Properties of Electrotonic Coupling in Inferior Olivary Network

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Introduction

Electrotonic coupling in the inferior olivary (IO) complex is known to play a crucial role in generating the subthreshold membrane potential oscillations in olivary neurons, and in synchronizing climbing fiber input into the cerebellar cortex. Hence we present a systematic study of the strength and spatial distribution of the coupling in 138 pairs of olivary neurons, using both electrophysiological and morphological methods in brain slice preparations. Electrotonic coupling was observed in 50% of the cell pairs, while most of the pairs were weakly coupled. The coupling was voltage-independent but showed a certain degree of asymmetry.

Methods

Simultaneous double patch recordings were performed in sagittal brain stem slices of 9-31 day old rats. The pipettes were filled with the intracellular solution containing in mM: 4 NaCl, 10 KCl, 140 CaCl2, 40 K-glutamate, 4 Mg-ATP, 10 Hepes, pH 7.2. In a few experiments the CsCl and 0.5 mM CaCl2 were added to the intracellular solution to prolong the high threshold Ca2+ spike. Neurobiotin (Sigma) was often added to the intracellular solution in a concentration of 0.5% for intracellular staining.

To compare the strength of electrotonic coupling between different pairs of IO neurons, we calculated the coupling coefficient (CC) from the voltage responses of pre- and post-junctional cells. The coupling resistance $R_c$ was calculated using Vf and CC measures upon current injection into each one of the cells in a pair.

Prevalence of coupling

A. Possibility of finding a coupled cell pair as a function of distance between the cells. Separation distance was the minimal distance measured from the cell membrane of one cell body to that of the other cell body. B. Distribution of coupling coefficients (CC) in 20 pairs of electrotonically coupled neurons. The CC varied between 0.02 and 1.7. In many of 65% of pairs the CC was less than 0.85. Both CC upon current injection into cell 1 (CC1) and into cell 2 (CC2) are plotted in the same graph.

C. Coupling coefficient (CC) as a function of distance between the cells, measured in 20 coupled pairs. Each pair is denoted by a different symbol. Each symbol appears twice in the figure, indicating coupling during current injection into cell 1 (CC1) and into cell 2 (CC2). Strongly coupled pairs show clear divergence of CC1 and CC2 values, indicating a directional preference of the coupling.

D. Distribution of coupling resistances ($R_c$) in 17 cell pairs. The $R_c$ varied between 0.7 to 19.8 GΩ and 68% of the values fell between 0.7 to 8 GΩ. Both $R_c$1 and $R_c$2 are plotted.

Asymmetry in coupling of olivary neurons

A. 200 msec negative current pulses of various amplitudes (bottom traces) were injected into cell 1 (left) and cell 2 (right). Averaged voltage responses ($n=30$) are shown for both conditions. The CC was less than 0.05. Both CC upon current injection into cell 1 (CC1) and into cell 2 (CC2) are plotted in the same graph.

B. Current-voltage relationship of cell 1 (squares) and cell 2 (triangles) shows similar input resistance.

C. Post-junctional voltage as a function of post-junctional current (transfer resistance) shows rectification reflecting the pre-junctional current-voltage relationship. Note the asymmetry.

D. Distribution of coupling resistances ($R_c$) in 17 cell pairs. The $R_c$ varied between 0.7 to 19.8 GΩ and 68% of the values fell between 0.7 to 8 GΩ. Both $R_c$1 and $R_c$2 are plotted.

Coupling during an action potential

A. Neuronal density of 3±2 cells in 50x50x25 μm cube (5x10⁴ cells/mm³) was measured using Cresyl Violet stained olivary sections. Only large cell bodies represent neurons. In some neurons are denoted by asterisks. An arrow and an arrowhead mark two inter-cellular links, round and elongated respectively. Scale bar, 20 μm.

B. The number of cells coupled to the cell at (0,0) is calculated based on the data shown in figure 1A as follows: A two-dimensional projection of a sphere with a radius of 85 μm is binned into 7 sphere-inside-sphere volumes. In each one of the volumes the number of cells is calculated (square). A number of cells in each volume coupled to a cell in the middle, calculated according to figure 1A, is denoted by triangles. This calculation shows that each olivary neuron is coupled to at least 50 other neurons.

C. Dye injection into one straight neuron resulted in indirect labeling of another neuron, two of them are shown in the figure. A darkly stained dendrite belongs to the cell that was labeled directly. The indirectly labeled cell bodies are indicated as well. Scale bar, 20 μm.

D. Two distinct morphological types of olivary neurons, “curly” and “straight” cells, can be distinguished on the basis of their dendritic morphology. Arrows denote examples of interconnections of different cells, possible locations of gap junctions.

Conclusions

Each olivary neuron is directly coupled to at least 50 other neurons. Electrotonic coupling between olivary neurons is asymmetric (this asymmetry can generate a condition where information within the nucleus flows in a directionally selective way).

Under normal experimental conditions a spike in one olivary neuron does not trigger spikes in coupled interneurons. Two distinct morphological types of olivary neurons, “curly” and “straight” cells, form two distinct non-interconnected networks.

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