Available online at www.sciencedirect.com
SCIENCE DIRECT
Brain Research 989 (2003) 122–127
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Short communication

Glycine mediated alterations in intracellular pH

Joshua S. Green[a], Vibhakar C. Kotak[a], Dan H. Sanes[a,b,*]

[a]*Center for Neural Science, New York University, New York, NY 10003, USA*
[b]*Department of Biology, New York University, New York, NY 10003, USA*

Accepted 30 June 2003

Abstract

Glycinergic transmission shapes the coding properties of the lateral superior olivary nucleus (LSO). We investigated intracellular pH responses in the LSO to glycine using BCECF-AM in brain slices. With extracellular bicarbonate, glycine produced an alkalinization followed by an acidification while, in the nominal absence of bicarbonate, glycine produced acidifications. Separately, in whole-cell recordings from LSO neurons, glycine caused hyperpolarization followed by long-lasting depolarization. While the bicarbonate-dependent intracellular alkalinization could be related to chloride/bicarbonate exchange, bicarbonate-independent acidification may be triggered by depolarization.
© 2003 Elsevier B.V. All rights reserved.

Theme: Neurotransmitters, transporters and receptors

Topic: Other neurotransmitters

Keywords: Inhibition; Glycine; pH; Auditory

Neural activity modulating functional properties often involves ligand- or voltage-gated conductance. Neurotransmitters can affect pH [7] leading to modified ion channel or receptor and intracellular enzyme function [7,19]. The present study examined the effect of glycine on intracellular pH. The LSO receives inhibitory afferents from the medial nucleus of the trapezoid body (MNTB) [2,4,20,24]. Although this innervation is glycinergic and GABAergic [16], we focused on glycine because unlike GABA [9,16,17], it binds only to ionotropic receptors [1]. Furthermore, glycine receptors are primarily postsynaptic, with one exception [3,30].

Gerbils (*Meriones unguiculatus*) aged 7–13 days postnatal were anaesthetized with chloral hydrate (350 mg/kg body weight), and 300 μm brainstem slices were incubated for 20 min and treated with the acetoxymethoxy ester of 2′,7′-bis(carboxyethyl)-5,6-carboxyfluorescein (BCECF-AM; Molecular Probes, 100 μg in 20% pluronic acid–dimethylsulfoxide in aCSF: dye concentration 20 μM) in the dark for 45 min. The aCSF contained (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 (pH 7.3). Slices were kept at 23±1 °C throughout all experiments. Slices were superfused at 7 ml/min with aCSF (95% O_2–5% CO_2 bubbling). Dye efficacy was verified by fluorescence ratio changes following acidic (50 μl 0.1 M acetate) or alkaline (50 μl 0.1 M NaOH) challenges. All glycine superfusions were 5 mM for 1 min. In three experiments, HEPES was substituted for bicarbonate (100% O_2 bubbling) and after a 10–30 min equilibration period, glycine was superfused during HEPES–aCSF treatment. To investigate $GABA_A$ receptor involvement, muscimol (50 μM) was used.

Presumptive neurons were distinguished morphologically [23]. Excitation wavelengths were 440 and 495 nm while images were collected at 0.016–0.33 Hz. The fluorescent signal was obtained at 530 nm using a GenSysII intensifier and a CCD72 camera (Dage-MTI). ACQUISITION software (Ratiotool, Inovision) was used to collect and store data. Off-line analyses were performed using ORIGIN (MicroCal). Averaged responses were plotted against time and durations were obtained from this plot as the time from when pH changed from the baseline to the beginning of recovery. Response magnitudes extracted

*Corresponding author. Tel.: +1-212-998-3924; fax: +1-212-995-4348.
E-mail address: sanes@cns.nyu.edu (D.H. Sanes).

0006-8993/03/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0006-8993(03)03340-7

from the average response were analyzed as the percentage change from baseline ratio ($F - F_0/F_0$).

For electrophysiology experiments, P9–13 gerbils were used to make 300 μM slices through the LSO. The aCSF (pH 7.3 when bubbled with 95% O_2–5% CO_2) was superfused at 4 ml/min (23 °C). Whole-cell current-clamp recordings were obtained from LSO neurons [15]. Hyperpolarizing current (50 pA, 300 ms) at 0.5 Hz was injected during glycine treatments to obtain input resistance. The internal recording solution contained (mM) 127.5 potassium gluconate, 0.6 EGTA, 10 HEPES, 2 $MgCl_2$, 5 KCl, 2 ATP, and 0.3 GTP (pH 7.2). Tonic effects of glycine (5 mM) were assessed by bath perfusion for 50 s ($n=8$) and its focal effect by 100 ms duration puffs ($n=3$). Data were recorded and analyzed on Axotape (Axon). All animal experiments were in accord with NIH guidelines.

In 31/43 slices, glycine superfusion for 1 min produced

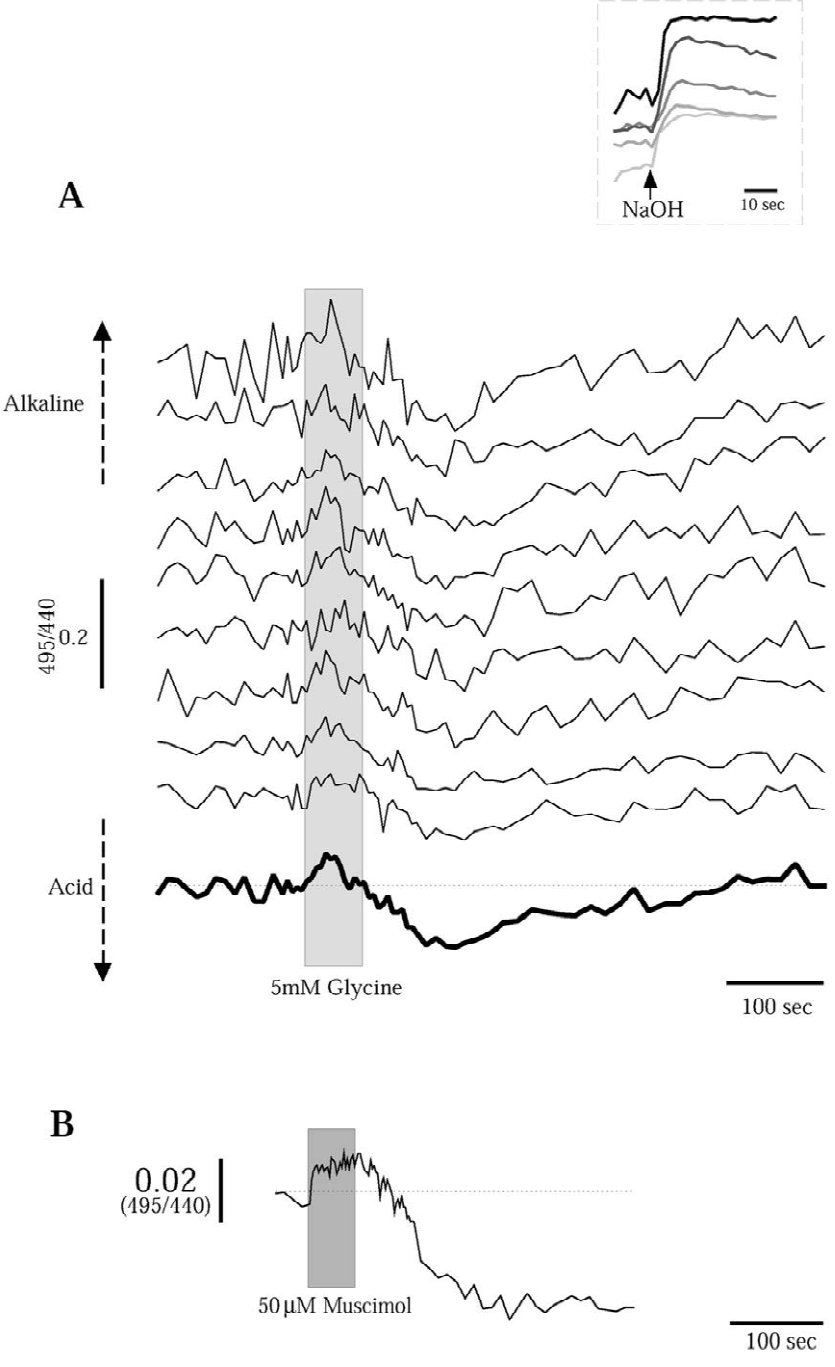

Fig. 1. Effect of glycine on intracellular pH. (A) Response of nine neurons (average at bottom) in the presence of bicarbonate shows an alkalinization and acidification. Inset: NaOH (0.1 M) increases the ratiometric response. Calibration bars are in units of ratiometric dye response at 495 nm/response at 440 nm. (B) Muscimol (50 μM, 50 s) elicited a biphasic response: an alkalinization followed by acidification.

a biphasic response; an alkalinization followed by acidification (no change in 2/43 slices). Of 41 responsive slices, 39 exhibited acidification and 33 showed alkalinization (Fig. 1). Eight slices showed acidification only and two responded only with alkalinization. The average response durations were 54±36 s for alkalinization (mean±S.D.; $n=33$) and 210±95 s for acidification (mean±S.D.; $n=23$; sixteen acidifications were nonreversible, one experiment was interrupted during acidification). Muscimol superfusion resulted in alkalinizations in 5/8 slices (44±22 s; mean±S.D.), acidifications in 7/8 slices (297±74 s mean±S.D.; $n=5$ slices in which minimal recovery permitted duration calculation), or both in 5/8 slices; alkalinization preceded the acidification (Fig. 1B). Muscimol evoked acidifications were significantly longer than glycine evoked acidifications ($P=0.02$, Wilcoxon–Mann–Whitney rank sum test) but the durations of alkalinizations did not significantly differ ($P=0.85$, Wilcoxon–Mann–Whitney rank sum test). The absence of an uptake mechanism for muscimol may impede recovery to baseline pH.

To assess whether these pH transients were dependent upon trans-membrane flux of bicarbonate, we superfused glycine in nominally bicarbonate-free aCSF. In all cases, this abolished the alkalinization ($n=3$ slices; Fig. 2) while acidification persisted; the difference from slices in normal aCSF was significant ($P=0.02$, Fisher's exact test). Responses in these slices always had alkalinizations when treated in normal aCSF ($n=2$ slices tested before switching to bicarbonate-free medium, $n=2$ slices tested after restoring bicarbonate). The durations of acidification in bicarbonate-free buffer were 554 s and 111 s (one acidifi-

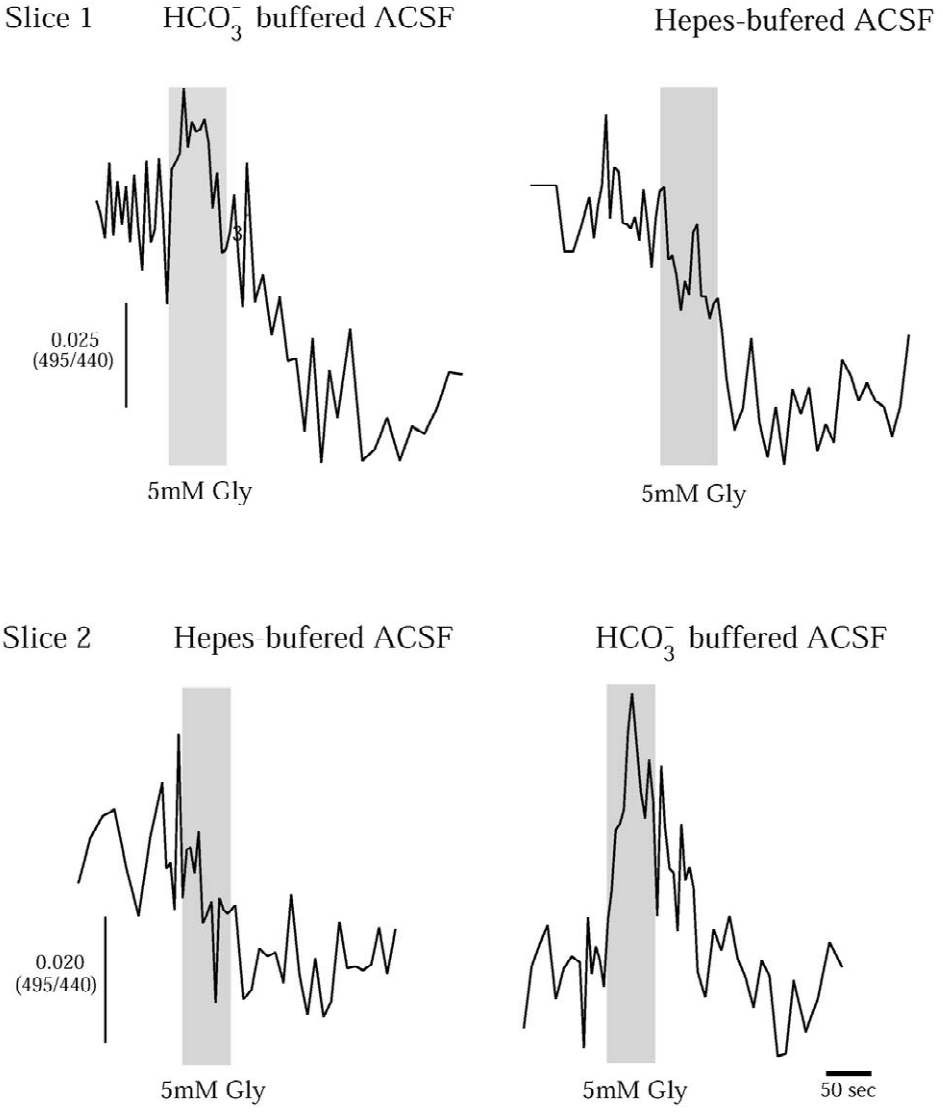

Fig. 2. Intracellular change in pH is bicarbonate dependent. Top: glycine elicits a biphasic response in the presence of bicarbonate (left) but causes acidification in nominally bicarbonate-free buffer (right; average responses). Bottom: in nominally bicarbonate-free buffer (left), acidification was observed. The alkaline response returned when bicarbonate was restored (right).

cation interrupted by an early return to bicarbonate medium).

Application of glycine (50 s) evoked a biphasic membrane potential response (Fig. 3). A hyperpolarization (1–5 mV, 2–15 s) was accompanied by up to 25% decreased membrane resistance; this was not significant because of high baseline variance [R_{input} before glycine treatment: 328±32 MΩ; vs. during peak hyperpolarization: 265±22 MΩ; (mean±S.E.M.); pairwise comparison of means by Student's *t*-test: $P>0.05$, $n=8$)]. This response was followed by a reversible depolarization (up to 15 mV, up to 10 min). In contrast to hyperpolarization, depolarization was accompanied by a significant drop in input resistance (up to 90%; input resistance before glycine treatment: 328±32 MΩ; vs. during peak depolarization: 97±14 MΩ; mean±S.E.M.; pairwise comparison of mean by Student's *t*-test; $P<0.001$, $n=8$). However, focal application of glycine (50 ms) via a pipette close to the recording site (200 μM) produced only hyperpolarizations (9.8±1.4 mV, $n=3$).

Glycine elicited a biphasic pH response in LSO neurons; an initial bicarbonate-dependent alkalinization was fol-

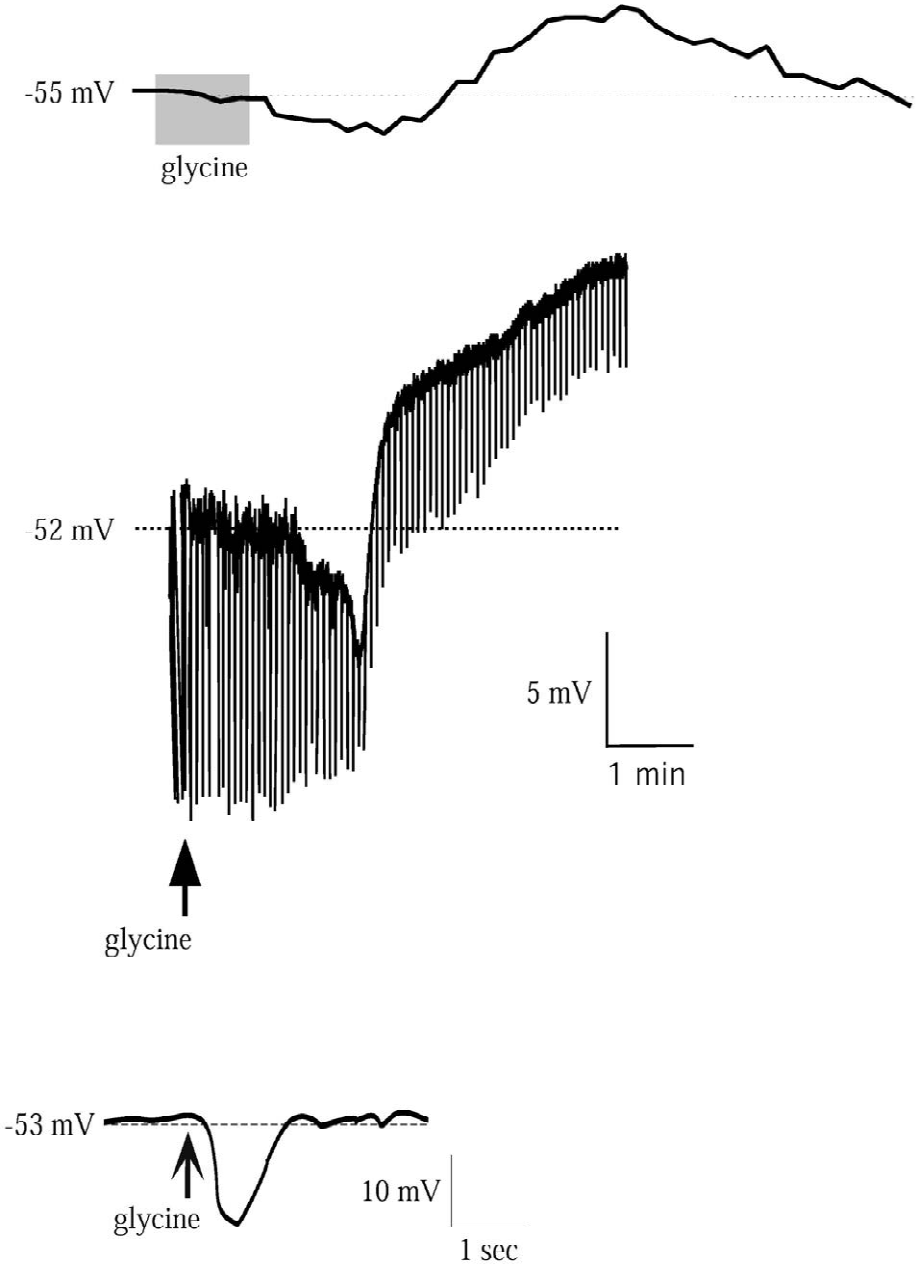

Fig. 3. Glycine induces a biphasic change in V_m. Top: whole cell recording from an LSO neuron showed that tonic application of glycine (5 mM, 50 s, gray bar) produced hyperpolarization followed by reversible depolarization. Resting potentials appear on left. Middle: another recording shows decreased membrane resistance during both phases. Bottom: whole cell recording showing focal application of glycine (5 mM, 50 ms) by a puffer pipette elicited a brief hyperpolarization. In this, physiological levels of intracellular chloride may be altered by chloride in the internal recording solution.

lowed by an extended acidification (Fig. 1). Although measured separately, we predict that this biphasic response may be related to biphasic membrane potential change by glycine. We propose that the alkalinization may be caused by chloride/bicarbonate exchange whereas the acidification may be a linked to membrane depolarization.

Glycine and GABA receptors are permeable to bicarbonate in addition to chloride [1,11,14]. Hence, glycine-induced changes in pH could result by a flux of bicarbonate across the membrane. While this is valid for GABA-evoked acidifications in some preparations [5,6,10,12,13,27,31,32], the current glycine and muscimol related transients seem different for two reasons. The equilibrium potential for bicarbonate favors an outward flux [7] that would not support an alkalinization. Second, the acidifications in the nominal bicarbonate rules out bicarbonate efflux causing acidification. Since alkaline transients require extracellular bicarbonate, bicarbonate influx into the cytosol is implicated in the alkalinization. One way bicarbonate may enter the cell is via the chloride/bicarbonate exchanger whose activation may result from an initial glycine dependent entry of chloride.

We speculate that the acidification is a consequence of membrane depolarization (Fig. 3) leading to calcium influx. The depolarization could occur during glycine exposure due to a reversal of the chloride gradient. Existing evidence supports a link between depolarization, increases in intracellular $[Ca^{2+}]_i$ and intracellular acidification [33]. When $GABA_A$ receptors are activated, inferior colliculus neurons display an initial hyperpolarization followed by extended depolarizations [18]. Such depolarizations are accompanied by increased intracellular calcium measured ratiometrically. Therefore, it is reasonable to suspect that calcium/hydrogen counter-transport at the cell membrane [28] may contribute to the intracellular acidification we observe.

Ion channels and receptors such as NMDA, $GABA_A$ and intrinsic currents are affected by pH_o [8,22,29]. Given the dense glycinergic innervations in the LSO, it seems possible that glycine-evoked pH transients could affect synaptic function.

Another intriguing effect of pH changes concerns cell proliferation. For example, proliferation of astrocytes is sensitive to pH_i [21]. The possibility that pH changes lead to trophic effects is relevant because LSO dendrites and membrane currents are altered when glycinergic transmission is blocked [15,25,26]. The present report raises the possibility that a potent inhibitory transmitter in the auditory brainstem may exert its influence via intracellular pH shifts.

Acknowledgements

Supported by NSF IBN-9630544. We thank Dr. Mitch Chesler for comments.

References

[1] J. Bormann, O.P. Hamill, B. Sakmann, Mechanism of anion permeation through channels gated by glycine and γ-aminobutyric acid in mouse cultured spinal neurones, J. Physiol. 385 (1987) 243–286.
[2] J.C. Boudreau, C. Tsuchitani, Binaural interaction in the cat superior olive S segment, J. Neurophysiol. 31 (1968) 442–454.
[3] S. Brenowitz, J. David, L. Trussell, Enhancement of synaptic efficacy by presynaptic GABA(B) receptors, Neuron 20 (1998) 135–141.
[4] R.H. Browner, D.B. Webster, Projections of the trapezoid body and the superior olivary complex of the Kangaroo rat (*Dipodomys merriami*), Brain Behav. Evol. 11 (1975) 322–354.
[5] J.C. Chen, M. Chesler, A bicarbonate-dependent increase in extracellular pH mediated by $GABA_A$ receptors in turtle cerebellum, Neurosci. Lett. 116 (1990) 130–135.
[6] M. Chesler, J.C. Chen, Alkaline extracellular pH shifts generated by two transmitter-dependent mechanisms, Can. J. Physiol. Pharmacol. 70 (Suppl.) (1992) S286–292.
[7] M. Chesler, K. Kaila, Modulation of pH by neuronal activity, Trends Neurosci. 15 (1992) 396–402.
[8] K.L. Coulter, F. Perier, C.M. Radeke, C.A. Vandenberg, Identification and molecular localization of a pH-sensing domain for the inward rectifier potassium channel HIR, Neuron 15 (1995) 1157–1168.
[9] P. Dutar, R.A. Nicoll, Pre- and postsynaptic $GABA_B$ receptors in the hippocampus have different pharmacological properties, Neuron 1 (1988) 585–591.
[10] W. Jarolimek, U. Misgeld, H.D. Lux, Activity dependent alkaline and acid transients in guinea pig hippocampal slices, Brain Res. 505 (1989) 225–232.
[11] K. Kaila, P. Paalasmaa, T. Taira, J. Voipio, pH transients due to monosynaptic activation of $GABA_A$ receptors in rat hippocampal slices, Neuroreport 3 (1992) 105–108.
[12] K. Kaila, J. Saarikoski, J. Voipio, Mechanism of action of GABA on intracellular pH and on surface pH in crayfish muscle fibres, J. Physiol. 427 (1990) 241–260.
[13] K. Kaila, J. Voipio, Postsynaptic fall in intracellular pH induced by GABA-activated bicarbonate conductance, Nature 330 (1987) 163–165.
[14] J.S. Kelly, K. Krnjeviá, M.E. Morris, G.K. Yim, Anionic permeability of cortical neurones, Exp. Brain Res. Exp. Hirnforschung. Exp. Cereb. 7 (1969) 11–31.
[15] V.C. Kotak, C. DiMattina, D.H. Sanes, GABA(B) and Trk receptor signaling mediates long-lasting inhibitory synaptic depression, J. Neurophysiol. 86 (2001) 536–540.
[16] V.C. Kotak, S. Korada, I.R. Schwartz, D.H. Sanes, A developmental shift from GABAergic to glycinergic transmission in the central auditory system, J. Neurosci. 18 (1998) 4646–4655.
[17] V.C. Kotak, D.H. Sanes, Developmental influence of glycinergic transmission: regulation of NMDA receptor-mediated EPSPs, J. Neurosci. 16 (1996) 1836–1843.
[18] Y.J. Lo, S.C. Rao, D.H. Sanes, Modulation of calcium by inhibitory systems in the developing auditory midbrain, Neuroscience 83 (1998) 1075–1084.
[19] W. Moody Jr., Effects of intracellular H^+ on the electrical properties of excitable cells, Annu. Rev. Neurosci. 7 (1984) 257–278.
[20] M.J. Moore, D.M. Caspary, Strychnine blocks binaural inhibition in lateral superior olivary neurons, J. Neurosci. 3 (1983) 237–242.
[21] C.A. Pappas, N. Ullrich, H. Sontheimer, Reduction of glial proliferation by K^+ channel blockers is mediated by changes in pH_i, Neuroreport 6 (1994) 193–196.
[22] M. Pasternack, S. Smirnov, K. Kaila, Proton modulation of functionally distinct $GABA_A$ receptors in acutely isolated pyramidal neurons of rat hippocampus, Neuropharmacology 35 (1996) 1279–1288.

[23] N.A. Ritucci, J.S. Erlichman, J.B. Dean, R.W. Putnam, A fluorescence technique to measure intracellular pH of single neurons in brainstem slices, J. Neurosci. Methods 68 (1996) 149–163.
[24] D.H. Sanes, An in vitro analysis of sound localization mechanisms in the gerbil lateral superior olive, J. Neurosci. 10 (1990) 3494–3506.
[25] D.H. Sanes, P. Chokshi, Glycinergic transmission influences the development of dendrite shape, Neuroreport 3 (1992) 323–326.
[26] D.H. Sanes, S. Markowitz, J. Bernstein, J. Wardlow, The influence of inhibitory afferents on the development of postsynaptic dendritic arbors, J. Comp. Neurol. 321 (1992) 637–644.
[27] T. Taira, P. Paalasmaa, J. Voipio, K. Kaila, Relative contributions of excitatory and inhibitory neuronal activity to alkaline transients evoked by stimulation of Schaffer collaterals in the rat hippocampal slice, J. Neurophysiol. 74 (1995) 643–649.
[28] S. Trapp, M. Luckermann, K. Kaila, K. Ballanyi, Acidosis of hippocampal neurones mediated by a plasmalemmal $Ca^{2+}/H+$ pump, Neuroreport 7 (1996) 2000–2004.
[29] S.F. Traynelis, S.G. Cull-Candy, Proton inhibition of *N*-methyl-D-aspartate receptors in cerebellar neurons, Nature 345 (1990) 347–350.
[30] R. Turecek, L.O. Trussell, Presynaptic glycine receptors enhance transmitter release at a mammalian central synapse, Nature 411 (2001) 587–590.
[31] J. Voipio, P. Paalasmaa, T. Taira, K. Kaila, Pharmacological characterization of extracellular pH transients evoked by selective synaptic and exogenous activation of AMPA, NMDA and $GABA_A$ receptors in the rat hippocampal slice, J. Neurophysiol. 74 (1995) 633–642.
[32] J. Voipio, M. Pasternack, B. Rydqvist, K. Kaila, Effect of γ-aminobutyric acid on intracellular pH in the crayfish stretch-receptor neurone, J. Exp. Biol. 156 (1991) 349–360.
[33] D. Willoughby, C.J. Schwiening, Electrically evoked dendritic pH transients in rat cerebellar Purkinje cells, J. Physiol. 544 (2002) 487–499.