Towards an Understanding of Functional, Morphological and Molecular Properties of Bushy Cells of the Rat Ventral Cochlear Neurons

In the master project, the cells in the ventral cochlear nucleus (VCN), mainly bushy cells and multipolar cells, were studied from a morphological, functional and molecular aspect. The bushy and multipolar cells in the VCN are known to have distinguishing morphological and functional characteristics. Bushy cells give rise to thin dendrites which intensively branch to form a bush-like dendritic tree surrounding the soma, and generate one or two brief action potentials at the onset of a depolarizing current injection. On the other hand, the multipolar cells send several dendrites without branching close to the soma, and generate repetitive action potentials upon current injection.

Whole-cell patch-clamp recordings from \( n = 42 \) neurons were made in transverse slices of the VCN from postnatal 8-10 days old rats. During the recording, a subset of cells was filled with AlexaFluor488 via the patch pipette, and the cell morphology was reconstructed by confocal laser scanning microscopy after fixation of the slice. At the same time, the firing behavior and the membrane properties were recorded under current clamp and the currents present in the cell were investigated with drugs (TTX and Cadmium) and voltage-clamp protocols. The functional properties demonstrated some of the important features of the VCN neurons, such as the fast membrane time constant (~10 ms on average), strong membrane rectification (~35%), a relatively depolarized resting membrane potential (~-55 mV) and pronounced \( I_h \) in some cells. However, the firing behavior and \( K^+ \) current analysis alone did not allow us to develop clear criteria to distinguish bushy cells from multipolar cells. Single-cell PCR was established to detect the expression of some genes of interest in the various VCN neurons. The calcium binding proteins, calbindin (CB), calretinin (CR), Parvalbumin (PV), and the Synaptotagmin isoforms 1 and 2 (Syt1 and Syt2) were examined. PV and Syt1 were both found consistently expressed and the expression of CR and Syt2 was heterogeneous between cells.

The results suggest that at P8-P10, the cells giving rise to the calyx of Held, classically thought to be the globular bushy cells, cannot easily be distinguished from neighboring cells (like multipolar cells) simply on the basis of firing properties. This might be caused by the relatively immature developmental stage. In the future, similar experiments should be performed in older animals (P15-P20) to obtain the developmental regulation of these features. Together with retrograde labelling by injection into the medial nucleus of the trapezoid body (MNTB), it will be possible to use the method established in this project to study the specificity of the neurons giving rise to the calyx of Held, and their gene expression pattern by using single-cell RT PCR.