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# Granule and Purkinje-like cells of the rat dorsal cochlear nucleus—a study using combined application of electrophysiology and imaging techniques

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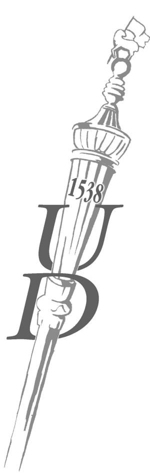


**UNIVERSITY OF DEBRECEN**
**DOCTORAL SCHOOL OF MOLECULAR MEDICINE**
**Physiology and Neurobiology PhD program**

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# Granule and Purkinje-like cells of the rat dorsal cochlear nucleus—a study using combined application of electrophysiology and imaging techniques


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# Introduction

The cochlear nucleus (CN) is situated on the laterodorsal surface of the brain stem, at the boundary separating the medulla and the pons. The CN is divided into ventral and dorsal cochlear nuclei (VCN and DCN, respectively). Either part of the CN is contacted by acoustic fibres originating from the ipsilateral spiral ganglion; i.e., they have monaural inputs. The CN also contacted by fibres originating from the colliculus inferior, auditory cortex, contralateral CN, vestibular ganglion and nuclei, trigeminal nucleus, and cuneate nucleus. Collaterals from the olivocochlear bundle reach the CN, too.

All primary acoustic fibres terminate within the CN and the incoming activity patterns are transferred to several cells. In turn, the activity entering the CN is projected to the inferior colliculus via several parallel channels. These pathways converge in the inferior colliculus, from where the auditory information is passed to the auditory cortex via the medial geniculate nucleus.

The CN has complex cytoarchitecture. Lorente de Nó estimated that the number of cell types in this nucleus is about 40–50. Detailed analyses of the various cell types were carried out using Nissl and Glees staining as well as Golgi impregnation. Early studies established that the most prominent projection neurons of the VCN are the spherical and globular bushy, octopus, and stellate cells. Beside the projection cells, several types of interneurones have also been identified, including Golgi and granule cells. The projection neurones of the DCN are the pyramidal (fusiform) and the giant cells. Several interneurones can also be found in the DCN, such as the cartwheel, Golgi, and granule cells. Another cell type, found primarily in the DCN and having characteristic morphology, is the Purkinje-like cell (PLC). At present there is no consensus regarding the nature of the PLCs; i.e., whether their axons leave the CN or not—in other words whether they are projection- or interneurones.

The axons of both the pyramidal and giant cells travel via the dorsal acoustic stria and target other nuclei of the auditory pathway. Pyramidal neurones mainly project to the contralateral inferior colliculus and to the medial geniculate nucleus. However, some of these axons may also reach the ipsilateral inferior colliculus. Giant cells mainly project to the contralateral inferior colliculus but some of the axons target the ipsilateral inferior colliculus and the contralateral CN as well.

The DCN shows a layered organisation which is evident in both coronal and sagittal sections. The axons of the granule cells (i.e., parallel fibres), the apical dendrites of the pyramidal cells, the cell bodies of some granule cells, and the PLCs are located in the outermost *molecular layer*. The *pyramidal cell layer* is located beneath the molecular layer, accommodating the cell bodies of the pyramidal neurons, the somata of most granule cells, and those of the cartwheel and Golgi cells. The *deep layer* contains the cell bodies of the giant neurones and the basal dendritic trees of the pyramidal cells.

Two types of excitatory inputs reach the pyramidal and giant cells. The acoustic fibres make contact with the basal, whereas the parallel fibres terminate on the apical dendritic trees. No noteworthy synaptic plasticity has been detected in the synapses between the acoustic fibres and the basal dendrites of the projection neurones. In contrast, prominent two-way plasticity (i.e., long-term potentiation and depression) was described in the synapses formed between the parallel fibres and the apical dendrites. The parallel fibre network is known to transmit somatosensory and vestibular information regarding the position of the auricles, neck, and head.

Granule cells may be capable of integrating information originating from multiple sources. One of their most important inputs is the somatosensory information arriving via the cuneate nucleus. Particularly strong field potential can be recorded from the DCN when the dorsal root of the second cervical nerve is stimulated. This spinal nerve is the proprioceptive nerve of the muscles that

move the auricles. While stretching the muscles that move the ear greatly influences the action potential (AP) firing frequency of the DCN projection neurones, the stimulation of the skin or hair of the auricles does not influence their activity. These data indicate that the discharge pattern of these neurones is not only influenced by auditory stimuli but by the position of the auricles as well. This mechanism may be important in locating the position of the sound source. The granule cells also receive inputs from higher auditory centres, such as the inferior colliculus and the auditory cortex. In addition, the activity of the projection neurones of the DCN is also influenced by inhibitory inputs. The cartwheel cells, which receive excitatory inputs from the granule cells, are particularly important inhibitory interneurones in the DCN. Their axons make synaptic contacts with both the pyramidal and giant cells.

Sound stimuli with spectral notches have strong influence on the activity of the projection neurones of the DCN. Parts of the outer ear introduce specific spectral notches to sound stimuli—a phenomenon explained by the shadowing effect of the pinna. The characteristics of the spectral notches change according to the relative positions of the pinna and the sound source, thus the frequency of the spectral-gap (or 'spectral clue') depends on the horizontal and vertical positions of the sound source. Accordingly, one of the possible functions of the DCN is sound source localization through spectral clues. This function may be assisted by incoming somatosensory information, too.

The morphology of PLCs differs from all other cell types of the DCN. As their name suggests, the rich arborisation of their dendritic trees resembles that of the cerebellar Purkinje cells. PLCs, similar to Purkinje cells, present strong calbindin-specific immunopositivity. These cells are mostly present just beneath the surface of the DCN, though some PLCs have been described in the deeper layers of the DCN and in the VCN, too. The cell bodies of the PLCs are usually globular or slightly elongated. Their axons—or axon collaterals—probably leave the DCN. In sharp contrast with their morphological features, no information is

available about the electrophysiological and membrane characteristics, synaptic inputs, possible neurotransmitter, or cerebellin expression of the PLCs.

There are several lines of evidence indicating that cholinergic mechanisms can substantially influence the function of the CN. The presence of muscarinic cholinergic receptors and vesicular acetylcholine transporter in the nucleus has been already described. It has been also shown that cholinergic fibres originating from various brain regions—including the superior olive as well as the pedunculopontine and laterodorsal tegmental nuclei—terminate in the CN. *In vitro* and *in vivo* experiments showed that 90% of the spontaneously active neurones of the DCN modify their activity when cholinergic agonists are applied.

Besides the aforementioned general observations, there are some data about cell type-specific effects of cholinergic modulation in the DCN. In one of our earlier studies we have described cholinergic modulation of the giant cells. We provided evidence that the neurotransmission between the parallel fibres and giant cell dendrites is modulated by the activity of presynaptic muscarinic receptors, belonging to the M3 subclass. This finding is in harmony with earlier findings demonstrating the presence of several cholinergic nerve terminals in the granule cell domain of the CN. Although our preliminary results raised the possibility that granule cells may also be subjects of cholinergic modulation, no specific experiments have been hitherto carried out to prove or reject this hypothesis.

# Aims

The dorsal cochlear nucleus (DCN) is suggested to integrate auditory and somatosensory information. It may also be involved in sound source localisation, although its precise function is not fully understood yet. Two cell types of the DCN have been studied in the present work; namely, the Purkinje-like neurones and the granule cells. In general, our aim was to provide new data which may help us to better understand the organisation and function of the DCN.

As for the **Purkinje-like cells**, the following tasks have been pursued:

- Detailed examination of the morphology to ensure their unambiguous recognition in functional experiments;
- Identification of their neurotransmitter and determination of their inhibitory or excitatory nature;
- Characterisation of their membrane characteristics and firing properties;
- Functional examination of their synaptic inputs to determine their possible contribution to the overall neuronal network accommodated by the cochlear nucleus; and
- Investigation of their cerebellin expression pattern to assess the degree of similarity between Purkinje-like cells and cerebellar Purkinje neurones.

**Granule cells** are the smallest and most numerous cells in the cochlear nucleus. They have important roles in both multimodal sensory integration and regulation of the firing pattern of the projection neurones. It has been described that cholinergic agonists modify the activity of several cell types of the cochlear

nucleus, including pyramidal and cartwheel cells. It has been also noticed that cholinergic nerve terminals are present in areas of the cochlear nucleus known to accommodate granule cells in high density. However, there has been no investigation of the presence and nature of cholinergic modulation affecting the granule cells. Because the cholinergic modulation in the cochlear nucleus may have an amplification function, and dysfunction of the cholinergic network may result in tinnitus, any new information about the cholinergic modulation of the granule cells may have practical significance. For this reason, the second part of our work had two general aims:

- Investigation of the cholinergic modulation of the granule cells using functional measurements and immunohistochemistry. Because intracellular calcium imaging in brain slices allows simultaneous monitoring of the activity of a large number of cells, this has been our primary method of choice in the functional experiments.
- Identification of the receptors mediating the cholinergic effects, using receptor subtype-specific immunohistochemistry and subtype-selective antagonists.

# Materials and methods

## Animals, chemicals, and solutions

All experiments were conducted on Wistar rats. The functional experiments were carried out in an artificial cerebrospinal fluid (aCSF). In order to block neurotransmission (i.e., to functionally 'uncouple' DCN neurones), all major chemical synaptic pathways were inhibited by applying a glutamatergic-glycinergic-GABAergic neurotransmission blocking cocktail (referred to as '3G cocktail' hereafter). For fluorescent calcium imaging, the acetoxymethylester form of the Oregon Green 488 BAPTA 1 fluorescent calcium indicator dye (OGB-AM) was employed.

## Functional experiments

Sagittal slices—with a thickness of 200 µm—were cut from the DCN. The slices were transferred into an aCSF-filled incubation chamber and they were continuously bubbled with a mixture of 95% $O_2$ and 5% $CO_2$ (37°C).

Brain slices were viewed with a microscope equipped with differential interference contrast optics and a 63× water immersion objective. The slices were placed into the recording chamber, and they were continuously perfused with aCSF bubbled with a mixture of 95% $O_2$ and 5% $CO_2$. Neuronal action potential firing was monitored using whole-cell patch-clamp. The cells were filled with biocytin during the experiments, the slices fixed overnight, permeabilised, washed, and incubated with streptavidin-conjugated Alexa488 dye to visualise the labelled neurones.

Intracellular calcium concentration changes were monitored using fluorescent calcium imaging. Cells were loaded with OGB-AM. Visualisation of the changes in the fluorescence signal intensity was achieved using a fluorescence imaging system. For processing the $Ca^{2+}$ imaging data, the recorded frames were exported into 16-bit TIF-movies that were viewed and

analysed using the ImageJ software (National Institute of Health). Data analysis started with manual selection of regions of interest (ROIs). The mean fluorescence intensity values of the ROIs were determined as a function of time. The resting fluorescence intensity of a given ROI was determined by averaging data points obtained during activity-free periods. To process large quantities of data a software capable of noise filtration, semi-automatic detection, and description of the kinetic features of the $Ca^{2+}$ transients was developed.

## Immunohistochemistry

The rats were euthanized and perfused through the left ventricle with 4% paraformaldehyde. The brains were postfixed and cryoprotected in 30% sucrose-buffer overnight. Serial sections (both sagittal and coronal) were cut at 40 µm. The sections containing the CN were consecutively harvested and immunohistochemically labelled using M1-specific and M3-specific primary antibodies. In the cases of the PLCs, sagittal sections were cut at 80 µm and a similar procedure was performed. The fluorescent images were acquired with confocal microscope.

## Statistics

For statistical analysis of the data, tests suitable for processing data sets showing non-normal distribution were used. When comparing averages obtained from independent populations, the Mann-Whitney test was applied. On comparing averages obtained from the same population, the Wilcoxon signed-rank test was used. Results are given as mean ± S.E.M.

# Results

## Investigation of the Purkinje-like cells of the dorsal cochlear nucleus

### *Morphological characteristics of the Purkinje-like cells*

PLCs are known to present particularly significant calbindin expression, thus calbindin-specific immunolabelling was performed to assess their morphology. The greatest diameter of the cell body in the sagittal plane was 28 ± 4 μm (n = 33). The length of the axis perpendicular to the sagittal plane was very similar to this value (25 ± 2 μm). The volume of the PLC cell bodies was 7,200 ± 650 fL.

On the basis of the shape and arrangement of the dendritic trees, PLCs fell into three categories. Three PLCs possessed dendritic trees that extended radially from the cell body, giving a roughly 'globular' appearance to the cell. These cells were found in the deep parts of the DCN. The remaining two classes of PLCs consisted of cells situated in the subependymal region of the DCN. Some of these neurones had dendritic branches running towards the centre of the nucleus in a more or less parallel fashion (columnar or centripetal arborisation); forming the second morphologically distinct subgroup of the PLCs. Cells belonging to the third subtype had characteristic fan-like appearance. In these cells the initial segments of the major dendrites proceeded in parallel with the surface of the nucleus, while the secondary branches travelled towards the centre of the DCN. The proportion of the above two subtypes of the PLCs was the same, accounting for 45%-45% of the total number of cells (n = 33).

The proximal dendrites of the PLCs were spineless and in the majority of cases their diameters were between 0.7–1.25 μm. All proximal processes gave rise to extensively branching dendritic trees tufted with spines. The average number of proximal dendrites was also determined. PLCs had 11 ± 1

appendages, but there was a considerable variability between the individual cells: the lowest and highest numbers were 4 and 19, respectively. All the aforementioned morphological parameters are suitable for assisting reliable identification of the PLCs during functional experiments.

### *Purkinje-like cells possess $I_h$*

PLCs had large whole-cell capacitance (89 ± 5 pF) and a resting membrane potential of -53 ± 1 mV (n = 12). Eleven out of twelve PLCs did not present spontaneous AP firing. When subjected to depolarizing currents, all the investigated PLCs showed non-adapting AP firing. In voltage-clamp, when subjected to hyperpolarizing stimuli, all PLCs produced a slowly activating inward current. The amplitude of the current was higher when stronger hyperpolarizations were applied. The density of the current was 13.2 ± 4.5 pA/pF (at -140 mV; n = 4) and it could be blocked by 10 µM ZD7288. The slow activation and the ZD7288 sensitivity of the hyperpolarization-activated inward current indicated that this component corresponded to the hyperpolarization-activated non-specific cation current ($I_h$).

### *Spontaneous postsynaptic currents recorded from the Purkinje-like cells*

In the next step of the functional experiments, we asked if PLCs are ‘wired’ to the rest of the nucleus. In these experiments the voltage-clamp configuration of the patch-clamp technique was employed, the membrane potential was set to -60 mV, and the spontaneous postsynaptic currents reaching the cell bodies of the PLCs were recorded. Under these conditions spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded with a frequency of 14 ± 2 Hz. The frequency was markedly reduced when voltage-gated $Na^+$ channels were blocked (to 0.4 ± 0.1 Hz; n = 4), suggesting sIPSCs are the consequences of presynaptic AP firing. Similar observation could be made in other experiments, when lesions severing the connections between the PLCs and the deeper layers of the nucleus were applied. The latter observation pointed at the significance of

deep inhibitory interneurones in evoking the inhibitory postsynaptic activity recorded from the PLCs. Using traditional blockers of the GABA- and glycinergic synapses (bicuculline and strychnine, respectively), it was established that the majority of the sIPSCs were mediated via glycinergic synapses (76%), whereas the remaining 24% was the result of the activation of GABAergic synapses (n = 4). The reduction of the frequency of the spontaneous IPSCs also revealed the presence of spontaneous excitatory postynaptic currents (sEPSCs), which were otherwise masked by the more prominent—and higher frequency—IPSCs. We assume that some of the sEPSCs reached the PLC cell bodies via the parallel fibre system, which is located in the most superficial region of the DCN.

### *Immunohistochemistry*

The functional data obtained from the PLCs indicated that these cells were parts of the synaptic circuits in the DCN. This finding was further substantiated using synaptophysin- and calbindin-specific double immunolabelling. Because synaptophysin is an established marker of presynaptic terminals, juxtapositions between the synaptophysin and calbindin positive structures suggest the presence of synaptic contacts between various nerve terminals and the PLCs. Synaptophysin positive boutons were observed in the close vicinity of both the somata and dendrites of the PLCs. The dendrites were also heavily covered with these boutons in many cases. Although electron microscopy is necessary for the unambiguous morphological identification of synapses, the confocal microscopic and functional data together strongly suggest that PLCs are synaptically coupled to other neurones of the DCN.

One of the possible neurotransmitters that PLCs might produce and utilise is GABA. To ascertain if PLCs are GABAergic neurones, calbindin- and glutamate decarboxylase- (GAD-) specific double immunolabelling was performed. GAD is an enzyme necessary for GABA biosynthesis, thus it is an

established marker of GABAergic neurons. All PLCs demonstrated strong GAD positivity.

Using conventional wax-embedded preparations, the presence of cerebellin has been reported in both cerebellar Purkinje cells and in PLCs of the CN. In our work we sought if confocal microscopy—in combination with cerebellin- and calbindin-specific labelling—reveals differences in the subcellular localization of cerebellin in these two cells. In PLCs, the cerebellin labelling was the most prominent in the somata, whereas the dendrites rarely showed immunopositivity. In contrast, the cerebellar Purkinje-cells always possessed labelling in both their cell bodies and dendrites.

## Cholinergic modulation of the granule cells of the dorsal cochlear nucleus

### *Characterisation of calcium transients recorded from a DCN slice-preparation*

Although the time courses of the calcium concentration changes recorded from DCN slices were heterogeneous, the transients fell in to two broad categories. Some cells produced rapid, repetitive calcium signals, whereas others showed rather slow calcium concentration changes. In other brain regions, rapid calcium transients are known to be AP-coupled events. To prove the suspected AP-coupled nature of the rapid transients in the DCN, the intracellular calcium concentration changes and electrical activity of individual cells were simultaneously monitored using calcium imaging in combination with 'loose patch' recording. All rapid calcium transients were preceded by current spikes, indicating the AP -coupled nature of the $Ca^{2+}$ transients. In a different type of experiments, the AP firing was prevented by blocking the voltage-gated $Na^{+}$ channels. In these instances the fast calcium transients immediately disappeared.

### ***Cholinergic stimulation increases the frequency of the rapid calcium transients in the granule cells of the DCN***

Carbamylcholine (CCh), an established cholinergic agonist, was employed for cholinergic stimulation. Before its application, the neurones were functionally disconnected by blocking the glutamatergic, glycinergic, and GABAergic neurotransmissions. Application of CCh increased the frequency of rapid calcium transients from $0.37 \pm 0.11$ $min^{-1}$ to $6.31 \pm 0.43$ $min^{-1}$ ($p < 0.001$). In order to identify the receptors responsible for mediating the CCh effect, various cholinergic antagonists were used. The general muscarinic receptor antagonist atropine diminished the CCh-induced increase of the $Ca^{2+}$ transient frequency. In contrast, the nicotinic receptor antagonist hexamethonium did not exert appreciable effects.

### ***Granule neurones possess both M1 and M3 muscarinic receptors***

Considering some data available in the literature and the results of our own preliminary experiments, it was reasonable to assume that the cholinergic effects observed in the present work were most likely mediated by M3 and/or M1 receptors. For this reason, M1- and M3 receptor-specific immunohistochemistry was employed in combination with confocal microscopy. Granule cells presented strong immunopositivity for both subunits. To reveal the functional significance of these receptor subtypes, $Ca^{2+}$ imaging experiments were performed in the presence of M1 and/or M3 receptor-specific antagonists. As opposed to the M3-specific 4-DAMP, which prevented the CCh-induced increase of the $Ca^{2+}$ transient frequency, the M1-specific pirenzepine had no appreciable effect. This result suggested that the observed effects of CCh were mediated via M3 receptors.

# Discussion

## Purkinje-like cells of the dorsal cochlear nucleus

For the first time in the literature, we recorded spontaneous postsynaptic currents from the PLCs, proving that these cells are integral parts of the neuronal network in the CN. We also showed that PLC-s express GAD, suggesting that they are GABAergic neurones.

Detailed morphological characterization of the PLCS has already been attempted in earlier studies, using classical histological methods. The novelty of our present work is that we employed confocal microscopy, thus a more accurate description of the morphology of the PLCs was provided. Because of the extensive dendritic arborisation of the PLCs, it is practically impossible to determine the accurate number of the proximal dendrites in classical histological sections. Confocal microscopy, however, makes this possible. According to our results, the average number of the proximal dendrites is 11. This feature makes the PLC-s significantly different from mature Purkinje cells, which possess only one proximal dendrite.

Cerebellin was first described as a Purkinje cell-specific protein. Later on, its presence was shown in cartwheel and in some other cells, showing preferential subependymal localisation and subsequently referred as 'ectopic Purkinje cells' (i.e., Purkinje-like cells). We also documented strong cerebellin positivity of cerebellar Purkinje cells, PLCs, and cartwheel cells. However, in cerebellar Purkinje cells both the cell body and dendrites showed cerebellin-specific immunolabelling, whereas in the PLC-s the labelling was almost exclusively confined to the soma.

Our results also showed that although some of their functional characteristics resemble those of the cerebellar Purkinje cells, PLCs are not spontaneously active. Nevertheless, both cell types respond with continuous AP firing to suprathreshold depolarization, they both possess $I_h$, and spontaneous inhibitory postsynaptic currents can be recorded from both cell types. The

inhibitory postsynaptic currents recorded from the PLCs had both glycinergic and GABAergic origins. Because our experimental design ensured that the sources of all IPSCs were within the DCN, we suggest that cartwheel cells may provide inhibitory inputs for the PLCs. Further, the results of synaptophysin-specific immunolabelling suggested that a great number of synaptic terminals make contacts with the cell bodies and dendrites of the PLCs. Considering that some authors regard PLCs 'misplaced Purkinje cells', it is important that we provide functional and morphological evidence, indicating that PLCs are active parts of the neuronal network of the DCN.

## Granule cells of the dorsal cochlear nucleus

Calcium transients with varying time courses were recorded from cochlear nucleus sections. On the basis of their kinetic parameters, the $Ca^{2+}$ transients were classified as either rapid or slow events. We have shown that the fast calcium transients have an AP -coupled nature—thus changes in the frequency of the fast calcium signals reflect neuronal activity changes.

Our results show that cholinergic stimulation directly increases the activity of granule cells, even if the synaptic transmission is blocked. This effect could be prevented by atropine, indicating the involvement of muscarinic receptors. This observation is in harmony with earlier studies, suggesting that muscarinic receptors mediate cholinergic stimulation-evoked activity changes in the DCN and pointing out the likely involvement of granule cells in the process. Our immunohistochemistry experiments demonstrated that granule cells express both M1 and M3 muscarinic receptors. We have shown that the M3 receptors have primary importance in mediating the cholinergic modulation targeting the granule cells. Because hexamethonium did not prevent the effect of CCh, the involvement of nicotinic receptors is unlikely.

Our results show that activation of the M3 receptors increases the frequency of the rapid calcium transients of the granule cells. An important question to be answered concerns the possible link between the muscarinic

activation and the increased $Ca^{2+}$-transient frequency. Muscarinic activation may release calcium from intracellular stores and/or may close M-type $K^{+}$-channels. In the latter case depolarization of the cell surface membrane results in depolarization, AP firing, and $Ca^{2+}$ entry through voltage-dependent calcium channels. Because we proved the AP-coupled nature of the rapid calcium transients as well as the essential role of $Ca^{2+}$ entry from the extracellular space, the second theory appears to be more likely.

According to some authors, hyperfunction of the cholinergic modulation may lead to tinnitus. In a noise load-induced animal model of tinnitus, increased acetylcholine-esterase enzyme activity was described. Moreover, an increased CCh- sensitivity of some DCN cell types was also shown. Most authors agree that tinnitus is accompanied by neuronal hyperactivity at multiple levels of the auditory pathway, including the cochlear nucleus. Increased spontaneous activity of pyramidal and cartwheel cells of the DCN has been described in an experimental model of tinnitus. Tinnitus may also be initiated or affected by somatosensory stimulation originating from the head or neck. Because somatosensory information reaches the neuronal network of the DCN via granule cells, cholinergic modulation of the granule neurones may have significance in the development of tinnitus. This theory is in harmony with the fact that tinnitus is accompanied with an increased spontaneous activity of pyramidal and cartwheel cells, especially as granule cells can alter the activity of either cell type. If one accepts that tinnitus is associated with a cholinergic modulation-mediated hyperactivity of some DCN neurons, the possible involvement of the granule cells is likely.

## Summary

Our knowledge about the function of either the cochlear nucleus or its individual cell types is still obscure. Particularly little information is available about the granule cells that represent the smallest, yet most numerous cell type of the cochlear nucleus. Even less is known about the Purkinje-like cells (PLCs). PLCs are mostly located in the dorsal cochlear nucleus and show highly characteristic morphology. It is still not clear, for example, whether PLCs are integrant parts of the neuronal network of the cochlear nucleus or they are mere ectopic Purkinje neurons, without function. For these reasons, the present work aimed at investigating the granule neurones and the PLCs of the rat dorsal cochlear nucleus. The experiments have been carried out by combined application of electrophysiological and imaging techniques.

Using double immunolabelling and confocal microscopy; we demonstrated that PLCs express glutamate-decarboxylase, an enzyme which is essential for GABA biosynthesis. This observation suggests that the PLCs are GABAergic inhibitory neurones. Using whole-cell patch-clamp in a brain slice preparation, we revealed the action potential firing pattern of the PLCs. We also showed that PLCs possess the hyperpolarisation-activated non-specific cationic current ($I_h$). Analysis of postsynaptic currents recorded from the somata of PLCs indicated that this cell type is an active member of the neuronal network situated in the dorsal cochlear nucleus. We also pointed out that although cerebellar Purkinje-neurones and PLCs are similar in terms of their strong cerebellin-1 immunopositivity, the intracellular distribution of cerebellin-1 is different in these cell types.

One of the neurones whose activity may significantly modify the function of all other cell types of the dorsal cochlear nucleus — including PLCs — is the cochlear granule cell. Granule neurones are particularly important because they receive somatosensory information, too. In the second part of the present work,

we have been studying the effects of cholinergic stimulation on the intracellular calcium concentration of the granule neurones. The experiments were carried out on calcium-sensitive fluorescent dye-loaded granule cells situated in a thin slice preparation. We showed that cholinergic stimulation significantly increases the activity of the granule cells and the frequency of their rapid calcium transients. Using specific antagonists, we demonstrated that this cholinergic influence is mediated by muscarinic receptors. We showed that although granule neurones express both M1 and M3 muscarinic receptors, the functional effects are primarily mediated by the activation of M3 receptors.

Our results help us to understand better how granule neurones and PLCs are integrated into the complex neuronal network of the cochlear nucleus. Consequently, as the result of our observations, one may have more comprehensive information depicting the function of this highly complicated structure without which central processing of the auditory information would be impossible.

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## List of publications related to the dissertation

1. **Kőszeghy, Á.**, Vincze, J., Rusznák, Z., Fu, Y., Paxinos, G., Csernoch, L., Szűcs, G.: Activation of muscarinic receptors increases the activity of the granule neurones of the rat dorsal cochlear nucleus: A calcium imaging study.
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2. **Kőszeghy, Á.**, Pál, B., Pap, P., Pocsai, K., Nagy, Z., Szűcs, G., Rusznák, Z.: Purkinje-like cells of the rat cochlear nucleus: A combined functional and morphological study.
   *Brain Res. 1297*, 57-69, 2009.
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   IF:2.463

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3. Kecskés, S., **Kőszeghy, Á.**, Szűcs, G., Rusznák, Z., Matesz, K., Birinyi, A.: Three-dimensional reconstruction and quantitative morphometric analysis of pyramidal and giant neurons of the rat dorsal cochlear nucleus.
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   DOI: http://dx.doi.org/10.1007/s00429-012-0457-7
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4. Lontay, B., Pál, B., Serfőző, Z., **Kőszeghy, Á.**, Szűcs, G., Rusznák, Z., Erdődi, F.: Protein phosphatase-1M and Rho-kinase affect exocytosis from cortical synaptosomes and influence neurotransmission at a glutamatergic giant synapse of the rat auditory system.
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*Hear. Res. 255* (1-2), 73-83, 2009.
DOI: http://dx.doi.org/10.1016/j.heares.2009.05.006
IF:2.177

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