Excitatory and inhibitory transmission in the superior olivary complex.

Ian D. Forsythe, Matt Barker, Margaret Barnes-Davies, Brian Billups, Paul Dodson, Fatima Osmani, Steven Owens and Adrian Wong. Department of Cell Physiology and Pharmacology, University of Leicester, Leicester LE1 9HN. UK.

The timing and pattern of action potentials propagating into the brainstem from both cochleae contain information about the azimuth location of that sound in auditory space. This binaural information is integrated in the superior olivary complex. This part of the auditory pathway is adapted for fast conduction speeds and the preservation of timing information with several complimentary mechanisms (see Oertel, 1999; Trussell, 1999). There are large diameter axons terminating in giant somatic synapses that activate receptor ion channels with fast kinetics. The resultant postsynaptic potentials generated in the receiving neuron are integrated with a suite of voltage-gated ion channels that determine the action potential threshold, duration and repetitive firing properties. We have studied presynaptic and postsynaptic mechanisms that regulate efficacy, timing and integration of synaptic responses in the medial nucleus of the trapezoid body and the medial and lateral superior olives.

Presynaptic calcium currents in the calyx of Held.
The calyx of Held is a giant synaptic terminal that forms around the soma of principal cells in the Medial Nucleus of the Trapezoid Body (MNTB) (Forsythe, 1994). Each MNTB neuron receives a single calyx. Action potentials propagating into the synaptic terminal trigger the opening of P-type calcium channels (Forsythe et al. 1998) which in turn trigger the release of glutamate into the synaptic cleft (Borst et al., 1995). Physiological studies show that the calyx of Held/MNTB synapse can transmit APs at rates of 600 Hz.

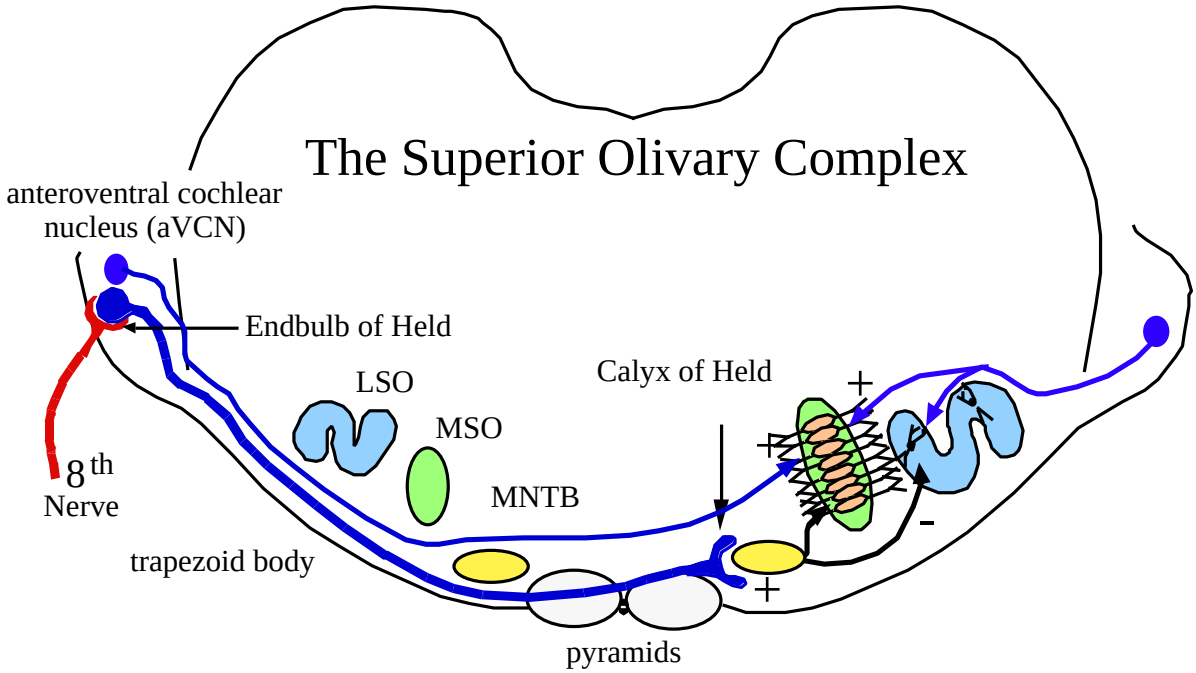

Figure 1. The fidelity of transmission along the tri-neuronal circuit of the binaural auditory pathway is essential to decode azimuth location in auditory space. Spherical and globular bushy cells in the aVCN project to principal cells of the medial nucleus of the trapezoid body (MNTB), the medial (MSO) and lateral superior olivary (LSO) nuclei ("+" shows glutamatergic and "-" glycinergic projections).

Maintenance of high transmission rates is a major physiological problem since it causes severe depletion of the pool of readily releasable synaptic vesicles. Consequently, there is considerable depression in the number of vesicles released following each sequential action potential of the train. This leads to a smaller EPSP in the postsynaptic MNTB neuron and an increase in the latency variability in generating an action potential. Recent studies suggest that there are around 2000 release sites on each calyx (Schneggenberger and Neher, 2000). The role of calcium and calcium sequestration mechanisms in regulating exocytosis and vesicle recycling are major areas of interest which will have a major impact on information transmission at this site (Helmchen et al., 1997).

Integration of the synaptic currents with postsynaptic voltage-gated currents.
Synaptic release of glutamate activates both AMPA and NMDA receptors generating a dual component excitatory postsynaptic current (EPSC) at the MNTB (Barnes-Davies and Forsythe 1995). The fast component is mediated by AMPA receptors while a slow component is mediated NMDA receptors. The NMDA receptor mediated component makes little or no contribution to generation of action potentials. The postsynaptic AMPA receptors are dominated by GluRD subunits while there are relatively lower expression levels of the RNA edited GluRB subunits (Geiger et al., 1995; Ravindranathan et al., 2000). All the glutamate receptor subunits in the MNTB contain the flop cassette. The lower expression of GluRB subunits means that the AMPA receptor ion channels are calcium permeable (Otis et al., 1995) with fast kinetics and generate a synaptic current that is 10-20 times that required to trigger an action potential in the postsynaptic neuron.

MNTB neurons respond to sustained depolarisation with a single action potential (Banks and Smith 1992) due to expression of low voltage-activated potassium channels that suppress the multiple firing. Using specific antibodies we can show that the rat MNTB expresses high levels of several *shaker*-related potassium channels. In addition there are toxins which selectively block voltage-gated currents generated by ion channels containing Kv1.1 or Kv1.2 (tityustoxin; toxin-K, respectively). Application of either toxin blocked a large component of the low voltage activated currents, suggesting that many channels contain both Kv1.1 and Kv1.2 subunits. These currents make a major contribution ot the fidelity of information transmission across the brainstem, since without them, the MNTB neuron will generate multiple action potentials for each calyx of Held giant EPSP. High voltage-activated Kv3.1 channels serve to accelerate repolarisation and minimise action potential duration (Brew and Forsythe, 1995; Wang et al., 1998).

The output of the MNTB to the MSO (and LSO).
The MSO receives binaural excitatory projections from the aVCN and an inhibitory projection from the MNTB. In neonatal rats this is mediated by both GABA and glycine receptors, but after 1 week of age glycine predominates. At around the onset of hearing in rats (day 11/12) there is a dramatic acceleration in the decay kinetics of the glycinergic IPSC. Grothe and Sanes (1994) demonstrate that this inhibition plays a role in temporal coding. From avian systems it seems likely that this inhibition serves in a general way to refine coincidence detection (Funabiki et al., 1998) but the adaptations preserving timing in this pathway suggest that the inhibition may play a more specific role in refining the ITDs (Grothe and Park, 1998). We have recently started investigating synaptic transmission and the role of the MNTB projection to the chopper cells and delay neurons of the LSO.

We have taken a cellular approach to the study of auditory processing in the brainstem. Numerous adaptations at both presynaptic and postsynaptic sites can be recognised which together function in a concerted manner to refine the ability of this pathway to maintain the pattern and timing of the incoming auditory activity. Modelling of the MNTB onset responses shows the pathway seems well

adapted for preserving the timing information, but later in an action potential train, the timing precision is traded for preservation of a supra-threshold response. This suggests that the MNTB is switching emphasis in the presentation of timing information which perhaps reflects differences in the function of inhibition within interaural timing and level discrimination pathways.

References:

Banks, M.I. & Smith, P.H. (1992). Intracellular recording from neurobiotin-labelled cells in brain slices of the rat medial nucleus of the trapezoid body. Journal of Neuroscience **12**, 2819-2837.

Barnes-Davies, M. and Forsythe I. D. (1995) Pre- and post-synaptic glutamate receptors at a giant excitatory synapse in rat auditory brain stem slices. Journal of Physiology, **488**: 387-406.Borst, J.G.G., Helmchen, F. & Sakmann, B. (1995). Pre-and postsynaptic whole-cell recordings in the medial nucleus of the trapezoid body of the rat. Journal of Physiology **489**, 825-840.

Brew, H.M. & Forsythe, I.D. (1995). Two voltage-dependent K^+ conductances with complementary functions in postsynaptic integration at a central auditory synapse. J. Neuroscience **15**, 8011-8022.

Forsythe, I.D. (1994). Direct patch recording from identified presynaptic terminals mediating glutamatergic EPSCs in the rat CNS, in vitro. Journal of Physiology **479**, 381-387.

Funabiki, K., Koyano, K. & Ohmori, H. (1998). The role of GABAergic inputs for coincidence detection in the neurons of nucleus laminaris of the chick. Journal of Physiology **508**, 851-869.

Geiger, J.R.P., Melcher, T., Koh, D.-S., Sakmann, B., Seeberg, P.H., Jonas, P. & Monyer, H. (1995). Relative abundance of subunit mRNAs determines gating and Ca^{2+} permeability of AMPA receptors in principal neurons and interneurons in rat CNS. Neuron **15**, 193-204.

Grothe, B. & Sanes, D.H. (1994). Synaptic inhibition influences the temporal coding properties of medial superior olivary neurons: An *in vitro* study. Journal of Neuroscience **14**, 1701-1709.

Grothe, B. & Park, T.J. (1998). Sensitivity to interaural time differences in the medial superior olive of a small mammal, the Mexican free-tailed bat. Journal of Neuroscience **18**, 6608-6622.

Helmchen, F., Borst, J.G.G. & Sakmann, B. (1997). Calcium dynamics associated with a single action potential in a CNS presynaptic terminal. Biophysical Journal **72**, 1458-1471.

Oertel, D. (1999) The role of timing in the brain stem auditory nuclei of vertebrates Annu. Rev. Physiol. **61**:497-519.

Otis, T.S., Raman, I.M. & Trussell, L.O. (1995). AMPA receptors with high Ca^{2+} permeability mediate synaptic transmission in the avian auditory pathway. Journal of Physiology **482**, 309-315.

Raman, I.M., Zhang, S. & Trussell, L.O. (1994). Pathway-specific variants of AMPA receptors and their contribution to neuronal signaling. Journal of Neuroscience **14**, 4998-5010.

Ravindranathan, A., Donevan, S.D., Sugden, S.G., Grieg, A., Rao, M.S. & Parks, T.N. (2000). Contrasting molecular composition and channel properties of AMPA receptors on chick auditory and brainstem neurons. Journal of Physiology **523**, 667-684.

Schneggenburger, R. & Neher, E. (2000). Intracellular calcium dependence of transmitter release rates at a fast central synapse. Nature **406**, 889-893.

Trussell, L.O. (1999) Synaptic mechanisms for coding timing in auditory neurons. Annu. Rev. Physiol. **61**:477-496

Wang, L.-Y., Gan, L., Forsythe, I.D. & Kaczmarek, L.K. (1998). Contribution of the Kv3.1 potassium channel to high frequency firing in mouse auditory neurons. Journal of Physiology **509**, 183-194.