

Commissural glycinergic inhibition of bushy and stellate cells in the anteroventral cochlear nucleus

Alexandre L. Babalian^{CA}, Anne-Valerie Jacomme, John R. Doucet,¹ David K. Ryugo¹ and Eric M. Rouiller

Institute of Physiology, University of Fribourg, Rue du Musée 5, CH-1700 Fribourg, Switzerland; ¹Departments of Otolaryngology-HNS and Neuroscience, Johns Hopkins University, Baltimore, MD, USA

^{CA}Corresponding Author

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Synaptic inputs from one cochlear nucleus (CN) to the other can play an important role in modulating the activity of CN neurons. Using the isolated whole brain preparation of the guinea pig, we tested the effects of electrical stimulation of the contralateral auditory nerve (AN) on intracellularly recorded and stained neurons of the anteroventral cochlear nucleus. Stimulation of the contralateral AN evoked only inhibitory postsynaptic potentials (IPSPs) in 63% of recorded neurons, including bushy and stellate

cells. The latency of most IPSPs (88%) was in the range 3.3–7.6 ms, consistent with mono- and disynaptic transmission from the contralateral CN. The IPSPs had an average amplitude of 2.6 ± 1.9 mV and were blocked by strychnine suggesting their glycinergic nature. These data, together with our similar findings in other CN subdivisions, indicate that principal cells of the CN contribute to binaural interactions at earliest stages of acoustic processing. *NeuroReport* 13:555–558 © 2002 Lippincott Williams & Wilkins.

Key words: Binaural interactions; Guinea pig; Intracellular recording and staining; IPSP; Isolated whole brain

INTRODUCTION

Functional interactions between the cochlear nuclei (CN) could be an important step in binaural integration underlying directional hearing. Most available evidence for commissural interactions of two CN lack, however, direct correlation between the functional properties of involved pathways and targets of their actions in the CN. Previous physiological studies, conducted mainly on the dorsal cochlear nucleus of intact animals, have demonstrated that extracellularly recorded neurons can be both inhibited [1–4] and excited [4,5] by sounds applied to the contralateral ear. However, limitations of the extracellular recording technique have not permitted precise determination of the nature and timing of underlying synaptic events in conjunction with the identification of CN neurons affected by contralateral stimulation. On the other hand, anatomical demonstrations of direct projections from one CN to the other [6–13] have provided limited information about functional strength of the connections and their target neurons in the CN. Moreover, purely anatomical methods may fail to demonstrate the possible existence of indirectly operating pathways, which can be functionally more powerful than direct ones. Many of above mentioned limitations can be circumvented by using the *in vitro*, isolated whole brain (IWB) preparation which allows direct assessment of the functional and pharmacological properties of electrically activated inputs to intracellularly recorded and stained CN

neurons. Using this approach, we have recently characterized the inputs from the contralateral CN to the principal cell types in the postero-ventral (PVCN) and dorsal (DCN) subdivisions of the CN [14]. However, the physiological properties of possible commissural inputs to the anteroventral subdivision of the CN (AVCN) are not known. The AVCN contains two major neuronal classes, bushy and stellate cells [15], projecting to higher auditory centers. These two cell types in the AVCN, especially bushy cells, have morphological, connective, and physiological properties distinct from those of neurons in other CN subdivisions (for review see [16]; see also [17]) suggesting that AVCN neurons might also exhibit a specific relationship with the contralateral CN. In the present study, we provide direct evidence for efficient commissural inputs to identified neurons of the AVCN.

MATERIALS AND METHODS

Experiments were conducted in compliance with the guidelines for animal care of the Swiss Veterinary Authorities. The methods used to prepare and maintain the IWB preparation *in vitro* were similar to those described in previous studies [14,18]. Briefly, pigmented guinea pigs were injected with a lethal dose of pentobarbital (120–150 mg/kg, i.p.) and perfused through the heart with Ringer's solution for 5–6 min. Following decapitation, the

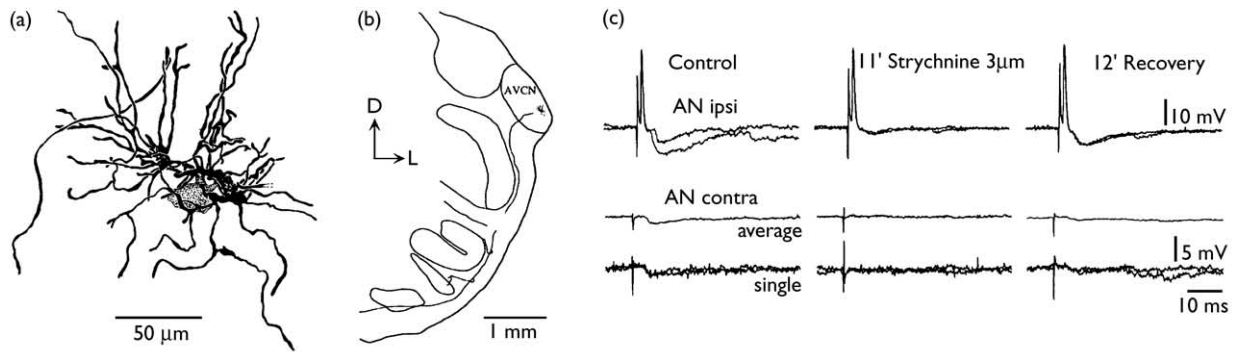


Fig. 1. Anatomical and physiological properties of a bushy cell in the anteroventral cochlear nucleus. High magnification reconstruction of the cell (a), and its schematic location on a frontal section of the brain stem (b). Orientation arrows: D, dorsal; L, lateral. (c) Intracellular recordings (at resting membrane potential of -62 mV) of responses induced by stimulation of the ipsilateral (first row) and contralateral (second and third rows) auditory nerve. First and third rows are superpositions of individual responses, whereas the traces of the second row are average records. Left column: responses in normal perfusion solution; middle column: modification of the responses after 11 min of brain perfusion with strychnine-containing solution; right column: partial recovery of initial responses 12 min after the perfusion with normal solution was resumed. On this and the following figure, note a blockade by strychnine of IPSPs evoked by the contralateral and ipsilateral AN stimulations.

brain was dissected from the skull and transferred to the perfusion/recording chamber. The brain was perfused with a Ringer's solution containing dextran through a fine metallic cannula inserted in one of the vertebral arteries. The second vertebral artery and the other major arteries cut during the dissection were ligated to minimize leaks of perfusate from the brain. While recording from some AVCN neurons, strychnine was added to the perfusate at a concentration of $3\ \mu\text{M}$ to block glycinergic synaptic transmission.

Intracellular recording and labeling of AVCN neurons was performed using glass micropipettes filled with 1–2% solution of the tracer Neurobiotin in 2M K-acetate. The auditory nerves (AN) were stimulated with single electrical pulses delivered through stainless steel bipolar electrodes. Recorded neurons were iontophoretically injected with the tracer and subsequently revealed on transverse sections of the brain using standard histochemistry [19].

RESULTS

The quality of intracellular recordings was attested by the high level (at least -55 mV) and stability (± 2 mV) of the membrane potential (MP), as well as by a short duration of spikes showing no signs of deterioration during the entire recording time. We recorded the activity of 27 neurons in the AVCN. Fifteen of these neurons were subsequently recovered in histological sections and identified as bushy ($n=7$) or stellate cells ($n=8$). The remaining 12 neurons were either not recovered, only partially recovered, or could not be stained. This group of neurons, which will be referred to as unidentified cells, had similar physiological properties to bushy and stellate cells and were therefore included in the data base. The mean (\pm s.d.) MP of all recorded neurons was -61.1 ± 4.2 mV (range -55 to -73 mV; $n=27$), and it was not different across the three groups of cells ($p=0.52$, one-factor ANOVA).

Morphological and physiological properties of representative bushy and stellate cells are illustrated in Fig. 1 and Fig. 2, respectively. The neurons (Fig. 1a, Fig. 2a) were

located rostral to the entrance of the AN root in the CN (Fig. 1b and Fig. 2b). Axons of both cells left the CN through the trapezoid body (TB). The axon of the bushy cell gave rise to few axon collaterals directed to the nuclei of the ipsilateral superior olivary complex (SOC; Fig. 1b), whereas the axon of the stellate cell did not branch (Fig. 2b). All neurons for which the axonal trajectory could be traced ($n=14$), sent their axons to the TB and no recurrent axon collaterals were observed.

Stimulation of the ipsilateral AN induced monosynaptic excitation of bushy and stellate cells followed usually by a delayed inhibition (Fig. 1c, Fig. 2c, first row of the left column). The passive membrane properties and spike activation pattern of bushy *vs* stellate cells in the AVCN of guinea pig were generally similar to those of bushy/type II and stellate/type I cells in the AVCN of mice [20,21] and will be described elsewhere.

Stimulation of the contralateral AN often induced inhibitory postsynaptic potentials (IPSPs) in bushy and stellate cells (Fig. 1c, Fig. 2c, 2nd and 3rd row of the left column). Perfusion of the brain with a solution containing strychnine blocked the contralaterally-induced IPSPs as well as the delayed inhibition evoked by the ipsilateral AN stimulation (Fig. 1c, middle column; Fig. 2c, right column). These observations suggest that both types of inhibitory responses were mediated by glycinergic transmission. A gradual recovery of responses could be observed when the perfusion of the brain with normal solution was resumed (Fig. 1c, right column).

Stimulation of the contralateral AN induced IPSPs in 17 of 27 recorded cells (63%). No signs of excitation were observed. There was no significant difference between MP of cells exhibiting contralateral inhibition and neurons in which IPSPs were absent (60.2 ± 4 mV and 62.5 ± 4.3 mV, respectively; $p=0.09$). This observation, together with recordings performed at different levels of the membrane potential, indicate that the absence of inhibitory responses in some cells was true one and was not due to possible masking of IPSPs at membrane potentials close to their equilibrium potential. The IPSPs were observed in five of

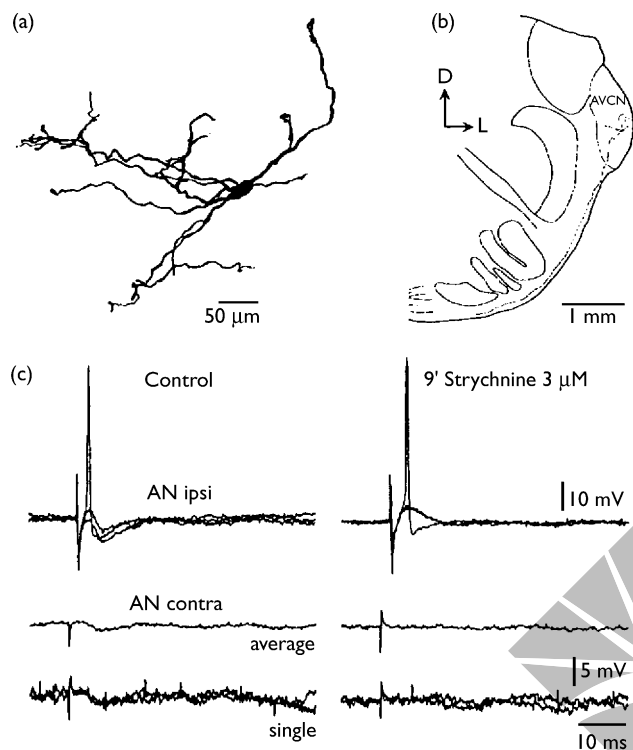


Fig. 2. Anatomical and physiological properties of a stellate cell in the anteroventral cochlear nucleus. High magnification reconstruction of the cell (a), and its schematic location on a frontal section of the brain stem (b). Orientation arrows: D, dorsal; L, lateral. (c) Intracellular effects of stimulation of the ipsilateral (first row) and contralateral (second and third rows) auditory nerve recorded in normal perfusion solution (left column) and 9 min after beginning of perfusion with strychnine-containing solution (right column). First and third rows are superpositions of individual responses, whereas the traces of the second row are average records. Note that the block of the ipsilateral delayed inhibition by strychnine reveals the shape of uncontaminated EPSP underlying the spike (first row). The recovery of responses in normal solution was not recorded in this cell. The resting membrane potential of the neuron was -56 mV.

seven bushy cells (71%), five of eight stellate cells (63%), and seven of 12 unidentified cells (58%). The IPSP latencies were measured on averaged responses thus excluding any possible confusion of evoked responses with spontaneous events. The mean latency of IPSPs was 6.2 ± 4 ms ($n=17$), with the majority of response latencies (15 of 17) ranging from 3.3 to 7.6 ms. The latency distribution of contralaterally induced IPSPs is shown in Fig. 3a. The IPSP latencies in bushy (7.3 ± 6.4 ms; $n=5$), stellate (5.4 ± 3.9 ms; $n=5$), and unidentified (5.9 ± 1.3 ms; $n=7$) cells were not statistically different ($p=0.18$, Kruskal-Wallis test). The amplitudes of IPSPs, measured in each cell as an average of 5–20 individual responses, ranged from 0.5 to 8 mV and had a mean value of 2.6 ± 1.9 mV ($n=17$). The proposed glycinergic nature of IPSPs (see above) and the fact that the equilibrium potential of glycinergic IPSPs in the cochlear nucleus is close to the resting MP of neurons ([22]; our polarization tests) suggest that the absolute value of IPSP amplitudes can strongly depend on the MP level. However, the absence of significant differences between the MP of the three groups of recorded cells allowed us to compare the

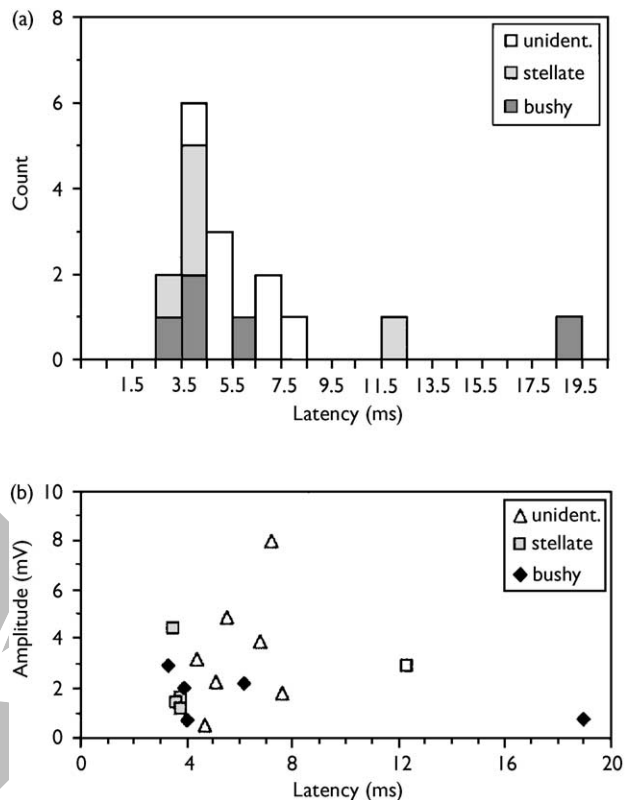


Fig. 3. Quantitative characteristics of IPSPs induced in anteroventral cochlear nucleus cells by stimulation of the contralateral auditory nerve. (a) Latency distribution for IPSPs in different cell types. Note the similarity of the response latencies across the different categories of cells. (b) Scatter diagram of IPSP amplitudes vs their latencies. The absence of significant correlation between the two parameters suggests that the strength of the inhibitory input is not related to the morphological properties of underlying pathways (see text for details).

relative strength of inhibition in different cell types. The IPSP amplitudes in bushy (1.8 ± 1 mV, $n=5$), stellate (2.3 ± 1.3 mV, $n=5$), and unidentified (3.5 ± 2.4 mV, $n=7$) cells were not statistically different ($p=0.3$, Kruskal-Wallis test).

Since the amplitudes of recorded IPSPs could potentially be related to the number of synapses and/or to the thickness of fibers in the pathways from the contralateral CN, the response amplitudes were plotted as a function of their latencies (Fig. 3b). The correlation between the two parameters was weak and not statistically significant ($r=0.05$, $p=0.84$) suggesting that the strength of the inhibitory input from the contralateral side was not related to the morphological features of the pathway.

DISCUSSION

The present experiments complete our study of the effects induced by stimulation of the contralateral AN on identified neurons in different subdivisions of the CN. In our previous study [14] we demonstrated that about 70% of neurons among the major cell types in the PVCN and DCN were inhibited by stimulation of the contralateral AN. The present study shows that contralaterally-induced effects in the neurons of AVCN are qualitatively and quantitatively

similar to those previously observed in PVCN and DCN. Indeed, the percentage of influenced cells, the exclusive inhibitory nature of these influences, the latencies and the amplitudes of IPSPs were similar in all three subdivisions of the CN. These results are consistent with morphological data in the guinea pig showing that commissural axons originating from the contralateral CN are distributed throughout much of the CN [13]. Such a widespread termination pattern could serve as a morphological substrate for relatively homogeneous effects on various CN cell types. The present data are the first direct demonstration that both principal cell types in the AVCN, bushy and stellate cells, receive functional inputs from the contralateral CN and that these inputs are inhibitory. The proportion of contralaterally influenced unidentified cells and patterns of their synaptic activity, similar to those of bushy and stellate cells, suggest that most, if not all, unidentified neurons also belonged to bushy or stellate cell categories.

The latencies of IPSPs evoked by stimulation of the contralateral AN ranged between 3.3 and 7.6 ms for the majority of neurons, and were similar to those of contralaterally-induced IPSPs in the PVCN and DCN [14]. As discussed previously [14], these latency values in the IWB preparation most likely correspond to a di- and trisynaptic transmission from the contralateral AN, or, respectively, to a mono- and disynaptic transmission from the contralateral CN. The possibility of monosynaptic influences from the contralateral CN is well supported by morphological studies demonstrating direct connections between the two CN [6-8,10,12,13]. A possible disynaptic pathway from the contralateral CN might operate via the SOC and/or through local inhibitory interneurons within the CN (see Discussion in [14]). It is not excluded, however, that longer-latency IPSPs are mediated directly through very thin slow-conducting commissural fibers. This possibility is supported by the observation that the diameters of commissural fibers connecting the two CN range from thick (2-4 μm) to thin (< 0.5 μm) [13]. The two IPSPs with very long latencies (> 12 ms), like similar responses in the PVCN and DCN [14], could be conveyed through polysynaptic pathways that include additional relays at the brainstem or higher brain levels.

IPSPs induced in the AVCN cells by stimulation of the contralateral AN were blocked by strychnine. Likewise, the inhibitory action of contralateral acoustic stimulation on the activity of some neurons in the ventral CN was blocked by a systemic administration of strychnine [1]. These results suggest that the neurons mediating inhibition from the contralateral CN use glycine as neurotransmitter. Recently, it has been demonstrated in the rat spinal cord slices that strychnine at concentrations used in the present study can block not only glycine receptors but also, partially, GABA-A receptors [23]. The fact that we observed a total block of IPSPs by strychnine rules out a possibility of any significant contribution of GABA-ergic transmission to the commissural inhibition. Our conclusion that glycinergic transmission plays a dominant role in the inputs from one CN to the other

is consistent with morphological data demonstrating that commissural projections are mostly immunoreactive for glycine [8,9,11]. Furthermore, physiological data indicate that glycine plays a major role in other inhibitory circuits of the CN [22,24,25], particularly in the pathway that mediates the delayed inhibition of CN cells in response to ipsilateral AN stimulation (our present results; [22,25]).

The contralaterally induced IPSPs are likely to be functionally significant. We observed that the IPSPs evoked by stimulation of the contralateral AN can inhibit the evoked activity of CN neurons as well as discharges of some spontaneously active neurons in the DCN. These effects exerted by small-amplitude IPSPs could be explained by significant increases in membrane conductivity usually accompanying inhibitory actions.

CONCLUSION

A large proportion of principal CN neurons are efficiently inhibited through pathways activated by the contralateral AN. This observation suggests that binaural interactions already take place at the level of CN, prior to the more well known circuits of the SOC. Thus the CN should be considered among other structures (SOC, nucleus of lateral lemniscus, inferior colliculus) as being potentially involved in binaural mechanisms of sound localization and directional hearing. Further behavioral and physiological experiments are needed to understand how the commissural inhibition is integrated in these mechanisms.

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