Synaptic responses relevant for binaural interactions in the medial superior olive studied in the isolated whole brain preparation of the guinea pig

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INTRODUCTION

It has been recently suggested that precise timing of glycinergic inhibition plays an important role in processing of interaural time differences (ITD) by neurons of the medial superior olive (MSO) in mammals. Experiments in the isolated whole brain preparation allow us to directly assess the contribution of inhibition in general and, specifically, contribution of glycinergic inhibition to synaptic responses of MSO cells produced by physiologically meaningful electrical activation of auditory nerves (AN).

METHODS

ISOLATION AND MAINTENANCE OF THE WHOLE BRAIN IN VITRO

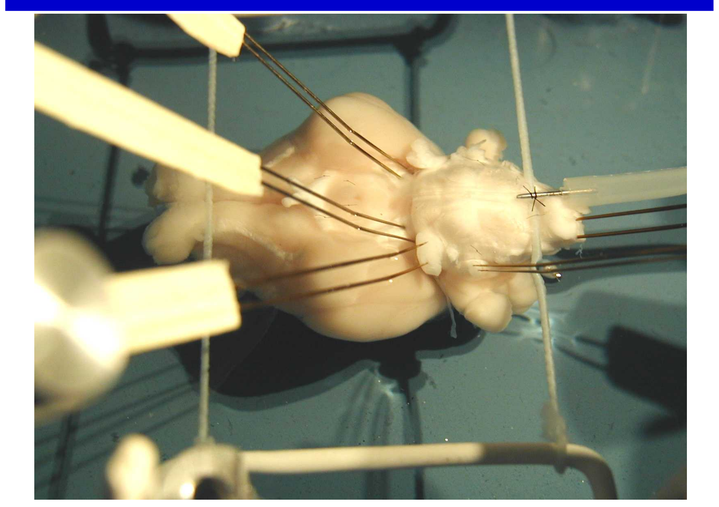
-Perfusion of anesthetized guinea-pigs through the heart with cold (10-12°C) Ringer's solution

-Dissection of the brain from the skull; transfer to the perfusion/recording chamber kept at 13°C

-Cannulation of one of the vertebral arteries and tying off the second vertebral artery. Start of brain perfusion

-Elimination of major arterial leaks by ligating vessels cut during the dissection

-Gradual warming of the brain to 29°C and progressive increase of the perfusion rate



RECORDING, STIMULATION and HISTOLOGY

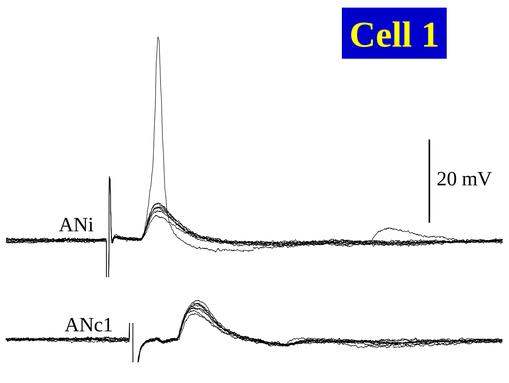
-Intracellular recordings and staining of SOC cells using glass micropipettes filled with a solution of 1-2% Neurobiotin in 2M K-acetate.

-Stimulation of the AN and inferior colliculus (IC) on both sides of the brain through bipolar metallic electrodes with rectangular electrical pulses of 0.2 ms duration

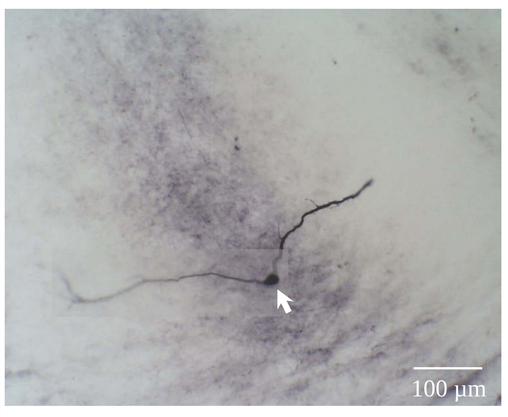
-Revealing of stained neurons on transverse sections of the brain (100 µm) using a standard ABC histochemistry.

Two MSO cells with contrasting pattern of synaptic responses from the auditory nerves

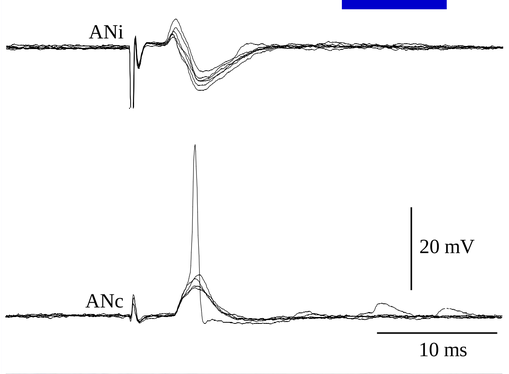
Cell 1



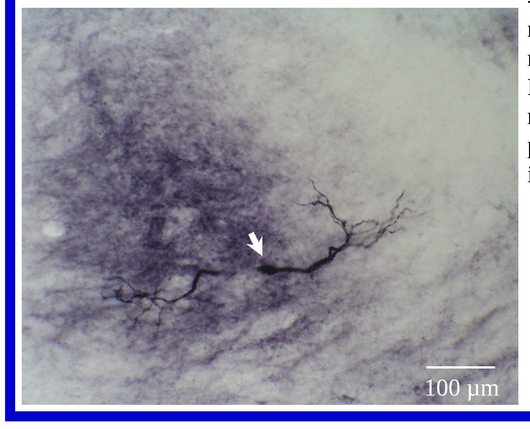
Cell 1. This cell exhibited purely excitatory response to stimulation of the ipsilateral auditory nerve (ANi) whereas stimulation of the contralateral auditory nerve (ANc) produced excitatory or mixed (excitatory – inhibitory) postsynaptic potentials at two slightly different stimulation intensities (ANc1 and ANc2, respectively). Subtraction of averaged ANc1 recordings from averaged ANc2 traces revealed the inhibitory component (ANc2 – ANc1) of the response from the ANc. Note that this inhibitory component is significantly delayed with respect to the latency of excitatory response from the ANi. The resting membrane potential of the neuron was -62 mV. The panel below recordings shows a microphotograph of this cell, which projected its axon to the ipsilateral inferior colliculus.



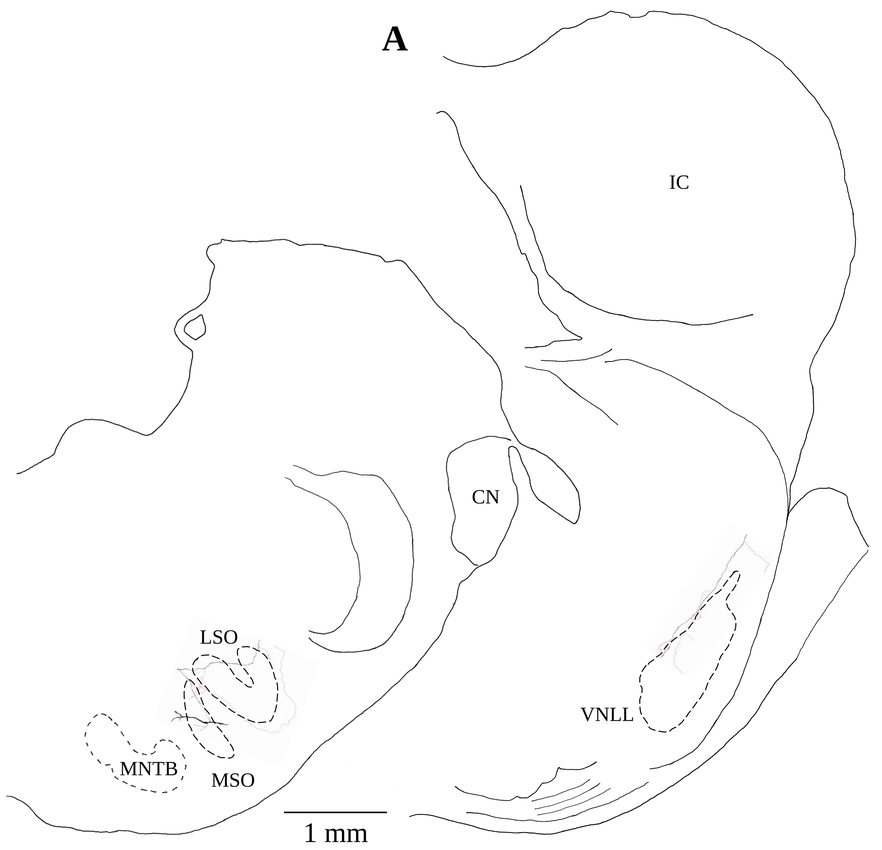
Cell 2



Cell 2. In contrast to cell 1, this cell responded with mixed (excitatory – inhibitory) postsynaptic potential to stimulation of the ipsilateral AN (ANi) and with pure EPSP to stimulation of the contralateral AN (ANc). The resting membrane potential of the neuron was -60 mV. Labeling of the neuron with neurobiotin revealed a typical bipolar MSO cell (panel below recordings) with an axon projecting to the ipsilateral inferior colliculus.

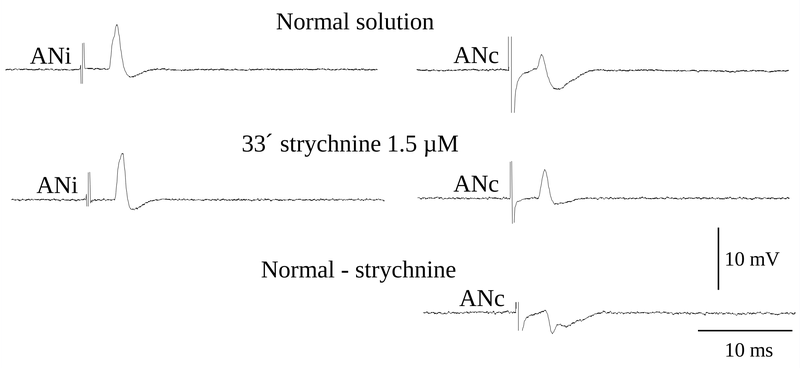


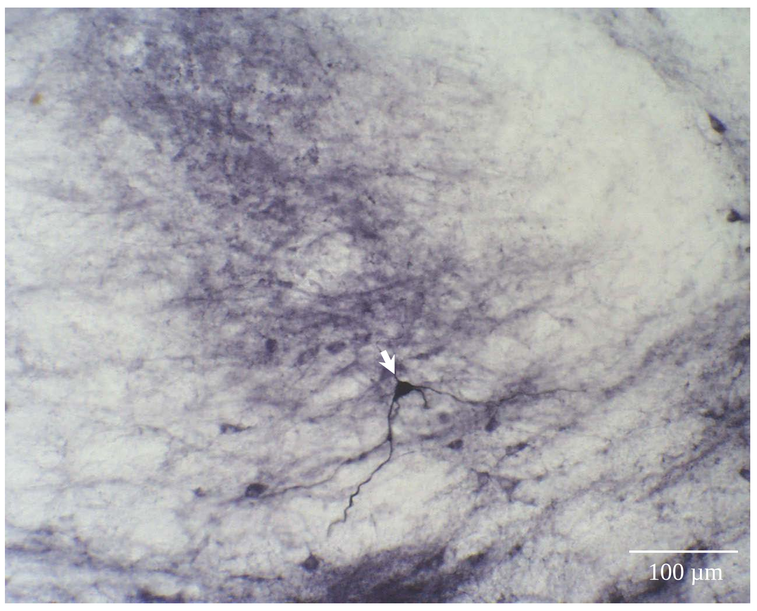
MSO cell with small inhibitory responses insensitive to strychnine



(A) Position of the cell on transverse sections of the brainstem. MNTB – medial nucleus of the trapezoid body; LSO – lateral superior olive; CN – cochlear nucleus; VNLL – ventral nucleus of lateral lemniscus. IC – inferior colliculus. (B) Intracellular recordings from the cell at a resting membrane potential of -58 mV. (B_1) Responses of the cell to ipsilateral (ANi) and contralateral (ANc) stimulation of auditory nerves in normal solution (first row) and in the solution containing 1µM strychnine. (B_2) Antidromic activation of the cell by stimulation of the ipsilateral inferior colliculus (ICi). (C) High-magnification reconstruction of the cell.

MSO cell with glycinergic inhibitory component in response to stimulation of the contralateral AN





Morpho-physiological characteristics of the cell. Intracellular (intradendritic?) recordings were made in the normal solution and in the solution containing strychnine. Responses of the cell to stimulation of ANi were not modyfied by strychnine, whereas there was a strychnine-sensitive (glycinergic) component in the response evoked by stimulation of the ANc (see subtraction trace Normal – strychnine).
The photomicrograph below recordings shows the labeled cell in the MSO (arrow). The axon of the cell could be followed through the lateral lemniscus to the ipsilateral inferior colliculus.

RESULTS & CONCLUSIONS

- Some of the neurons characterized as “bipolar” principal MSO cells projecting to inferior colliculus, which are considered to play the main role in ITD processing, exhibited very little, if any, contribution of glycinergic transmission to responses produced by stimulation of both auditory nerves.
- On the other hand, in neurons that exhibited glycinergic inhibition, the glycinergic inhibitory components were often significantly delayed with respect to excitatory components of mixed excitatory-inhibitory responses produced by activation of AN.
- This observations suggest that in many cells glycinergic inhibition can hardly influence bilateral excitatory synaptic interactions in physiologically significant interaural time intervals.
- Further experiments are needed to understand functional significance of glycinergic inhibition in the MSO and its possible role in encoding ITDs.

Supported by FNS grant 31-111925.1