (dark gray lines) to produce preparations containing both nuclei (left) or only the LSO (right). All sections were incubated on filter inserts. LSO plus MNTB pieces were grown in normal media or in the presence of SN, and the pieces of LSO alone were grown in normal media (see Materials and Methods).

Image Acquisition and Analysis

The reconstructions and anatomical analyses of dendritic arborizations within the brain slices and organotypic auditory brain stem preparation were performed on a morphometric system (Cellmate/Treemate; Canaday et al., 1990). A video image (Dage 68) of slide mounted material was generated and displayed on a monitor such that the object of interest was traced with a mouse-driven cursor. The system recorded the *X*, *Y*, and *Z* position of the microscope stage to produce a 3-dimensional structure for analysis. To estimate the area occupied by each dendritic tree, a multigon was drawn around the cell connecting tips of all dendritic branches. The analysis software computed length, area, and diameter of the contours (Sanes et al., 1990, 1992a; Sanes and Chokshi, 1992).

Brain Slice Physiology

Animals of 5–7 days postnatal age were first deeply anesthetized (hypothermia) and then decapitated. The brain was rapidly dissected free in oxygenated artificial cerebrospinal fluid (ACSF: NaCl, 123 m*M*; KCl, 4 m*M*; KH_2PO_4, 1.2 m*M*; $MgSO_4$, 1.3 m*M*; $NaHCO_3$, 28 m*M*; glucose, 15 m*M*; $CaCl_2$, 2.4 m*M*; L-ascorbic acid, 0.4 m*M*; pH 7.4) at 10–15°C. Vibratome sections of 300 μm were superfused at 8 mL/min in a recording chamber at room temperature (24 ± 1°C). A bipolar stimulating electrode was placed on the MNTB, and electrical stimuli were delivered under computer control while synaptic responses were digitized at 10 kHz and stored for off-line analysis (Sanes, 1993). Whole-cell patch clamp recordings were obtained with recording electrodes fabricated from 1.5-mm outer diameter (OD) borosylicate glass microcapillaries (4–6 MΩ). The composition of the internal pipette solution in millimoles was: 130 potassium gluconate, 0.6 EGTA, 10 Hepes, 2 $MgCl_2$, 5 KCl, 2 ATP, 0.3 GTP (pH 7.2). When a gigaohm seal was obtained, the stray capacitance was compensated and the membrane was broken with suction. Recordings were obtained under current clamp conditions (Warner Instruments 501A), and the access resistance (10–50 MΩ) was compensated throughout the experiment. When an MNTB-evoked IPSP was isolated, SN-containing ACSF (2 μ*M*) was superfused.

RESULTS

Neonatal Glycinergic Synapses Are Blocked by SN

To determine whether MNTB-evoked inhibitory synaptic potentials could be completely suppressed by 2 μ*M* SN at postnatal day 5, just prior to the

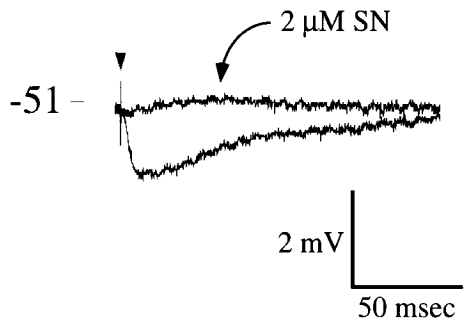

Figure 2 Strychnine blocks IPSPs at postnatal day 5. A whole-cell recording from an LSO neuron shows an MNTB-evoked IPSP (gray arrow denotes stimulus artifact) and its blockade in the presence of 2 μM SN. The resting potential was −51 mV.

earliest age of explantation, whole-cell recordings were made from LSO neurons in an acute brain slice preparation. As illustrated by the recording in Figure 2, SN at 2 μM, the concentration that was used for experimental cultures, was able to eliminate IPSPs elicited by electrical stimulation of the MNTB afferent pathway ($n = 3$).

Qualitative Description of Culture

The viability of organotypic cultures was assessed by placing biocytin crystals in the MNTB or the LSO. After 9 DIV, MNTB afferents continued to innervate the LSO in control and SN-treated sections [Fig. 3 (A,B)]. Depending upon the placement of biocytin, MNTB axons were labeled in the medial and lateral limbs of the LSO. When biocytin crystals were placed on the borders of the LSO, some neurons within the nucleus became fully labeled [Fig. 3 (C,D)]. While the dendritic processes of these LSO neurons were subjected to morphometric analysis (below), it was also of interest to determine if LSO axons sprouted within the nucleus borders. Some LSO neurons displayed thin processes of uniform caliber, consistent with an axonal morphology, that were found to branch and make boutonlike endings on neighboring LSO processes [Fig. 3 (E)]. For the purposes of morphometric analysis, all processes that displayed an uneven or tapering diameter were classified as dendrites.

Effect of SN on LSO Dendrites

The biocytin-filled neurons from cultures of LSO alone, LSO with MNTB, and LSO with MNTB plus 2 μM SN (Fig. 1), were identified and drawn with a computer-assisted morphometric system (Figs. 3, 4). Parametric comparisons were first made between neurons from control cultures and neurons from a previous data set of Golgi impregnated neurons obtained from gerbils at postnatal days 15–16 (from Sanes et al., 1992b). Table 1 shows values for number of branch points, total dendritic length, primary dendrites, and soma cross-sectional area, and contains details of the statistical comparisons. It was found that LSO neurons from control cultures of LSO + MNTB had significantly shorter dendrites and smaller somata compared to age-matched *in vivo* cells (postnatal days 15–16; Sanes et al., 1992a). The number of primary dendrites did not differ. Therefore, the deafferentation resulting from explantation apparently resulted in some neuronal atrophy.

As illustrated in Figure 4, control neurons had fewer branches compared to neurons from SN-treated cultures (Table 1). The number of branch points on neurons from SN-treated cultures was about 3 times greater compared to neurons in control cultures (18 ± 1.2 vs. 6.3 ± 1), and this difference was highly significant. The total dendritic length of neurons from SN-treated cultures was over 2 times greater compared to neurons in control cultures (1439 ± 92 vs. 676 ± 50). However, there was no significant difference among the groups for total number of primary dendrites nor was there a significant difference in soma cross-sectional area (Table 1). Although the cells shown in Figure 4 suggest that the control LSO neurons occupied a larger area, a quantitative analysis indicated otherwise. The mean area of multigons that surrounded dendritic trees was $18{,}477 \pm 2{,}702\ \mu m^2$ in control neurons ($n = 21$) and $22{,}150 \pm 2{,}476\ \mu m^2$ in SN-treated neurons ($n = 25$). These values were not significantly different ($t = 0.3217$; df $= 44$; $p > 0.05$).

Effect of Time *In Vitro*

To determine whether the duration of time spent in culture had an effect on dendritic morphology, slices were incubated for 6–13 days. For a given set of culture conditions, the number of dendritic branch points was compared between two durations (Table 2). For unmanipulated cultures, there was no significant difference between the number of branches from neurons grown for 7–9 DIV (6.8 ± 1.3) compared to those grown for 10–13 DIV (5.7 ± 1.5). For SN-treated neurons, there was no significant difference between the number of branches from neurons grown for 9 DIV (18.1

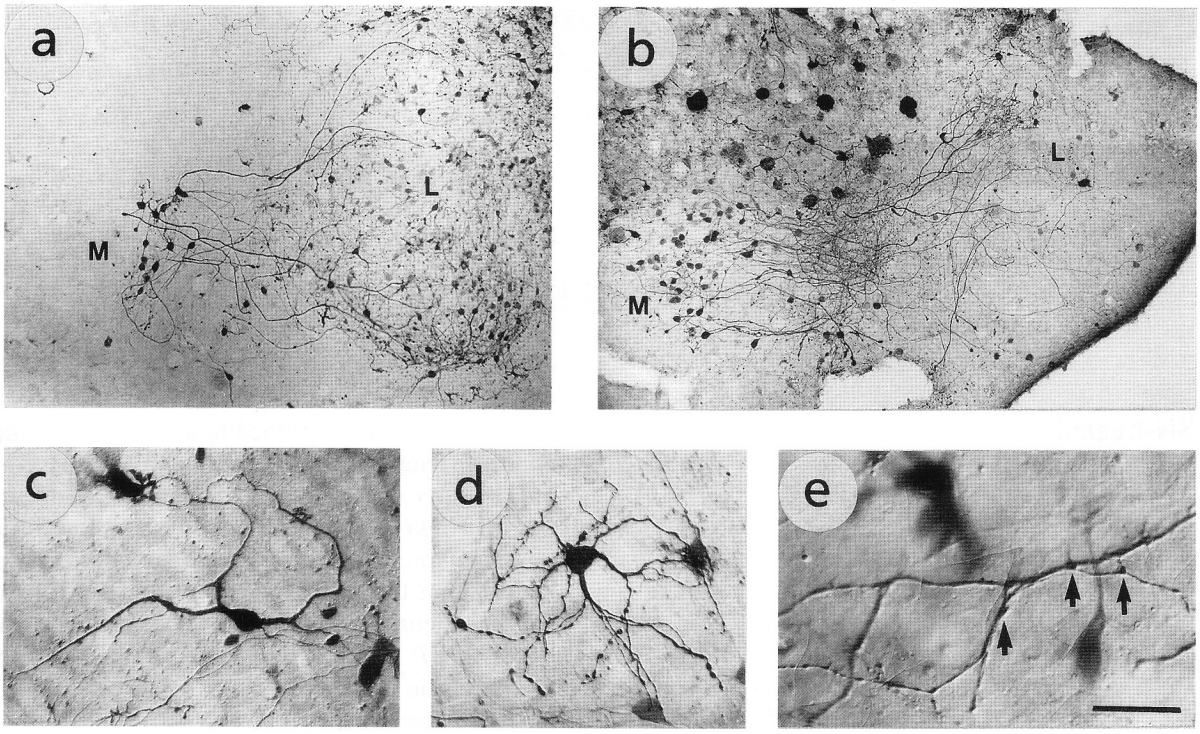

Figure 3 Viability of MNTB and LSO neurons in organotypic culture. (A) Biocytin-filled MNTB cell bodies and afferents to the LSO in an organotypic culture from a postnatal day 6 animal grown in normal media for 7 DIV. (B) Biocytin-filled MNTB cell bodies and afferents to the LSO in an organotypic culture from a postnatal day 7 animal grown in SN-containing media for 8 DIV. (C) Biocytin-filled LSO neuron in an organotypic culture from a postnatal day 6 animal grown in normal media for 10 DIV. (D) Biocytin-filled LSO neuron in an organotypic culture from a postnatal day 6 animal grown in SN-containing media for 9 DIV. (E) Boutonal contacts between an LSO axon (arrows) and an LSO dendrite in a control culture. M, medial nucleus of the trapezoid body; L, lateral superior olive. Scale bar = (A,B) 200 μm, (C,D) 50 μm, (E) 20 μm.

$\pm$ 1.7) compared to those grown for 10–11 DIV (17.9 $\pm$ 1.9). For LSO neurons grown without the MNTB, there was no significant difference between the number of branches from neurons grown for 6 DIV (5.8 $\pm$ 0.7) compared to those grown for 9 DIV (5.9 $\pm$ 0.9). Because no age effects were observed, we pooled all values within the same culture conditions.

Effect of MNTB Terminals

To determine whether the physical elimination of MNTB arbors could reproduce the effects of SN, we cultured explants of LSO alone (Fig. 1, right). As shown in Table 1, the length and branching of biocytin-filled LSO dendrites was not significantly different compared to neurons from control sections (MNTB + LSO). However, there was a significant difference in the soma cross-sectional area. Elimination of the MNTB led to a 37% increase in LSO soma size compared to cultures of LSO with MNTB (268 $\pm$ 18 vs. 197 $\pm$ 13) (see Table 1).

DISCUSSION

The present results support and extend our previous *in vivo* findings suggesting that glycinergic transmission regulates postsynaptic dendrite morphology during development (Sanes et al., 1992a; Sanes and Chokshi, 1992; Aponte et al., 1996). In contrast to the *in vivo* studies, the organotypic preparation allows complete blockade of glycine-gated transmission. This was verified in the acute postnatal day 5 brain slice preparation by demonstrating that MNTB-evoked IPSPs were completely blocked by 2 μM SN (Fig. 2). Such a manipulation would induce seizures and death in the

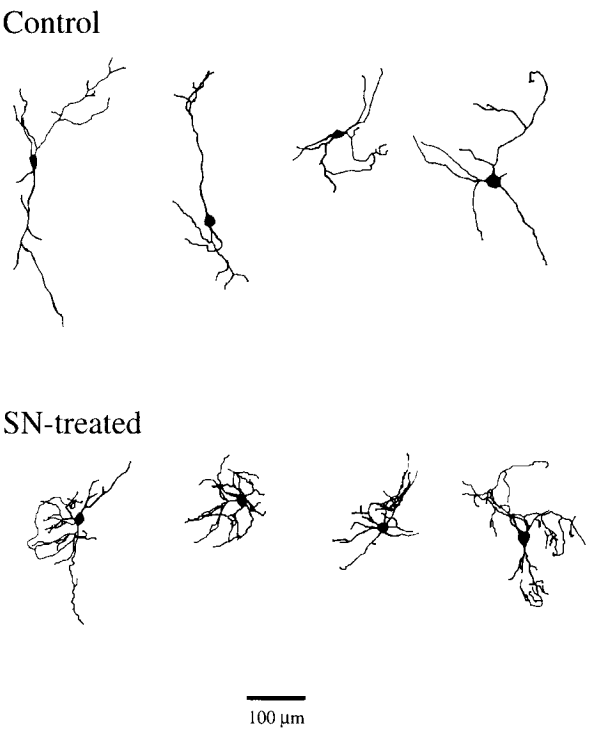

Figure 4 Tracings of control and SN-treated LSO neurons. The SN-treated neurons displayed highly branched dendritic patterns.

animal. The survival of the inhibitory MNTB neurons and their target LSO neurons in culture was evident from the biocytin labeling. MNTB afferents [Fig. 3 (A,B)], and LSO dendritic arborizations [Fig. 3 (C,D)] were readily labeled after 6–13 days *in vitro*.

In SN-treated explants, there was a threefold increase in LSO dendritic branching compared to cultured control neurons (Table 1). This extensive branching was accompanied by an increase in total dendritic length (Table 1), although LSO neurons did not appear to occupy a greater area than control neurons (Fig. 4). These results are fully consistent with previous *in vitro* experiments demonstrating that the inhibitory transmitter GABA can regulate neurite outgrowth (Michler-Stuke and Wolff, 1987; Mattson and Kater, 1989). Despite the dendritic hypertrophy, SN treatment did not alter LSO soma size (Table 1).

A second experimental culture, in which the glycinergic MNTB projection was physically removed, did *not* mimic the effects of SN treatment. However, a large fraction of LSO neurons are themselves glycinergic (Helfert et al., 1989; Saint Marie et al., 1989; Wenthold et al., 1987; Henkel and Brunso-Bechtold, 1995; D.H.S., unpub. observ.); and clear examples of local sprouting were found in the cultured LSO. Therefore, it is likely that LSO neurons innervated one another, thus providing a source of glycinergic innervation in the absence of the MNTB. However, the present

Table 2 Number of Dendritic Branch Points

	n	Days *In Vitro*	Branches ($x \pm$ S.E.)
Control	12	7–9	6.8 ± 1.3
	9	10–13	5.7 ± 1.5
LSO alone	12	6	5.8 ± 0.7
	18	9	5.9 ± 0.9
SN treated	14	9	18.1 ± 1.7
	11	10–11	17.9 ± 1.9

Table 1 Parametric Comparisons

	Branch Points[1]	Total Length[2]	Primary Dendrites[3]	Soma Area[4]
Control (n = 21)	6.3 ± 1.0	676 ± 79	3.5 ± 0.3	197 ± 17*
SN treated (n = 25)	18.0 ± 0.9†	1439 ± 72†	5.1 ± 0.3	203 ± 16*
LSO alone (n = 30)	5.9 ± 0.9	818 ± 66	4.3 ± 0.3	269 ± 14
PND 15–16 (n = 47) (Golgi-stained sections)	9.0 ± 0.7§	930 ± 52	4.3 ± 0.4	266 ± 9.5

PND, postnatal day.

[1] one-way ANOVA ($p < 0.00001$, df = 3, $F = 35.10$).

[2] One-way ANOVA ($p < 0.00001$, df = 3, $F = 35.10$).

[3] One-way ANOVA ($p > 0.05$, df = 3, $F = 2.06$).

[4] One-way ANOVA ($p < 0.00001$, df = 3, $F = 35.10$).

* Tukey-Kramer HSD: significantly different from LSO alone ($p < 0.01$, $t = 3.18$) and PND 15–16 ($p < 0.01$, $t = 3.18$).

† Tukey-Kramer HSD: significantly different from control ($p < 0.00001$, $t = 7.29$), LSO alone ($p < 0.00001$, $t = 7.29$), and PND 15–16 ($p < 0.00001$, $t = 7.29$).

‡ Tukey-Kramer HSD: significantly different from control ($p < 0.00001$, $t = 6.65$), LSO alone ($p < 0.001$, $t = 4.49$), and PND 15–16 ($p < 0.001$, $t = 4.49$).

§ Tukey-Kramer HSD: significantly different from SN treated ($p < 0.00001$, $t = 7.29$) and LSO alone ($p < 0.05$, $t = 2.60$).

experiments do not exclude the possibility that the SN effects were mediated by GABA or acetylcholine receptors (Trombley and Shepherd, 1994; Erostegui et al., 1994).

The extent of growth was also assessed with respect to previously published *in vivo* measures from Golgi impregnated LSO neurons (Sanes et al., 1992b). Clearly, the following conclusions must be treated with caution due to the difference in technique (*in vivo:* Golgi; present study: biocytin). Control explants displayed significantly atrophic dendrites compared to postnatal days 15–16 cells *in situ,* but SN-treated neuronal dendrites were hypertrophic (Table 1). It is interesting that LSO soma size does not seem to depend on glycinergic transmission. The maximum soma area of control or SN-treated LSO neurons was significantly smaller compared to postnatal days 15–16 cells *in situ* (Table 1). However, explants in which MNTB afferents were removed exhibited normal LSO soma size. These results suggest that the physical presence of MNTB arbors restricts LSO soma growth, particularly when the normal complement of excitatory afferents is absent (i.e., in the explant cultures) and the signal is not glycine. Regulation of glycine receptors in goldfish Mauthner neurons also seems to depend on innervation by glycinergic terminals, but not transmission. Whereas partial removal of glycine-containing terminals alters the distribution of the 93-kDa glycine receptor associated protein, SN exposure has no affect (Seitanidou et al, 1992).

The cellular mechanism by which inhibitory synapses regulate postsynaptic shape remains unknown, and the following discussion is speculative. First, a decrease in glycinergic inhibition may effect postsynaptic pH, as demonstrated for GABAergic systems (Kaila and Voipio, 1987; Kaila et al., 1990, 1992). Second, inhibitory transmission may also lead to increases or decreases of the intracellular free Ca^{2+} concentration (Yuste and Katz, 1991; Obrietan and van den Pol, 1995; Callaway et al., 1995; Lo et al., 1995). For example, Kandler and Friauf (1995) showed that MNTB stimulation produces depolarizing IPSPs during the first postnatal week in the rat LSO. Third, inhibitory transmission may influence the synthesis of trophic factors (Zafra et al., 1991).

Finally, inhibitory transmission may effect changes at excitatory synapses. For example, NMDA receptor-mediated conductances are increased by depolarizing GABAergic potentials (Staley et al., 1995) and by the extracellular alkalization resulting from HCO_3^- efflux through the GABA-gated chloride channel (Traynelis and Cull–Candy, 1991; Vyklicky et al., 1990; Chen and Chesler, 1992; Kaila et al., 1993). In fact, we recently demonstrated that decreasing glycinergic transmission for as little as 24 h dramatically alters postsynaptic physiology, including an enhanced NMDA receptor-mediated response (Kotak and Sanes, 1996). These results suggest that inhibitory afferents may regulate cell shape indirectly by modifying excitatory synaptic strength. The organotypic preparation should allow us to assess each of these hypothetical mechanisms and their relationship to dendrogenesis.

We thank Dr. Vibhakar Kotak for performing the acute brain slice recordings. This work was supported by NSF IBN-9308377 and NIH DC00540.

REFERENCES

APONTE, J. E., KOTAK, V. C., SANES, D. H. (1996). Decreased synaptic inhibition leads to dendritic hypertrophy prior to the onset of hearing. *Aud. Neurosci.,* **2:** 235–240.

BENES, F. M., PARKS, T. N., and RUBEL, E. W. (1977). Rapid dendritic atrophy following deafferentation, an EM morphometric analysis. *Brain Res.* **122:**1–13.

BERRY, M., MCCONNELL, P., and SIEVERS, J. (1980). Dendritic growth and the control of neuronal form. *Curr. Topics Dev. Biol.* **15:**67–101.

CALLAWAY, J. C., LASSER-ROSS, N., and ROSS, W. N. (1995). IPSPs strongly inhibit climbing fiber-activated $[Ca2+]_i$ increases in the dendrites of cerebellar Purkinje neurons. *J. Neurosci.* **15:**2777–2787.

CANADAY, M., HILLMAN, D. E., and MAHONEY, G. (1990) Neuronal arbor recording and analysis. In: *Three-Dimensional NeuroImaging.* A. Toga, Ed. Raven, New York, pp. 87–100.

CANT, N. B. and CASSEDAY, J. H. (1986). Projections from the anteroventral cochlear nucleus to the lateral and medial superior olivary nuclei. *J. Comp. Neurol.* **247:**457–476.

CHEN, J. C. and CHESLER, M. (1992). Modulation of extracellular pH by glutamate and GABA in rat hippocampal slices. *J. Neurophysiol.* **67:**29–36.

DEITCH, J. S. and RUBEL, E. W. (1984). Afferent influences on brain stem auditory nuclei of the chicken. Time course and specificity of dendritic atrophy following deafferentation. *J. Comp. Neurol.* **229:**66–79.

EROSTEGUI, C., NORRIS, C. H., and BOBBIN, R. P. (1994). *In vitro* pharmacologic characterization of a cholinergic receptor on outer hair cells. *Hearing Res.* **74:**135–147.

FENG, A. S. and ROGOWSKI, B. A. (1980). Effects of monaural and binaural occlusion on the morphology

of neurons in the medial superior olivary nucleus of the rat. *Brain Res.* **189**:530–534.

GLENDENNING, K. K., HUTSON, K. A., NUDO, R. J., and MASTERTON, R. B. (1985). Acoustic chiasm II. Anatomical basis of binaurality in lateral superior olive of cat. *J. Comp. Neurol.* **232**:261–285.

HARRIS, R. M. and WOOLSEY, T. A. (1981). Dendritic plasticity in mouse barrel cortex following postnatal vibrissa follicle damage. *J. Comp. Neurol.* **196**:357–376.

HELFERT, R. H., BONNEAU, J. M., WENTHOLD, R. J., and ALTSCHULER, R. A. (1989). GABA and glycine immunoreactivity in the guinea pig superior olivary complex. *Brain Res.* **501**:269–286.

HENKEL, C. K. and BRUNSO-BECHTOLD, J. K. (1995). Development of glycinergic cells and puncta in nuclei of the superior olivary complex of the postnatal ferret. *J. Comp. Neurol.* **354**:470–480.

HORIKAWA, K. and ARMSTRONG, W. E. (1988). A versatile means of intracellular labeling, injection of biocytin and its detection with avidin conjugates. *J. Neurosci. Methods* **25**:1–11.

KAILA, K., PAALASMAA, P., TAIRA, T., and VOIPIO, J. (1992). pH transients due to monosynaptic activation of $GABA_A$ receptors in rat hippocampal slices. *NeuroReport* **3**:105–108.

KAILA, K., SAARIKOSKI, J., and VOIPIO, J. (1990). Mechanism of action of GABA on intracellular pH and on surface pH in crayfish muscle fibres. *J. Physiol.* **427**:241–260.

KAILA, K. and VOIPIO, J. (1987). Postsynaptic fall in intracellular pH induced by GABA-activated bicarbonate conductance. *Nature* **330**:163–165.

KAILA, K., VOIPIO, J., PAALASMAA, P., PASTERNAK, M., and DEISZ, R. A. (1993). The role of bicarbonate in $GABA_A$ receptor-mediated IPSPs of rat neocortical neurons. *J. Physiol.* **464**:273–289.

KALB, R. G. (1994). Regulation of motor neuron dendrite growth by NMDA receptor activation. *Development* **120**:3063–3071.

KANDLER, K. and FRIAUF, E. (1995). Development of glycinergic and glutamatergic synaptic transmission in the auditory brainstem of perinatal rats. *J. Neurosci.* **16**:6890–6904.

KENAN-VAKNIN, G., KATZ, H., and MALACH, R. (1992). Golgi-like staining of visual cortex cells obtained by extracellular biocytin application *in vitro*. *Brain Res.* **571**:338–341.

KITZES, L. M., HAGEYAMA, G. H., SEMPLE, M. N., and KIL, J. (1995). Development of ectopic projections from the ventral cochlear nucleus to the superior olivary complex induced by neonatal ablation of the contralateral cochlea. *J. Comp. Neurol.* **353**:341–363.

KOTAK, V. C. and SANES, D. H. (1995). Synaptically-evoked prolonged depolarizations in the developing central auditory system. *J. Neurophysiol.* **74**:1611–1620.

KOTAK, V. C., and SANES, D. H. (1996). Developmental influence of glycinergic inhibition: regulation of NMDA-mediated EPSPs. *J. Neurosci.* **16**:1836–1843.

LEVI-MONTALCINI, R. (1949). The development of the acoustico-vestibular centers in the chick embryo in the absence of the afferent root fibers and of descending fiber tracts. *J. Comp. Neurol.* **91**:209–241.

LO, Y.-J., RAO, S. C., and SANES, D. H. (1995). Modulation of Ca^{2+} levels in developing inferior colliculus by inhibitory neurotransmission. *Soc. Neurosci. Abstr.* **21.**

MATTSON, M. P. and KATER, S. B. (1989). Excitatory and inhibitory neurotransmitters in the generation and degeneration of hippocampal neuroarchitecture. *Brain Res.* **478**:337–348.

MICHLER-STUKE, A. and WOLFF, J. R. (1987). Facilitation and inhibition of neuron elongation by GABA in chick tectal neurons. In: *Neurotrophic Activity of GABA During Development. Neurology and Neurobiology,* Vol. 32. D. A. Redburn and A. Schousboe, Eds. Alan R. Liss, New York, pp. 253–266.

MOORE, M. J. and CASPARY, D. M. (1983). Strychnine blocks binaural inhibition in lateral superior olivary neurons. *J. Neurosci.* **3**:237–247.

OBRIETAN, K. and VAN DEN POL, A. N. (1995). GABA neurotransmission in the hypothalamus: developmental reversal from Ca^{2+} elevating to depressing. *J. Neurosci.* **15**:5065–5077.

PARKS, T. N. (1981). Changes in the length and organization of nucleus laminaris dendrites after unilateral otocyst ablation in chick embryos. *J. Comp. Neurol.* **202**:47–57.

RAKIC, P. (1972). Extrinsic cytological determinants of basket and stellate cell dendritic pattern in the cerebellar molecular layer. *J. Comp. Neurol.* **146**:335–354.

RAKIC, P. and SIDMAN, R. L. (1973). Organization of cerebellar cortex secondary to deficit of granule cells in weaver mutant mice. *J. Comp. Neurol.* **152**:133–162.

SAINT MARIE, R. L., OSTAPOFF, E. M., MOREST, D. K., and WENTHOLD, R. J. (1989). Glycine-immunoreactive projection of the cat lateral superior olive: possible role in midbrain ear dominance. *J. Comp. Neurol.* **279**:382–396.

SANES, D. H. (1990). An *in vitro* analysis of sound localization mechanisms in the gerbil lateral superior olive. *J. Neurosci.* **10**:3494–3506.

SANES, D. H. (1993). The development of synaptic function and integration in the central auditory system. *J. Neurosci.* **13**:2627–2637.

SANES, D. H. and CHOKSHI, P. (1992). Glycinergic transmission influences the development of dendritic shape. *NeuroReport* **3**:323–326.

SANES, D. H., GOLDSTEIN, N. A., OSTAD, M., and HILLMAN, D. E. (1990). Dendritic morphology of central auditory neurons correlates with their tonotopic position. *J. Comp. Neurol.* **294**:443–454.

SANES, D. H., MARKOWITZ, S., BERNSTEIN, J., and WARDLOW, J. (1992a). The influence of inhibitory

afferents on the development of postsynaptic dendritic arbors. *J. Comp. Neurol.* **321**:637–644.

SANES, D. H. and RUBEL, E. W. (1988). The ontogeny of inhibition and excitation in the gerbil lateral superior olive. *J. Neurosci.* **8**:682–700.

SANES, D. H., SONG, J., and TYSON, J. (1992b). Refinement of dendritic arbors along the tonotopic axis of the gerbil lateral superior olive. *Dev. Brain Res.* **67**: 47–55.

SANES, D. H. and TAKACS, C. (1993). Activity-dependent refinement of inhibitory connections. *Eur. J. Neurosci.* **5**:570–574.

SANES, D. H. and WOOTEN, G. F. (1987). Development of glycine receptor distribution in the lateral superior olive of the gerbil. *J. Neurosci.* **7**:3803–3811.

SEITANIDOU, T., NICOLA, M.-A., TRILLER, A., and KORN, H. (1992). Partial glycinergic denervation induces transient changes in the distribution of a glycine receptor-associated protein in a central neuron. *J. Neurosci.* **12**:116–131.

SPANGLER, K. M., WARR, W. B., and HENKEL, C. K. (1985). The projections of principal cells of the medial nucleus of the trapezoid body in the cat. *J. Comp. Neurol.* **238**:249–262.

STALEY, K. J., SOLDO, B. L., and PROCTOR, W. R. (1995). Ionic mechanisms of neuronal excitation by inhibitory $GABA_A$ receptors. *Science* **269**:977–981.

STOPPINI, L., BUCHS, P. A., and MULLER, D. (1991). A simple method for organotypic cultures of nervous tissue. *J. Neurosci. Methods* **37**:173–182.

TRAYNELIS, S. F. and CULL-CANDY, S. G. (1991). Pharmacological properties and H^+ sensitivity of excitatory amino acid receptor channels in rat cerebellar granule neurones. *J. Physiol.* **433**:727–763.

TROMBLEY, P. Q. and SHEPHERD, G. M. (1994). Glycine exerts potent inhibitory actions on mammalian olfactory bulb neurons. *J. Neurophysiol.* **71**:761–767.

TRUNE, D. R. (1982). Influence of neonatal cochlear removal on development of mouse cochlear nucleus: II. Dendritic morphometry of its neurons. *J. Comp. Neurol.* **209**:425–434.

VALVERDE, F. (1968). Structural changes in the area striata of the mouse after enucleation. *Exp. Brain Res.* **5**:274–292.

VAUGHN, J. E., BARBER, R. P., and SIMS, T. J. (1988). Dendritic development and preferential growth into synaptogenic fields: a quantitative study of Golgi-impregnated spinal motor neurons. *Synapse* **2**:69–78.

VOGEL, M. W. and PRITTIE, J. (1995). Purkinje cell dendritic arbors in chick embryos following chronic treatment with an *N*-methyl-D-aspartate receptor antagonist. *J. Neurobiol.* **26**:537–552.

VYKLICKY, L., VLACHOVA, V., and KRUSEK, J. (1990). The effect of external pH on responses to excitatory amino acids in mouse hippocampal neurones. *J. Physiol.* **430**:497–517.

WENTHOLD, R. J., HUIE, D., ALTSCHULER, R. A., and REEKS, K. A. (1987). Glycine immunoreactivity localized in the cochlear nucleus and superior olivary complex. *Neuroscience* **22**:897–912.

WU, S. H. and KELLY, J. B. (1992). Synaptic pharmacology of the superior olivary complex studied in mouse brain slice. *J. Neurosci.* **12**:3084–3097.

YUSTE, R. and KATZ, L. (1991). Control of postsynaptic Ca^{2+} influx in developing neocortex by excitatory and inhibitory neurotransmitters. *Neuron* **6**:333–344.

ZAFRA, F., CASTRÉN, E., THOENEN, H., and LINDHOLM, D. (1991). Interplay between glutamate and γ-aminobutyric acid transmitter systems in the physiological regulation of brain-derived neurotrophic factor and nerve growth factor synthesis in hippocampal neurons. *Proc. Natl. Acad. Sci.* **88**:10037–10041.

ZOOK, J. M. and DICAPRIO, R. A. (1988). Intracellular labeling of afferents to the lateral superior olive in the bat, *Eptesicus fuscus*. *Hearing Res.* **34**:141–148.