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SYNAPTIC CHANGES UNDERLYING THE STRENGTHENING OF GABA/glycinergic CONNECTIONS IN THE DEVELOPING LATERAL SUPERIOR OLIVE

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Abstract—Before hearing onset, the topographic organization of the auditory GABA/glycinergic pathway from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO) is refined by synaptic silencing and strengthening. The synaptic mechanisms underlying the developmental strengthening of maintained MNTB-LSO connections are unknown. Here we address this question using whole-cell recordings from LSO neurons in slices prepared from prehearing mice. Minimal and maximal stimulation techniques demonstrated that during the first two postnatal weeks, individual LSO neurons lose about 55% of their initial presynaptic MNTB partners while maintained single-fiber connections become about 14-fold stronger. Analysis of MNTB-evoked miniature events indicates that this strengthening is accompanied by a 2-fold increase in quantal amplitude. Strengthening is not caused by an increase in the probability of release because paired pulse ratios (PPRs) increased from 0.7 in newborn animals to 0.9 around hearing onset, indicating a developmental decrease rather than increase in release probability. In addition, a possible soma-dendritic relocation of MNTB input seems unlikely to underlie their strengthening as indicated by analysis of the rise times of synaptic currents. Taken together, we conclude that the developmental strengthening of MNTB-LSO connections is achieved by a 2-fold increase in quantal size and an 8-fold increase in quantal content.
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Interaural sound level differences are a major cue by which mammals determine the direction of incoming sound. In the CNS, interaural sound level differences are processed for the first time by binaural neurons in the lateral superior olive (LSO). Lateral superior olive neurons receive excitatory inputs from the ipsilateral ear via a glutamatergic projection from the ipsilateral cochlear nucleus (CN) and receive inhibitory inputs from the contralateral ear via a glycinergic projection from the medial nucleus of the trapezoid body (MNTB; Boudreau and Tsuchitani, 1968; Cant and Casseday, 1986; Sanes and Rubel, 1988; Bledsoe et al., 1990; Sommer et al., 1993). Both projections are tonotopically organized and aligned such that binaural LSO neurons receive excitatory and inhibitory inputs that are tuned to the same sound frequency (for review see Tollin, 2003).

The precise tonotopic organization and physiological properties of the inhibitory MNTB-LSO pathway emerge gradually during development. The initial formation of the MNTB-LSO pathway takes place prenatally (Sanes and Siverls, 1991; Kandler and Friauf, 1993, 1995) and is followed by a series of anatomical and physiological changes that occur both in presynaptic MNTB neurons and postsynaptic LSO neurons (Sanes and Friauf, 2000; Friauf, 2004; Kandler and Gillespie, 2005; Kandler et al., 2009). The majority of these changes occur before animals can hear airborne sound and thus occur independently of auditory experience. In rats and mice, topographic refinement of the MNTB-LSO pathway before hearing onset of the first two postnatal weeks (Geal-Dor et al., 1993) is characterized by silencing of most connections and a strengthening of the maintained ones (Kim and Kandler, 2003; Noh et al., 2010). The magnitude of this pre-hearing reorganization is quite remarkable because single LSO neurons lose approximately 75% of their initial presynaptic MNTB partners while maintained connections become about 12-fold stronger. While these changes before hearing onset are crucial for the ability of LSO neurons to encode interaural intensity differences right at hearing onset (Sanes and Rubel, 1988), the mechanisms that underlie the pre-hearing strengthening of MNTB-LSO connections are unknown.

Work conducted in other brain areas indicated that the major mechanisms to increase the amplitude of GABAergic or glycinergic postsynaptic currents (PSCs) elicited by a single axon include an increase in quantal amplitude (Singer and Berger, 1999; Awatramani et al., 2005), in number of release sites (Juttner et al., 2001; Morales et al., 2002), or in presynaptic release probability (Kobayashi et al., 2008). To investigate whether and to what degree these pre- and postsynaptic mechanisms contribute to the strengthening of individual connections in the MNTB-LSO pathway, we compared the properties of MNTB-evoked synaptic responses in LSO neurons in slices prepared from newborn mice and mice around hearing onset. Our results demonstrate that the degree of refinement of the MNTB-LSO pathway in mice is highly similar to rats and further indicate that the strengthening of the maintained MNTB inputs is achieved by an approximate 2-fold increase in quantal amplitude and an over 8-fold increase in the quantal content of individual MNTB inputs.

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Abbreviations: CV, coefficient of variation; LSO, lateral superior olive; MNTB, medial nucleus of the trapezoid body; mPSCs, miniature PSCs; PPD, paired pulse depression; PPRs, paired pulse ratios; PSCs, postsynaptic currents.

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EXPERIMENTAL PROCEDURE

Animals, slice preparation, and electrophysiology

Experimental procedures were in accordance with NIH guidelines and were approved by the IACUC at the University of Pittsburgh. Particular effort was made to reduce the number of animals and their suffering. All experiments were performed in brainstem slices prepared from mouse pups of the strain 129S6/SvEv aged between postnatal day P1 and P12. Although the 129S6/SvEv strain has a deletion variant of the Disc1 gene which causes slight abnormalities in working memory and other behavioral tests (Koike et al., 2006; Ishizuka et al., 2007), Disc 1 is not expressed in auditory brainstem neurons (Schurov et al., 2004) and therefore its mutation is unlikely to influence early development of LSO circuitry.

Brainstem slices were prepared as described previously (Kim and Kandler, 2003; Kullmann and Kandler, 2008). Briefly, animals were anesthetized by hypothermia (P1–P3) or isoflurane (Minrad Inc., Bethlehem, PA, USA) (P10–12) before decapitation. Coronal slices (300 μm) were cut on a vibrating microtome (DTK-1500E, Ted Pella, Redding, CA, USA). Slices containing both the LSO and the MNTB were selected and allowed to recover in an interface-type chamber under 95% O_2/5% CO_2 atmosphere for 1~2 h at RT (23 °C). For slice preparation and incubation, 1 mM kynurenic acid was present in the ACSF (artificial cerebrospinal fluid, composition in mM: NaCl 124, $NaHCO_3$ 26, Glucose 10, KCl 5, KH_2PO_4 1.25, $MgSO_4$ 1.3, $CaCl_2$ 2, pH=7.4 when bubbled with 95% O_2/5% CO_2). For recordings, slices were transferred to a submerged-type chamber mounted to an upright microscope (BX50, Olympus) and superfused with oxygenated ACSF at RT at a rate of ~3–4 ml/min. Unless noted otherwise, all chemicals were purchased from Sigma (St. Louis, MO, USA).

Electrophysiological recordings

Whole-cell voltage clamp recordings were obtained at RT from visually identified LSO principal neurons in the medial half of the LSO using an Axopatch 1 D amplifier. Recording electrodes (2–3 MΩ) contained (in mM): 76 Cs-methanesulfonate, 56 CsCl, 10 EGTA, 1 $MgCl_2$, 1 $CaCl_2$, 2 ATP-Mg, 0.3 GTP-Na, 5 Na_2-phosphocreatine, and 10 HEPES (pH=7.3, 290 mosM). With the internal and external solution, E_{Cl} was −20 mV and LSO neurons were held at −70 mV (corrected for −5 mV liquid junction potential), resulting in −50 mV driving force for Cl^-. Patch electrodes with larger tip diameters (~1 MΩ) were used for electrical stimulation at the lateral edge of the MNTB. Constant current pulses (0.2 ms) were delivered using a stimulation isolation unit (IsoFlex, AMPI). All evoked postsynaptic currents (PSCs) including paired pulse responses were elicited at 0.2 Hz. Data were filtered at 2 kHz (Axopatch 1D) and acquired at 10 kHz using custom-written Labview data acquisition software (Kullmann and Kandler, 2001).

Minimal stimulation

Minimal stimulation techniques were used to record responses elicited by single MNTB axons (Stevens and Wang, 1994; Kim and Kandler, 2003). A stimulus-response relation was obtained and the first plateau was determined at which the failure rate decreased without increasing the mean amplitude of successful responses (see Fig. 3B, D). Responses during this plateau were regarded as inputs by single-fibers, and 20–100 responses were evoked at 0.2 Hz. Only responses with latencies that fell into a 1 ms long window were accepted.

Evoked miniature PSC

To examine miniature PSCs (mPSCs) that are generated by MNTB terminals, MNTB fibers were stimulated in ACSF in which Ca^{2+} was replaced by equimolar Sr^{2+}. Under this condition, asynchronous release is promoted, and asynchronous events represent quantal events (Goda and Stevens, 1994; Behrends and ten Bruggencate, 1998). In 2 mM Sr^{2+}/0 mM Ca^{2+}, PSCs evoked by electrical stimulation were markedly smaller than in normal 2 mM Ca^{2+} ACSF, and asynchronous events were observed. However, because in the LSO, single stimuli in Sr^{2+} ACSF rarely elicited asynchronous events we applied stimulus trains (2–10 pulses at 100 Hz). These train stimuli effectively induced asynchronous events at all ages examined. A 1 s long baseline was recorded before the stimulus train and another 1 s long trace was recorded after the train. To prevent contamination by spontaneous events, cells that showed high spontaneous activity during the baseline period were excluded from analysis.

Data analysis

Synaptic responses were analyzed using custom written Labview (National Instruments, TX, USA) and MATLAB (MathWorks, MA, USA) programs. Evoked mPSCs were identified and collected from a 3 to 400-ms window after the stimulus train using a sliding template method (Clements and Bekkers, 1997) implemented in MATLAB. The detection thresholds were set low, and false positives (events that did not exhibit a rapid increase followed by a typical decay time) were rejected by visual inspection. In P1–3 animals, asynchronous events often appeared only during the decay phase of the stimulus-locked responses. For these events, peak amplitude was measured after the baseline was adjusted by fitting a straight line to the baseline immediately before the peak (Lu and Trussell, 2000). Paired pulse ratios (PPRs) were computed from paired PSCs evoked by two stimuli with intervals of 10, 20, 50, 100, 200, and 500 ms. At least 20 trials were averaged and then the mean second PSC peak amplitude was divided by the first (Kim and Alger, 2001). At short inter-pulse intervals (10, 20, and 50 ms in P1–3; 10 and 20 ms in P10–12), there was a significant overlap between the first and the second PSCs (Fig. 5A). To accurately measure the peak amplitude of the second PSCs, a mean PSC from a longer interval was scaled and subtracted from the paired-pulse responses, isolating the second PSC. The coefficient of variation (CV) for peak amplitudes of paired PSCs was computed from 15 to 30 individual trials in each cell (Fig. 5C). Throughout the text and figures, errors are expressed as standard error.

RESULTS

Developmental changes in single-fiber and maximal MNTB inputs

Synaptic currents evoked by electrical stimulation of MNTB fibers were recorded from visually identified, uni- or bipolar mouse LSO neurons using whole-cell voltage clamp technique. Because recording electrodes contained a high Cl^- concentration (E_{Cl}: −20 mV), MNTB-elicited PSCs were inwardly directed. To isolate PSCs mediated by GABA and glycine receptors, all recordings were performed in the presence of the ionotropic glutamate receptor antagonist Kynurenic acid (1 mM). MNTB-evoked PSCs in P1–3 animals were small and had a slow time course (Fig. 1A). As previously reported for rats and gerbils (Kotak et al., 1998; Nabekura et al., 2004), MNTB-evoked PSCs in the LSO of neonatal mice contained both bicuculline sensitive (10 μM) and strychnine sensitive (1 μM) components, indicating mixed release of GABA and glycine (Fig 1A, P1–2, n=7). Around hearing onset, PSCs were larger and faster and were blocked by 1 μM strychnine (Fig 1A, P10–11, n=5). Current-voltage relationships of MNTB-evoked PSCs were linear between −90 and +40 mV (r=0.998) with a reversal

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