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Dendritic Ca²⁺ responses in neonatal LSO neurons elicited by glycinergic/GABAergic synapses and action potentials

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Abstract

During development, GABA/glycinergic connections from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO) gradually change from being depolarizing to being hyperpolarizing. Previous studies have shown that depolarizing MNTB-LSO synapses can trigger action potentials and increase the concentration of intracellular calcium. In the present study we used confocal calcium imaging combined with whole-cell patch clamp recordings to investigate how depolarizing MNTB inputs increase the calcium concentration in the dendrites of LSO neurons. Our results show that subthreshold synaptic responses can elicit local dendritic calcium responses while suprathreshold responses reliably generate global calcium responses that are observed in all dendritic processes. The amplitude of global dendritic calcium responses increased with distance from the soma. Global calcium responses were blocked by tetrodotoxin and could not be recovered by somatic injection of action potential waveforms indicating that global calcium responses are generated by backpropagating sodium action potentials.

Keywords

Development; auditory; backpropagation; action potential; dendritic spikes; confocal imaging

Interaural sound level differences are a major cue by which animals determine the direction of incoming sound in the horizontal plane. In the mammalian brain, the first neurons which are specialized to extract and encode interaural sound level differences are located in the lateral superior olive (LSO). The LSO contains binaural neurons which are excited by sound at the ipsilateral ear and inhibited by sound at the contralateral ear (Boudreau and Tsuchitani, 1968; Tollin, 2003). LSO neurons receive their excitatory inputs from glutamatergic bushy and multipolar cells in the ipsilateral cochlear nucleus (CN) (Glendenning et al., 1985; Cant and Casseday, 1986; Thompson and Thompson, 1987; Doucet and Ryugo, 2003). The inhibitory input to the LSO arises from glycinergic neurons in the ipsilateral medial nucleus of the

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trapezoid body (MNTB) (Spangler et al., 1985; Smith et al., 1989; Kuwabara and Zook, 1991; Kandler and Friauf, 1995; Henkel and Gabriele, 1999) which in turn are innervated by neurons in the contralateral CN (Warr, 1972; Glendenning et al., 1985; Smith et al., 1991; Kandler and Friauf, 1993). Both of these pathways are tonotopically organized and aligned so that individual LSO neurons are excited and inhibited by the same sound frequency (Boudreau and Tsuchitani, 1968; Sanes and Rubel, 1988; Tollin, 2003).

The precise organization of synaptic circuitry in the LSO emerges gradually during development and involves a large degree of functional and structural reorganization of the MNTB-LSO pathway (Sanes and Friauf, 2000; Kandler, 2004). Before hearing onset, most MNTB-LSO connections become functionally eliminated (silenced) and sustained connections become about 12-fold stronger, a process which increases the topographic precision of functional MNTB-LSO connectivity (Kim and Kandler, 2003). After hearing onset, an increase in topographic accuracy is evident by the pruning of terminal arbors of MNTB axons and dendritic branches of LSO neurons (Sanes and Siverls, 1991; Sanes et al., 1992b; Rietzel and Friauf, 1998). Structural and functional maturation of the LSO circuitry requires normal levels or patterns of afferent neuronal activity because cochlear ablation results in decreased axonal pruning and abnormal functional synaptic properties in the LSO (Sanes and Takacs, 1993; Kotak and Sanes, 1997). In line with this, during the period of synaptic silencing and pruning, MNTB-LSO synapses can undergo activity-dependent long term depression (Kotak and Sanes, 2000).

Similar to other brain areas, immature GABA/glycinergic MNTB-LSO synapses initially are depolarizing and excitatory and only gradually switch to being hyperpolarizing and inhibitory (Kandler and Friauf, 1995). The change from a depolarizing to a hyperpolarizing effect of MNTB-LSO synapses is caused by a gradual decrease in the intracellular chloride concentration of LSO neurons (Kakazu et al., 1999; Ehrlich et al., 1999) which results from a gradual increase in the activity of the potassium-chloride co-transporter KCC2 (Blaesse et al., 2006). Using calcium imaging in fura-2 labeled brainstem slices, we have previously shown that MNTB-LSO synapses can increase the intracellular calcium concentration $[Ca^{2+}]_i$ in the soma of neonatal LSO neurons (Kullmann et al., 2002). However, it remained unknown whether and how active MNTB inputs change $[Ca^{2+}]_i$ in LSO dendrites, the site of most synaptic inputs.

In this study we addressed this question by combining electrophysiological whole-cell recordings with confocal imaging of dendritic $[Ca^{2+}]_i$ in LSO neurons. Our results demonstrate that subthreshold MNTB-elicited responses can elicit spatially restricted dendritic Ca^{2+} responses, while suprathreshold responses generate widespread, global Ca^{2+} responses throughout the dendritic tree.

Experimental procedures

Animals and slice preparation

Experiments were performed in slices prepared from neonatal rats aged postnatal day (P) 2–4 (Sprague-Dawley, Charles River Laboratories, Wilmington, MA) and mice aged P0–P5 (C57Bl/6J, Jackson Laboratory, Bar Harbor, ME). At these ages GABA/glycinergic MNTB-LSO synapses are depolarizing and excitatory (Kandler and Friauf, 1995; Ehrlich et al., 1999; Kullmann and Kandler, 2001; Balakrishnan et al., 2003). Experimental procedures were in accordance with NIH guidelines and were approved by the IACUC at the University of Pittsburgh. Brainstem slices were prepared as described previously (Kullmann et al., 2002). Briefly, animals were anesthetized by hypothermia and decapitated. The brain was quickly removed and transferred to cold (4–8 °C) artificial cerebrospinal fluid (ACSF; composition in mM: NaCl 124, NaHCO₃ 26, Glucose 10, KCl 5, KH₂PO₄ 1.25, MgSO₄ 1.3, CaCl₂ 2, pH 7.4

when aerated with 95% O₂/5% CO₂). ACSF used for preparation and storage of slices additionally contained 1 mM kynurenic acid. Coronal slices of the brainstem (250 μm thick) were cut on a vibrating microtome (DTK-1500E, Ted Pella, Redding, CA) and kept in an interface chamber. Slices were allowed to recover for at least 1 h before being used for recordings.

Electrophysiological recordings

The pipette solution for whole-cell recordings consisted of (in mM): K⁺-Gluconate 98, KCl 24, MgCl₂ 2, HEPES 10, Na₂-Phosphocreatine 5, Mg-ATP 3, Na₂-GTP 0.3, pH adjusted to 7.2 with KOH. The Cl⁻ reversal potential of this solution, as given by the Nernst equation, is -40 mV at RT. In some experiments the internal chloride concentration was set to 42 mM by reducing K⁺-Gluconate to 84 mM and increasing KCl to 38 mM, giving a chloride reversal potential of -30 mV. In our experiments, we did not observe a change in calcium responses when increasing E_{Cl} by 10 mV. These reversal potentials are in the range of what has been reported for LSO neurons from neonatal rats or mice (Kandler and Friauf, 1995; Ehrlich et al., 1999; Kullmann and Kandler, 2001).

To load individual neurons with calcium indicator, the pipette solution contained 100 μM of the high-affinity Ca²⁺ indicator Calcium Green-1 (K_d = 190 nM; Molecular Probes, Eugene, OR). LSO neurons were targeted under infrared illumination and after gaining whole-cell access, the dye was given at least 20 min to diffuse into the cell before imaging was started. This was sufficient for equilibration of the dye within the cell as basal fluorescence, even of distal dendrites, did not increase further. Electrophysiological recordings were sampled at a rate of 5 kHz and analyzed using custom-written software (P.H.M.K.; pkullman@pitt.edu) running in the LabVIEW environment (National Instruments, Austin, TX). The MNTB-LSO pathway was stimulated with bipolar electrodes (two Tungsten microelectrodes [FHC, Bowdoinham, ME] separated by 100–200 μm) and placed in the ipsilateral MNTB. Single stimuli consisted of square current pulses with durations of 0.2 ms and amplitudes of 15–200 μA.

Calcium imaging

Confocal imaging was performed with Fluoview 300 (Olympus America, Melville, NY) on an upright microscope (BX50WI; Olympus) using a 60x water-immersion objective (NA 0.9). The 488 nm line of an Argon/Krypton laser (Model 643; Omnichrome, Chino, CA) was used for excitation. Emitted fluorescent light was highpass-filtered at 510 nm (BA510IF; Olympus). For xy-scans, rectangular boxes of 512×64 or 640×58 pixels were used at a frame rate of 5 Hz. All experiments were performed at room temperature (~21–24°C). In most experiments the ACSF contained 100 μM Trolox ((±)-6-Hydroxy-2,5,7,8-tetra-methylchromane-2-carboxylic acid; Sigma) or Glutathione (5 mM) to decrease phototoxicity.

Data analysis

Images were analyzed using either TillVision (T.I.L.L. Photonics, Graefelfing, Germany) or custom-written LabVIEW software (P.H.M.K.). Calcium responses were measured by calculating fluorescence changes relative to baseline fluorescence ($\Delta F/F$) and are expressed as percentage change relative to baseline ($\Delta F/F$). Images from xy-scans were first median-filtered (3×3 kernel) and, if necessary, translational movements in the xy-plane were corrected using TillVision. In some cases, bleach correction was performed which was followed by background subtraction using an area outside of the imaged structure as baseline. An increase in $\Delta F/F$ of two standard deviations above baseline was used as criterion for a Ca²⁺ response.

Line scans were median-filtered along the x-dimension (kernel of 3 pixels), if necessary, corrected for bleaching and background was subtracted. A $\Delta F/F$ image was constructed by

calculating, for each x-position along the scan line, the fluorescence change over time relative to the 200 to 300 ms preceding the stimulus. Peak amplitudes and decay time constants were determined by fitting a mono-exponential to the decay part of the response.

Distances from soma were measured in flattened xyz scans that were acquired at the end of each recording. A line following the course of the dendrite in the xy-plane from the soma to a dendritic location was drawn and the length of the line determined. These measurements do not take into account meandering of the dendrite in the z-plane and therefore slightly underestimate the true distance.

The level for statistical significance used is $p < 0.05$. If not expressed otherwise, average values are expressed as mean $\pm$ SEM.

Chemicals

TTX was purchased from Alomone Labs (Jerusalem, Israel) and Bicuculline Methiodide from Tocris (Ballwin, MO). All other chemicals were purchased from Sigma (St. Louis, MO).

Results

MNTB-elicited synaptic responses in LSO neurons resulted in an increase in dendritic $[Ca^{2+}]_i$ (Fig1). Ca^{2+} responses elicited by subthreshold postsynaptic potentials occurred were observed in 7 LSO neurons and these responses were confined to small segments of a dendrite. For a given dendritic location, responses occurred unreliably and were observed in only about 20% of the trials following MNTB stimulation. In contrast, suprathreshold postsynaptic responses generated Ca^{2+} responses which were highly reliable and could be observed throughout the whole dendritic segment (Fig 1c). Postsynaptic potentials and Ca^{2+} responses were blocked by the GABA_A and glycine receptor antagonists bicuculline (10 μ M) and strychnine (5 μ M) ($n=4$ neurons, data not shown), indicating that they resulted from the activation of GABA/glycinergic MNTB-LSO synapses.

To determine whether spike-elicited dendritic Ca^{2+} responses were restricted to one dendritic branch or whether they occurred throughout the whole dendritic tree of a neuron, Ca^{2+} responses in different dendrites were monitored successively by placing line scans through various dendritic branches. As illustrated in figure 2, spike-elicited Ca^{2+} responses were not restricted to single dendritic branches but could be observed in all dendritic arbors.

Using xy-scans, we further characterized the properties of these global Ca^{2+} responses in 25 LSO neurons. Imaging longer segments of dendrites that were located in a focal plane revealed that the amplitudes of spike-elicited Ca^{2+} responses increased as a function of soma distance. For a given dendritic branch the largest Ca^{2+} changes were always observed at the most distal location (Fig. 3D). Depending on the signal/noise ratio and the length of the dendritic segment within the field of view, we were able to analyze between 3 and 16 regions of interest (ROIs) per cell at locations up to a somatic distance of 145 μ m. For every neuron, there was an increase in peak $\Delta F/F$ with soma distance. In 16 neurons, in which linear regression analysis could be performed, this correlation was statistically significant ($p < 0.05$; Pearson Product Moment Correlation). Statistical evaluation of the pooled data from all 25 cells (226 ROIs) also demonstrated a significant increase of peak amplitude with somatic distance (Pearson Correlation Coefficient $r = 0.205$, $p = 0.002$; Fig. 4 A,B).

Because dendrites are tapered, larger Ca^{2+} responses at distal locations may reflect an increase in the surface-to-volume ratio. In support of this, the dendritic diameter of LSO neurons at the site of ROIs decreased with soma distance, (4C,D) which resulted in a positive correlation between the dendrite diameter and the amplitudes of Ca^{2+} responses (correlation coefficient =

0.409, $p < 0.001$, Pearson Product Moment Correlation; Fig. 4E). Consequently there was a correlation between surface/volume ratio and amplitudes of Ca^{2+} responses (Pearson Correlation Coefficient $r = 0.994$, $p < 0.0001$; Fig. 4F).

To explore the possibility that the increase of fluorescence changes with soma distance or smaller dendrite diameter is caused by incomplete filling of the neurons with calcium indicator, we analyzed the kinetics of fluorescence changes. The kinetic of a Ca^{2+} response is influenced by the concentration of the calcium indicator, which, due to its calcium buffering properties, can slow down the return to baseline (Regehr and Tank, 1992). To address whether the decay times of fluorescence changes is a function of soma distances or dendritic diameter, we measured fluorescence changes using line scans. Measurement of the time constant τ at soma distances up to 145 μm ($n=59$; 11 LSO neurons) showed a statistically significant, positive correlation between τ and distance ($R = 0.351$, $p=0.00648$, Pearson Product Moment Correlation; Fig. 5B) and no correlation between τ and dendrite diameter ($R = -0.006$; $p = 0.965$; Fig 5 C). The positive correlation between τ and distance is the opposite of what would be expected if the difference in the amplitude of the Ca^{2+} responses were due to higher dye concentrations in proximal dendrites rather than in distal dendrites.

The presence of synaptic spike induced Ca^{2+} responses throughout the dendritic tree of LSO neurons without decrement suggests that these responses reflect the active backpropagation of action potentials in LSO dendrites. To test this possibility, we first elicited action potentials in the soma by brief (50 ms), somatic current injections in current clamp mode and measured the resulting dendritic Ca^{2+} responses (Fig. 6). Under these conditions, the average peak Ca^{2+} response was $19.4 \pm 5.2\%$ $\Delta\text{F}/\text{F}$ ($n=6$ neurons, one dendrite per neuron; rat P3–P4, Fig. 6). Subsequently, we changed the recording mode to voltage clamp and imposed somatic spike-like depolarizations using one of the previously recorded action potential traces as the voltage command signal. The average peak of dendritic Ca^{2+} responses under these conditions was $15.6 \pm 3.5\%$ $\Delta\text{F}/\text{F}$ ($84.2 \pm 5.1\%$ of peak in measured in current clamp; $p = 0.12$, paired t-test). However, repeating the same voltage clamp protocol in the presence of the Na^+ channels blocker TTX (1 μM) resulted in a significant decrease of dendritic Ca^{2+} responses measured at the same locations (peak $\Delta\text{F}/\text{F}$: $3.4 \pm 0.8\%$). Responses in the presence of TTX were $19 \pm 4\%$ of the value when measured in current clamp ($p = 0.024$, paired t-test) and $23 \pm 4\%$ of the values in voltage clamp without TTX ($p = 0.011$; paired t-test). These results indicate that active backpropagation of Na^+ spikes underlies the generation of large, global dendritic calcium responses in immature LSO neurons.

Discussion

We have investigated MNTB-evoked Ca^{2+} responses in dendrites of developing LSO neurons and found that GABA/glycinergic postsynaptic responses give rise to two types of dendritic Ca^{2+} responses. Ca^{2+} responses generated by subthreshold synaptic responses occurred with low probability for a given site and were locally restricted, while Ca^{2+} responses generated by suprathreshold synaptic responses were reliable and occurred throughout the entire dendritic tree. Global Ca^{2+} responses were TTX-sensitive and depend on backpropagating action potentials.

While it is well established that depolarizing GABA and glycine can activate voltage-gated calcium channels and increase $[\text{Ca}^{2+}]_i$ in immature neurons (LoTurco et al., 1995; Obrietan and van den Pol, 1995; Kullmann et al., 2002), very few studies have investigated whether GABA/glycinergic synapses can increase $[\text{Ca}^{2+}]_i$ in neuronal dendrites. A recent study in hippocampal slice cultures from neonatal rats found that synaptically activated GABA_A receptors generate local Ca^{2+} responses in growing dendrites and filopodia (Lohmann et al., 2005). Our results indicate that developing GABA/glycinergic MNTB-LSO synapses can

induce similar responses postsynaptic LSO neurons. Future studies will be necessary to determine the specific mechanisms underlying the generation and spatial restriction of these responses in dendrites of LSO neurons which lack spines that could serve as spatial compartments (Rietzel and Friauf, 1998). It also will be interesting to find out whether glutamate which is co-released from immature GABA/glycinergic MNTB-LSO synapses contributes to these local responses by activating NMDA receptors (Gillespie et al., 2005).

We have previously shown that depolarizing GABA/glycinergic MNTB-LSO synapses can generate action potentials in immature LSO neurons (Kullmann and Kandler, 2001). The present study indicates that MNTB-elicited Na^+ spikes backpropagate and generate widespread, global calcium responses that engage the entire dendritic tree of an LSO neuron. In this respect, LSO neurons differ from other auditory brainstem neurons such as neurons in the dorsal cochlear nucleus (DCN) and medial superior olive (MSO). In DCN pyramidal and cartwheel cells, dendritic Na^+ channels are not required to generate dendritic Ca^{2+} responses because responses elicited by somatic depolarizations persist in TTX (Molitor and Manis, 2003). In the MSO, neurons are able to produce dendritic Na^+ spikes but these spikes are of low amplitude and are rapidly attenuated (Scott et al., 2007).

In LSO neurons, the amplitude of spike-elicited dendritic Ca^{2+} responses increased with increasing distance from the soma due to a distance-dependent decrease of dendrite diameter and an increase in the surface/volume ratio. This spatial pattern differs from pyramidal neurons in neocortex, hippocampus, and DCN in which the amplitude of spike-elicited Ca^{2+} responses decreases with soma distance (Yuste et al., 1994; Markram et al., 1995; Yuste and Denk, 1995; Svoboda et al., 1997; Magee et al., 1998; Molitor and Manis, 2003) but is more reminiscent of the global non-decremental Ca^{2+} responses that have been described more recently in interneurons (Goldberg et al., 2004; Rozsa et al., 2004; Cuna-Goycolea et al., 2008).

During the first postnatal week, LSO neurons undergo dendritic growth (Sanes and Siverls, 1991; Sanes et al., 1992a; Rietzel and Friauf, 1998) and the MNTB-LSO pathway is reorganized by processes which include synaptic silencing and strengthening (Kim and Kandler, 2003). It is likely that MNTB-elicited Ca^{2+} responses in postsynaptic LSO neurons play an important role in these processes. Moderate and local increases in $[\text{Ca}^{2+}]_i$ are generally associated with the induction of long term depression that may precede synapse elimination (LTD) (Sjostrom and Nelson, 2002). In LSO neurons, Ca^{2+} responses elicited by subthreshold MNTB inputs may underlie synapse-specific induction of LTD which at MNTB-LSO synapses is calcium-dependent but independent postsynaptic spiking (Kotak and Sanes, 2000). Backpropagating action potentials have been widely implicated in inducing long term potentiation (LTP) (Bi and Poo, 2001; Sjostrom and Nelson, 2002). Although LTP at MNTB-LSO synapses remains to be demonstrated, backpropagating action potentials in LSO dendrites may be involved in triggering LTP by amplifying synaptic Ca^{2+} responses through activation of voltage-dependent calcium channels or by removing the Mg^{2+} block from NMDARs allowing co-released glutamate to trigger NMDAR-mediated calcium influx.

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Reference List

- Bi G, Poo M. Synaptic modification by correlated activity: Hebb's postulate revisited. *Annu Rev Neurosci* 2001;24:139–166. [PubMed: 11283308]

- Blaesse P, Guillemín I, Schindler J, Schweizer M, Delpire E, Khiroug L, Friauf E, Nothwang HG. Oligomerization of KCC2 correlates with development of inhibitory neurotransmission. *J Neurosci* 2006;26:10407–10419. [PubMed: 17035525]
- Boudreau JC, Tsuchitani C. Binaural interaction in the cat superior olive S segment. *J Neurophysiol* 1968;31:442–454. [PubMed: 5687764]
- Caird D, Klinke R. Processing of binaural stimuli by cat superior olivary complex neurons. *Exp Brain Res* 1983;52:385–399. [PubMed: 6653700]
- Cant NB, Casseday JH. Projections from the anteroventral cochlear nucleus to the lateral and medial superior olivary nuclei. *J Comp Neurol* 1986;247:457–476. [PubMed: 3722446]
- Doucet JR, Ryugo DK. Axonal pathways to the lateral superior olive labeled with biotinylated dextran amine injections in the dorsal cochlear nucleus of rats. *J Comp Neurol* 2003;461:452–465. [PubMed: 12746862]
- Ehrlich I, Lohrke S, Friauf E. Shift from depolarizing to hyperpolarizing glycine action in rat auditory neurones is due to age-dependent Cl⁻ regulation. *J Physiol* 1999;520(Pt 1):121–137. [PubMed: 10517806]
- Gillespie DC, Kim G, Kandler K. Inhibitory synapses in the developing auditory system are glutamatergic. *Nat Neurosci* 2005;8:332–338. [PubMed: 15746915]
- Glendenning KK, Hutson KA, Nudo RJ, Masterton RB. Acoustic chiasm II: Anatomical basis of binaurality in the lateral superior olive of the cat. *J Comp Neurol* 1985;232:261–285. [PubMed: 3973093]
- Goldberg JH, Lacefield CO, Yuste R. Global dendritic calcium spikes in mouse layer 5 low threshold spiking interneurons: implications for control of pyramidal cell bursting. *J Physiol* 2004;558:465–478. [PubMed: 15146046]
- Henkel CK, Gabriele ML. Organization of the disynaptic pathway from the anteroventral cochlear nucleus to the lateral superior olivary nucleus in the ferret. *Anat Embryol (Berl)* 1999;199:149–160. [PubMed: 9930621]
- Kakazu Y, Akaike N, Komiyama S, Nabekura J. Regulation of intracellular chloride by cotransporters in developing lateral superior olive neurons. *J Neurosci* 1999;19:2843–2851. [PubMed: 10191302]
- Kandler K. Activity-dependent organization of inhibitory circuits: lessons from the auditory system. *Curr Opin Neurobiol* 2004;14:96–104. [PubMed: 15018944]
- Kandler K, Friauf E. Pre- and postnatal development of efferent connections of the cochlear nucleus in the rat. *J Comp Neurol* 1993;328:161–184. [PubMed: 8423239]
- Kandler K, Friauf E. Development of glycinergic and glutamatergic synaptic transmission in the auditory brainstem of perinatal rats. *J Neurosci* 1995;15:6890–6904. [PubMed: 7472446]
- Kim G, Kandler K. Elimination and strengthening of glycinergic/GABAergic connections during tonotopic map formation. *Nat Neurosci* 2003;6:282–290. [PubMed: 12577063]
- Kotak VC, Korada S, Schwartz IR, Sanes DH. A developmental shift from GABAergic to glycinergic transmission in the central auditory system. *J Neurosci* 1998;18:4646–4655. [PubMed: 9614239]
- Kotak VC, Sanes DH. Deafferentation weakens excitatory synapses in the developing central auditory system. *Eur J Neurosci* 1997;9:2340–2347. [PubMed: 9464928]
- Kotak VC, Sanes DH. Long-lasting inhibitory synaptic depression is age- and calcium-dependent. *J Neurosci* 2000;20:5820–5826. [PubMed: 10908623]
- Kullmann PH, Ene FA, Kandler K. Glycinergic and GABAergic calcium responses in the developing lateral superior olive. *Eur J Neurosci* 2002;15:1093–1104. [PubMed: 11982621]
- Kullmann PH, Kandler K. Glycinergic/GABAergic synapses in the lateral superior olive are excitatory in neonatal C57Bl/6J mice. *Brain Res Dev Brain Res* 2001;131:143–147.
- Kuwabara N, Zook JM. Classification of the principal cells of the medial nucleus of the trapezoid body. *J Comp Neurol* 1991;314:707–720. [PubMed: 1816272]
- Lohmann C, Finski A, Bonhoeffer T. Local calcium transients regulate the spontaneous motility of dendritic filopodia. *Nat Neurosci* 2005;8:305–312. [PubMed: 15711541]
- LoTurco JJ, Owens DF, Heath MJ, Davis MB, Kriegstein AR. GABA and glutamate depolarize cortical progenitor cells and inhibit DNA synthesis. *Neuron* 1995;15:1287–1298. [PubMed: 8845153]

- Magee J, Hoffman D, Colbert C, Johnston D. Electrical and calcium signaling in dendrites of hippocampal pyramidal neurons. [Review] [75 refs]. *Annu Rev Physiol* 1998;60:327–346. [PubMed: 9558467]
- Markram H, Helm PJ, Sakmann B. Dendritic calcium transients evoked by single back-propagating action potentials in rat neocortical pyramidal neurons. *J Physiol (Lond)* 1995;485:1–20. [PubMed: 7658365]
- Molitor SC, Manis PB. Dendritic Ca²⁺ transients evoked by action potentials in rat dorsal cochlear nucleus pyramidal and cartwheel neurons. *J Neurophysiol* 2003;89:2225–2237. [PubMed: 12612001]
- Obrietan K, van den Pol AN. GABA neurotransmission in the hypothalamus: developmental reversal from Ca²⁺ elevating to depressing. *J Neurosci* 1995;15:5065–5077. [PubMed: 7623135]
- Regehr WG, Tank DW. Calcium concentration dynamics produced by synaptic activation of CA1 hippocampal pyramidal cells. *J Neurosci* 1992;12:4202–4223. [PubMed: 1359030]
- Rietzel HJ, Friauf E. Neuron types in the rat lateral superior olive and developmental changes in the complexity of their dendritic arbors. *J Comp Neurol* 1998;390:20–40. [PubMed: 9456173]
- Rozsa B, Zelles T, Vizi ES, Lendvai B. Distance-dependent scaling of calcium transients evoked by backpropagating spikes and synaptic activity in dendrites of hippocampal interneurons. *J Neurosci* 2004;24:661–670. [PubMed: 14736852]
- Sanes DH, Friauf E. Development and influence of inhibition in the lateral superior olivary nucleus. *Hear Res* 2000;147:46–58. [PubMed: 10962172]
- Sanes DH, Markowitz S, Bernstein J, Wardlow J. The influence of inhibitory afferents on the development of postsynaptic dendritic arbors. *J Comp Neurol* 1992a;321:637–644. [PubMed: 1380523]
- Sanes DH, Siverls V. Development and specificity of inhibitory terminal arborizations in the central nervous system. *J Neurobiol* 1991;8:837–854. [PubMed: 1663990]
- Sanes DH, Song J, Tyson J. Refinement of dendritic arbors along the tonotopic axis of the gerbil lateral superior olive. *Dev Brain Res* 1992b;67:47–55. [PubMed: 1638742]
- Sanes DH, Takacs C. Activity-dependent refinement of inhibitory connections. *Eur J Neurosci* 1993;5:570–574. [PubMed: 8261131]
- Scott LL, Hage TA, Golding NL. Weak action potential backpropagation is associated with high-frequency axonal firing capability in principal neurons of the gerbil medial superior olive. *J Physiol* 2007;583:647–661. [PubMed: 17627992]
- Sjostrom PJ, Nelson SB. Spike timing, calcium signals and synaptic plasticity. *Curr Opin Neurobiol* 2002;12:305–314. [PubMed: 12049938]
- Smith PH, Joris PX, Carney LH, Yin TC. Projections of physiologically characterized globular bushy cell axons from the cochlear nucleus of the cat. *J Comp Neurol* 1991;304:387–407. [PubMed: 2022755]
- Spangler KM, Warr WB, Henkel CK. The projections of principal cells of the medial nucleus of the trapezoid body in the cat. *J Comp Neurol* 1985;238:249–262. [PubMed: 4044914]
- Svoboda K, Denk W, Kleinfeld D, Tank DW. In vivo dendritic calcium dynamics in neocortical pyramidal neurons. *N* 1997;385:161–165.
- Thompson AM, Thompson GC. Efferent projections from posteroventral cochlear nucleus to lateral superior olive in guinea pig. *Brain Res* 1987;421:382–386. [PubMed: 3690282]
- Tollin DJ. The lateral superior olive: a functional role in sound source localization. *Neuroscientist* 2003;9:127–143. [PubMed: 12708617]
- Warr WB. Fiber degeneration following lesions in the multipolar and globular cell areas in the ventral cochlear nucleus of the cat. *Brain Res* 1972;40:247–270. [PubMed: 5027165]
- Yuste R, Denk W. Dendritic spines as basic functional units of neuronal integration. *N* 1995;375:682–684.
- Yuste R, Gutnick MJ, Saar D, Delaney KR, Tank DW. Ca²⁺ accumulations in dendrites of neocortical pyramidal neurons: an apical band and evidence for two functional compartments. *Neuron* 1994;13:23–43. [PubMed: 8043278]

Figure 1.

Example of MNTB-elicited dendritic calcium responses in an LSO neuron. **A.** Maximal projection of Z-stack images of an LSO neuron filled through the recording pipette (rec) with 100 μ M Calcium Green-1. **B.** Sub-threshold synaptic responses in a different neuron elicit a local Ca^{2+} response. **C.** Supra-threshold responses elicit non-localized Ca^{2+} responses along the whole dendrite. Pseudocolor images show $\Delta F/F$ in the first frame after the stimulus which, in most cases, corresponds to the peak Ca^{2+} change. Distance to soma from the left edge of the field of view is 30 μ m. Numbers below membrane potential traces (VM) indicate resting membrane potential. The neuron in A as well as the neuron in B,C are from P2 mice. Time calibration bar in this and subsequent figures applies to both the electrophysiological and imaging traces.

Figure 2.

MNTB-elicited action potentials elicit a global Ca^{2+} response throughout the dendritic tree. MNTB-stimulation was given repeatedly and Ca^{2+} changes were monitored successively by positioning line scans at randomly chosen locations indicated by the red lines that are superimposed on a Z-stack image. A representative electrophysiological record with the same time scale as the fluorescence traces is shown in yellow ($V_{\text{rest}} -61 \text{ mV}$). Interval between linescans at different locations was 3–6 minutes. Slice was prepared from a P4 rat.

Figure 3.

The amplitudes of spike-elicited dendritic Ca^{2+} responses increase with somatic distance.

A: Confocal z-stack image of a mouse P3 LSO neuron. **B:** Single optical section image of the area outline in A. **C:** Pseudocolor image of peak fluorescence changes after an MNTB-elicited action potential. The largest changes occur at the most distal location. Traces on the right are from the ROIs outlined. The $\Delta F/F$ image of peak response and each $\Delta F/F$ trace are the average of 5 individual responses. The electrophysiological trace is from a single stimulation (V_{rest} : -59 mV). **D.** Peak $\Delta F/F$ values of all ROIs analyzed along the dendrite shown in B,C as a function of distance to soma. The 2 open diamonds represent the ROIs of the small side-branch visible in B and C.

Figure 4.

Spike-triggered Ca^{2+} responses increase with distance from soma and surface-volume ratio.

A. Response amplitudes are plotted against soma distance of ROIs. **B.** Means of data shown in A, binned in 10 μm segments. **C.** The dendritic diameter at ROI sites decreases with soma-distance (Pearson correlation coefficient $r = -0.32$, $p < 0.0001$). **D.** Means of data shown in C, binned in 10 μm segments. **E.** The amplitude of dendritic Ca^{2+} responses is inversely related to the diameter of the dendrite. **F.** The amplitude of dendritic Ca^{2+} responses increases with the surface/volume ratio (Pearson Correlation Coefficient $r = 0.994$, $p < 0.0001$).

Figure 5.

Decay time constants of spike-elicited Ca^{2+} responses do not decrease with somatic distance or dendrite diameter. **A.** Example fluorescence traces recorded in the same dendrite at distances of 15 μm and 60 μm from the soma. Traces are fit by single exponentials. The neuron was from a P3 mouse. **B.** Time constants are plotted against somatic distance of dendritic recording sites ($n=59$ recording sites in 11 neurons). There was a significant correlation of time constants with somatic distance ($R = 0.351$, $p = 0.006$). **C.** Time constants did not correlate with dendrite diameters ($R = 0.006$, $p = 0.965$; $n=59$ sites, 11 neurons).

Figure 6.

Global Ca^{2+} responses depend on backpropagating Na^{+} action potentials. **A** Stimulation protocol: *Left*: An action potential was first elicited in current clamp by a short depolarizing current injection. *Middle*: The voltage trace from left was used as command potential during voltage clamp. *Right*: The voltage clamp command is repeated after blocking sodium channels with TTX. **B**. Dendritic Ca^{2+} responses in the three conditions shown in A. The somatic distance of the left border of the dendritic segment is 33 μm . The average diameter of the dendrite is 0.95 μm . **C**. Summary data from 6 neurons.