# *Properties of Electrotonic Coupling in Inferior Olivary Network*

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## Introduction

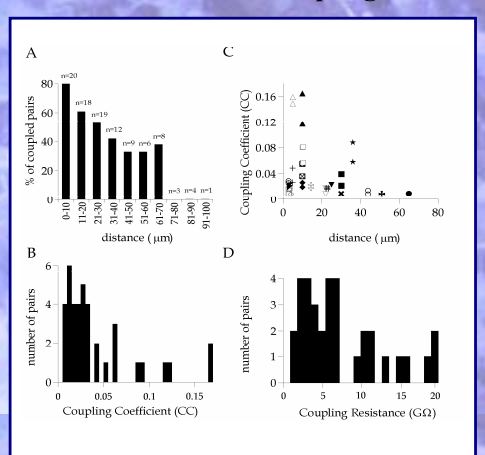
Electrotonic coupling in the inferior olivary (IO) nucleus is assumed to play a crucial role in generating the subthreshold membrane potential oscillations in olivary neurons, and in synchronizing climbing fiber input into the cerebellar cortex. Here we present a systematic study of the strength and 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. Neurobiotin injection into an olivary neuron produced indirect labeling of nearby neurons in 52% of staining experiments. We estimated that each olivary neuron is directly coupled to about 50 neurons, forming two independent networks of cells with distinct morphology.

## Methods

Simultaneous double patch recordings were performed in saggital brain stem slices of 9-31 day old rats. The pipettes were filled with the intracellular solution (containing in mM: 4 NaCl, $10^{-3}$ $CaCl_2$, 140 K-gluconate, $10^{-2}$ EGTA, 4 Mg-ATP, 10 Hepes; pH 7.2). In a few experiments 5mM EGTA and 0.5 mM $CaCl_2$ were added to the intracellular solution to prolong the high threshold $Ca^{2+}$ 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 to prolonged (150-250 msec), negative current pulses of various intensities. CC is defined as the ratio between voltage responses of the post- and the pre-juctional cell. The coupling resistance $R_c$ was calculated using V/I and CC measures upon current injection into each one of the cells in a pair.

## Prevalence of coupling



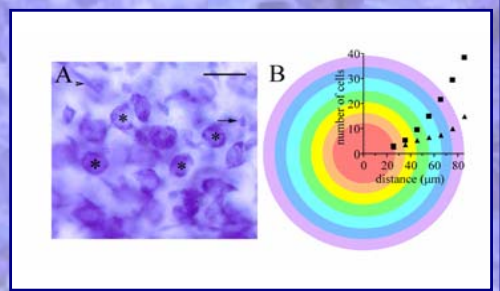

A. Possibility of finding a coupled cell pair as a function of distance between the cells. Separation distance was the minimal distance measured from the cell membrane of one cell body to that of the other cell body.

B. Distribution of coupling coefficient (CC) in 20 pairs of electrotonically coupled neurons. The CC varied between 0.002 and 0.17. In more than 75% of the pairs the CC was less than 0.05. Both CC upon current injection into cell 1 ($CC_1$) and into cell 2 ($CC_2$) 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 ($CC_1$) and into cell 2 ($CC_2$). Strongly coupled pairs show clear divergence of $CC_1$ and $CC_2$ values, indicating a directional preference of the coupling.

D. Distribution of coupling resistance ($R_c$) 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 $R_{c1}$ and $R_{c2}$ are plotted .

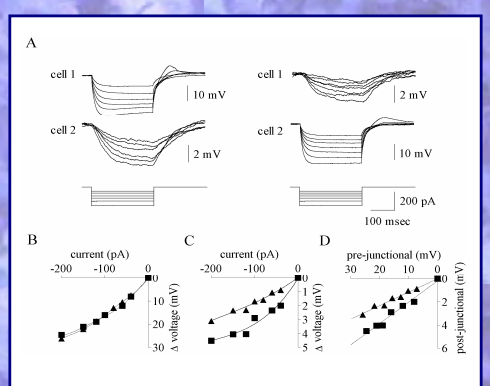
## Each neuron is coupled to at least 50 other neurons




A. Neuronal density of 3±2 cells in 50x50x25 μm cube ($5x10^4$ cells/$mm^3$) was measured using Cresyl Violet stained olivary sections. Only large cell bodies represent neurons. In-focus neurons are denoted by asterisks. An arrow and an arrowhead mark two in-focus glial cells, 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 (squares). 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.

## Asymmetry in coupling of olivary neurons



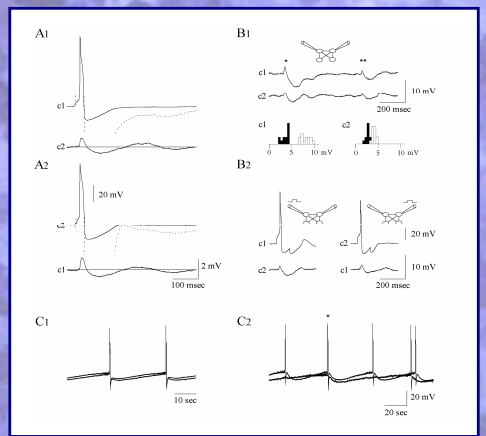

A. 200 msec negative current pulses of various amplitudes (bottom traces) were injected into cell 1 (left) and cell 2 (right). Averaged voltage responses (n=30) are shown for both conditions. This pair is denoted in the plot of CC as a function of distance by upward filled triangles.

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

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

D. Post-junctional voltage as a function of pre-junctional voltage. Note the linear relationship between the two and the asymmetry. Either cell 1 (squares) or cell 2 (triangles) was stimulated. The difference between the CC measured upon stimulation of cell 1 or cell 2 across 20 coupled pairs was 27±16%.

## Coupling during an action potential

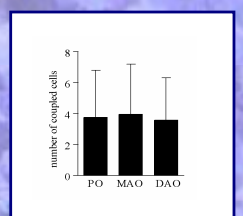



A. 20 msec 200 pA depolarizing current pulses injected into cell 1 or cell 2 elicited an action potential in the pre-junctional cell and a voltage deflection in the post-junctional cell. An action potential plotted at the same gain as the post-junctional response is indicated by a dashed line. Note that the initial post-junctional response is followed by an oscillation. In the pre-junctional cell the same oscillation is partially masked by voltage trajectory of the action potential and the associated conductances.

B. Two distinct spontaneous waveforms were recorded in a pair of coupled neurons (asterisk and double asterisk). The peak-to-peak amplitude distribution of these events (histograms, below) shows two distinct groups, where the black columns in cell 1 were associated with black columns in cell 2. The recorded pair was electrotonically coupled as demonstrated in B2. The insets illustrate possible connectivity.

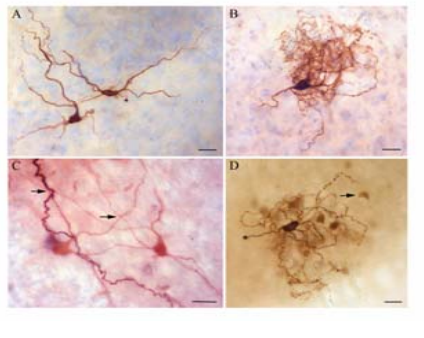
C. Artificially prolonged action potentials (by using 5 mM EGTA in the intracellular solution) elicited firing in the coupled cell.

## Coupling in uniform across olivary subnuclei




Mean (± SD) number of indirectly labeled cells per directly labeled cell in experiments in which at least one indirectly labeled cell was found. The data distinguish three main subnuclei of the IO complex (principal olive (PO), medial accessory olive (MAO), and dorsal accessory olive (DAO)).

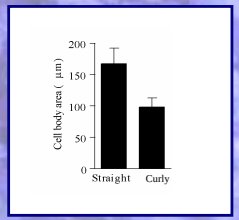
## "Straight" and "curly" neurons form two independent networks




A-B Two "straight" neurons (A) and one "curly" neuron (B) labeled directly through the patch pipette(s). No indirectly labeled cells were found. The sections were counterstained with Cresyl Violet.

C. Dye injection into one straight neuron resulted in indirect labeling of 9 additional neurons, two of them are shown in the figure. A darkly stained dendrite belongs to the cell that was labeled directly. The indirectly labeled neurons are straight as can be seen from their dendritic morphology. Arrows denote examples of intersections of dendrites of different cells, possible locations of gap junctions.

D. Dye injected into one curly neuron resulted in indirect labeling of an additional 11 neurons. Some of the cells are out of focus in the figure. Indirectly labeled cells had clearly stained cell bodies (e.g. arrow), but very weakly labeled dendrites. Scale bars, 20 μm.




The area of indirectly labeled cell bodies (mean ± SD) observed adjacent to directly stained straight or curly cells.

## 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 neurons.

Two distinct morphological types of olivary neurons, "curly" and "straight" cells, form two distinct non-interconnected networks.

## Acknowledgements

We are grateful to Hanoch Meiri for excellent technical assistance. This study was supported by the US-Israel Binational Science Foundation and the European Commission.