

THESIS

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Computational simulation and investigation of gap junction coupling of Deiters' cells in the hearing organ



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1 List of abbreviations

ATP = adenosine-triphosphate

AUC = area under the curve

BM = basilar membrane

CBX = carbenoxolone

cx26 = connexins 26

cx30 = connexins 30

DAG = diacylglycerol

DCs = Deiters' cells

ER = endoplasmic reticulum

GJC = gap junction channel

IHCs = inner hair cells

IP₃ = inositol 1,4,5-trisphosphate

IP₃R = inositol 1,4,5-trisphosphate
receptor

IP₃-3K = inositol-triphosphate 3-kinase

IP-5P = inositol-polyphosphate 5-
phosphatase

J_i = fluxes

MET = mechanoelectrical transduction

ODEs = ordinary differential equations

OGB-1 = Ca²⁺ indicator Oregon Green
488 BAPTA-1 hexapotassium salt

OHCs = outer hair cells

P2X = P2X receptor, ATP-gated cation
channel

P2Y = P2Y receptor, purinergic G-
protein coupled receptor

PIP₂ = phosphatidylinositol 4,5-
bisphosphate

PLC = phospholipase C

PMCA = plasma membrane Ca²⁺-ATPase

SERCA = sarco/endoplasmic reticulum
Ca²⁺-ATPase

SNHL = sensorineural hearing loss

TM = tectorial membrane

WHO = World Health Organization

2 Introduction

2.1 Sensorineural hearing loss

Hearing loss has become a major clinical and public health problem, affecting over 466 million people worldwide in 2018 according to WHO estimates. It is considered to be the fourth most significant cause of disability in the world. The rising prevalence is partly explained by the growth and ageing of the global population [1]. Although, several risk factors are associated to the increasing number of people suffering from hearing impairment, such as overexposure to noise, genetics, ototoxicity, ear infections [1, 2, 3].

Sensorineural hearing loss (SNHL) is one type of hearing impairment [4]. Regardless of the etiology, this biological disorder mainly results from the death or dysfunction of certain types of cells within the cochlea, the sensory organ for hearing [4, 5]. The common site of pathology of SNHL is the outer hair cells (OHCs) [2]. Hair cells are located in the cochlea and are responsible for the transduction of mechanical sound stimuli into electrical signals [4]. The degeneration of the OHCs can even lead to the damage of other cochlear components, such as inner hair cells (IHCs), supporting cells, and auditory nerve cells [2].

SNHL is a common clinical finding but effective treatments to improve or cure the condition remain limited [2, 4]. Hearing aids and cochlear implants are the currently used therapeutic options. While they do not restore normal hearing, they do help improve the condition, but this depends on the severity of SNHL [5]. Pharmacotherapy may cure SNHL in acute cases, although the options are still very limited. For this reason, researchers have actively sought the development of novel therapeutics [4].

There are various areas of research focusing on the mechanisms of hearing loss and the development of therapeutic treatments. For this purpose, it is crucial to provide a better understanding of the biology and functions of the different components of the mammalian cochlea, including mechanosensory hair cells and supporting cells, and their role in neuronal signal transduction [3, 6].

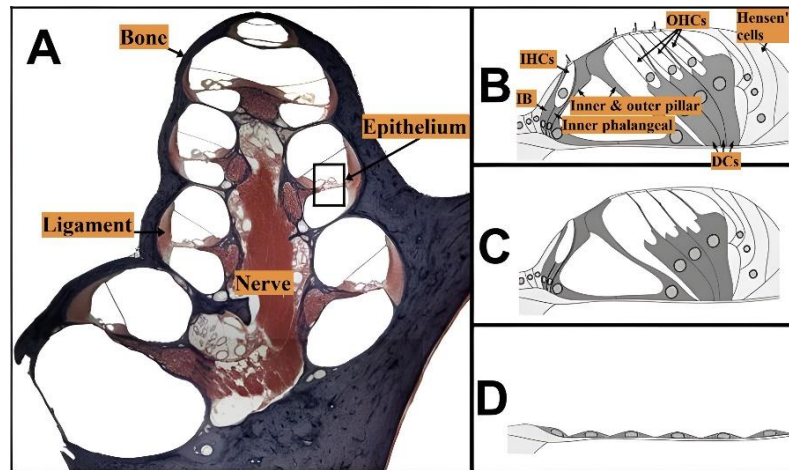


Figure 1 : Gross anatomy of the Mammalian cochlea. In this picture we can see a cross section of a guinea pig's cochlea. The main difference is that it has four cochlear turns whereas humans and mice have three. (A) Components of the cochlea : ligament, bone, sensory epithelium (box region), and nerve that sends electrical signals to the brain. (B) Schematic representation of the box region in A. It shows the sensory epithelium of the organ of Corti which consists of inner and outer hair cells (IHCs and OHCs), and their supporting cells (Deiters' cells (DCs), Hensen's cells and pillar cells). Hair cells are involved in transducing the mechanical motion of the basilar membrane into electrical signal that will travel to the brain through the nerve. IB : inner border cell. (C) Ototoxic damage (eg. exposure to noise or aminoglycoside antibiotics) causes hair cell loss and subsequent hearing loss. (D) In some cases, the ototoxic damage leads to a complete ablation of the organ of Corti. The remaining tissue cells leave a flat epithelial layer. Adapted from Parker MA (2011) "Biotechnology in the Treatment of Sensorineural Hearing Loss: Foundations and Future of Hair Cell Regeneration" [5].

2.2 Anatomy and function of the auditory apparatus

As mice have a cochlear morphology similar to humans and breed relatively quickly, they are considered an optimal animal model in hearing research [5]. The ear of mammals contains three main parts : the outer, middle and inner ears. The outer ear is made up of the pinna which acts as a funnel to direct the external sound signal towards the tympanic membrane. The middle ear contains the three ossicles (malleus, incus and stapes) forming a chain that allows air-borne sound waves to be transmitted from the tympanic membrane to the fluid-filled chambers of the inner ear. The inner ear consists of the bony labyrinth which is subdivided into the cochlea, the vestibule and the three semicircular canals. Those three cavities are filled with the perilymph which is rich in sodium but poor in potassium [7]. The membranous labyrinth, located within the bony labyrinth, is filled with the endolymph which is, on the contrary, rich in potassium and poor in sodium. The inner ear has two functional systems, one involving the cochlea, responsible for hearing, and the other

involving the vestibule and the three semicircular canals, responsible for the sense of balance [7].

The cochlea, in which the organ of Corti lies, has a characteristic spiral shape. In humans and mice, it can be divided into three cochlear turns : apical, middle and basal turns [8]. The cochlea has characteristic structural and mechanical arrangements along its turns that enable sound discrimination and ensure that the auditory nerve fibers fire in a frequency-coded manner. High-frequency sounds stimulate the base of the cochlea while low-frequency sounds stimulate the apex [9]. The basal membrane, which plays a central role in auditory signal transduction, is an important anatomical feature for such frequency selectivity. Its stiffness increases from apex to base, giving the cochlea a frequency-to-place (tonotopic) organization [10]. The characteristics of the sensory cells of the cochlea also vary tonotopically, with differences in size and properties along the turns [11].

When external sound waves enter the auditory canal, they cause the tympanic membrane to vibrate which in turn induces vibration of the ossicles. The mechanical signal is then transmitted to the cochlea through the oval window causing the perilymph to move. The motion of this fluid allows displacements between the tectorial membrane and basilar membrane and a consequent activation of hair cells by the bending of their stereocilia [12].

Hair cells are the primary auditory receptor cells in the organ of Corti. Their function is to transduce mechanical signals into electrochemical signals so that they can be transmitted to the brain through the auditory nerve [12]. When their stereocilia bend, due to movements between the tectorial membrane and basilar membrane, this induces the activation or deactivation of their surface mechanoelectrical transduction (MET) channels. When the channels open, potassium ions flow into the hair cells causing their depolarization. This is explained by the fact that the endolymph is rich in potassium (K^+) and calcium (Ca^{2+}) cations and has a positive potential [2]. The hair cells depolarization results in the opening of voltage-gated calcium channels that will then induce the release of glutamate [12], the neurotransmitter involved in synaptic transmission of the afferent auditory nerve terminals [2]. Another major function of the OHCs is to amplify sound-evoked vibrations primarily mediated by contraction mechanisms. When force is applied to the hair bundles, the MET channels open. The subsequent depolarizing receptor potential induces conformational changes in prestin motor protein leading to OHC contraction. This mechanism sharpens the frequency resolution and increase the sensitivity around the cell's characteristic frequency following the tonotopic arrangement of the cochlea [8]. Indeed, the properties of MET

currents in OHCs vary tonotopically, with increasing amplitude from apical to basal turn, and hence an increase in Ca^{2+} load. In addition, the tonotopic increase in MET current is accompanied by a decrease in cell size and hair bundle height. In IHCs, these tonotopic gradients are more limited [11]. Hair cells are arranged into three rows of OHCs and a single row of IHCs and are surrounded by their corresponding supporting cells (**fig. 1**) [12]. There are various types of supporting cells, such as Deiters', Hensen's and pillar cells [3, 13, 14].

The function and properties of the mechanosensory hair cells are widely investigated with the aim of studying their connection to impaired hearing function and potentially developing a therapeutical treatment [3, 15, 16]. However, fewer studies focus on investigating the physiological roles and cellular mechanisms of the inner ear's supporting cells, including Deiters' cells [3, 13, 15], although this topic is drawing increasing attention and is now believed to provide important information on the physiology of the cochlea [13, 16].

2.3 Deiters' cells as cochlear supporting cells

Deiters' cells provide a physical and metabolic support to the OHCs, modulating their electromotility and contributing to the amplification of the sound within the cochlea [15]. Besides, K^+ , released in the perilymph following mechanotransduction, requires recirculation in the inner ear. Deiters' cells help maintain ion homeostasis in the endolymph via active K^+ uptake, an essential prerequisite for a proper MET function [17]. Previous studies have also demonstrated that they exhibit functions similar to those of glial cells [3, 13].

The role and mechanism of action of Deiters' cells are not completely understood. Thus, the study of adenosine-triphosphate (ATP)-induced Ca^{2+} transients as well as Ca^{2+} homeostasis in Deiters' cells might provide insights into the regulation of their biological processes and intercellular communication [13, 15]. Using a targeted single-cell electroporation method in mouse hemicochlea preparations, one can obtain Ca^{2+} imaging of their two subcellular compartments (ie. soma and phalangeal process), thus allowing the investigation of their Ca^{2+} signalling pathways in the different cochlear turns (apical, middle and basal turns) [6, 15].

2.4 Calcium signalling

Calcium ions are involved in many signal transduction pathways in the cells and are the most narrowly regulated ions among the membrane-bound organisms. Ca^{2+} signalling is a key element of the cell's life and death [18]. In the mammalian cochlea, calcium signalling in non-sensory cells plays multiple roles in many biological processes such as supporting hair cell maturation, proper development of OHC afferent innervation and biochemical communication with the cochlear sensory epithelium modulating cellular activity [19, 20]. Ca^{2+} ions are implicated in all steps of the transduction process, including MET function and neurotransmitter release. It is also involved in the process of cochlear amplification and ion homeostasis in the endolymph [17].

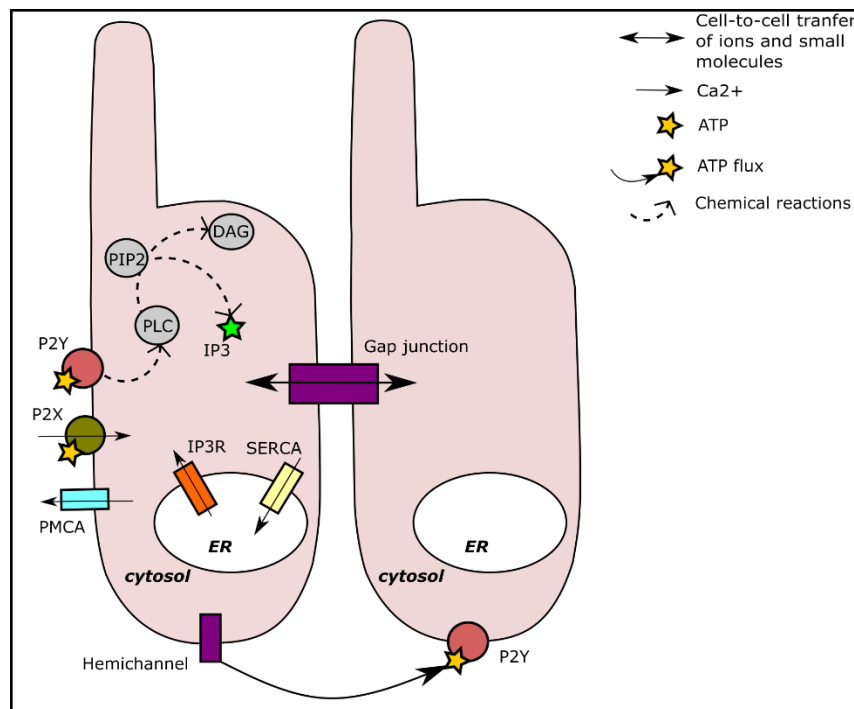


Figure 2 : Schematic representation of the main cellular processes involved in ATP-evoked Ca^{2+} signalling in Deiters' cells. ATP stimulates both P2X and P2Y receptors. Activation of P2X leads to an influx of Ca^{2+} across the plasma membrane. Activation of P2Y induces the cleavage of PIP_2 catalyzed by PLC, releasing IP_3 and DAG. Transport of Ca^{2+} from the cytosol into the endoplasmic reticulum (ER) by SERCA pumps. Outflux of Ca^{2+} across the plasma membrane through PMCA pumps. Ca^{2+} release from the ER through IP_3R activated by IP_3 . Transport of ions and small molecules through gap junctions between neighboring Deiters' cells, including second messengers such as IP_3 . Hemichannels release ATP that will activate P2Y receptors in the adjacent cell, propagating the Ca^{2+} signals intercellularly (paracrine signalling).

In non-sensory cells of the cochlea, oscillation of the cytosolic free Ca^{2+} concentration and intercellular Ca^{2+} waves are regulated by several processes (**fig. 2**) [19]. ATP has a crucial role to play in the organ of Corti. It is the main paracrine mediator involved in the propagation of calcium signals through supporting and epithelial cells. It acts through a highly sensitive purinergic/ IP_3 -mediated signalling pathway [17]. Previous studies have confirmed the involvement of two types of purinergic receptors in Deiters' cells : ionotropic P2X and metabotropic P2Y receptors. Both receptor types induce intracellular Ca^{2+} transients when stimulated by extracellular ATP [6, 13, 15]. In P2Y receptors, which are G-protein-coupled receptors located on the plasma membrane, this is mediated by the activation of phospholipase C (PLC). This results in the cleavage of phosphatidylinositol 4,5-bisphosphate (PIP_2) releasing inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG) [19]. IP_3 is a Ca^{2+} -mobilizing second messenger [21]. It induces the release of Ca^{2+} from internal stores [17, 20] after binding to intracellular IP_3 receptors (IP_3R) [19]. On the other hand, calcium signalling mediated by P2X receptors is extracellular Ca^{2+} -dependent [13]. As calcium ions play an important role in the regulation of cellular activity, its intracellular concentration is critical. There are several mechanisms that can lower cytoplasmic calcium concentration. The plasma membrane Ca^{2+} -ATPase (PMCA) can remove calcium from the cell [22], while the sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) allows transport of calcium from the cytosol to internal stores [13].

Non-sensory cells, including supporting cells, form a distinct intercellular network coupled by gap junctions. It allows the propagation of Ca^{2+} waves via IP_3 diffusion between adjacent cells [17]. Cochlear supporting cells are coupled via gap junction channels which are primarily made up of connexins 26 (cx26) and connexins 30 (cx30). It allows the intercellular transfer of Ca^{2+} -mobilizing second messengers IP_3 . Cx26 and cx30 also assemble to form unpaired connexons (hemichannels) which permits the extracellular release of ATP. Later on, ATP will act on the neighbour purinergic receptors to propagate the calcium signal to other supporting cells [23]. This ATP-induced ATP-release [21, 24] is a type of paracrine signalling pathway [17]. In order to investigate the gap junction coupling mechanism, carbenoxolone (CBX) can be used to block non-selectively the connexin channels [23, 25]. Though, it is also thought to block IP_3 -mediated Ca^{2+} release by inhibiting IP_3R activity [26].

2.5 Mathematical modelling

A computational model is a simplified representation of a complex system. It is a valuable tool in cell biology, especially when experimental study of the system of interest is time-consuming or even unfeasible. It can be used to describe intracellular dynamics such as biochemical reactions/transport, as well as the propagation of intercellular signals [27].

Calcium signalling can be simulated with deterministic or stochastic approaches [28]. Historically, biological systems, including Ca^{2+} signalling, were first described with deterministic models that use ordinary differential equations (ODEs) to represent biochemical reactions [27]. It has the advantage of being a simpler mathematical description than stochastic models [28]. It assumes that space is homogenous, molecules are evenly distributed in the system and fluctuations in the cellular environment are negligible [27]. This method has already provided good results, agreeing with the experimental findings [28]. However, at low concentrations or small volumes, fluctuations may not be neglected, so that stochastic approaches would be preferable [27, 28]. In this case, each biochemical reaction is considered as a probabilistic event [27].

A wide variety of mathematical models have been developed to describe calcium dynamics. ‘Minimal’ models, containing two variables, were originally used [28]. Li-Rinzel model is one well-known two-variable model used as reference for developing new models. The latter includes cytoplasmic Ca^{2+} and inactive IP_3R as variables [29]. Recently, the so-called ‘extended’ models have been mostly used. They involve three or more variables such as IP_3 concentration which seem to be coupled with Ca^{2+} oscillation in certain cell types [28].

In this thesis, we introduce and investigate a mathematical model for ATP-evoked Ca^{2+} transients in Deiters’ cells of the apical and middle turns. Our model is an extended model considering cytosolic Ca^{2+} concentration, inactive IP_3R and IP_3 dynamics as variables. It is based on Taheri et al. [30] and De Pittà et al. [31] models. As Deiters’ cells are connected via gap junctions and IP_3 can diffuse through them, we also explore the coupling strength for IP_3 employing Wu et al. [32] model. However, in the previously performed experiment that serves as a guidance for building a mathematical model, CBX was used to block gap junctions. Since CBX may also inhibit IP_3R activity [26], we investigate both hypotheses.

3 Aims

The aim of this thesis is to provide a better understanding of Deiters' cells' role and mechanism of action as supporting cells of the sensory receptor cells in the inner ear. Improving knowledge about the latter is believed to be of crucial importance in pharmacology and therapeutic area for the treatment of hearing disorders.

For this purpose, Ca^{2+} imaging experiment has been used to study ATP-evoked Ca^{2+} signalling and Ca^{2+} -induced gap junctional opening and closing mechanisms in Deiters' cells. Calcium ions being known to play a significant role in cell communication via regulatory effects on certain enzymes and proteins, and can also participate in signal transduction pathways through the activation of ion channels or as second messengers.

The cochlea consists of a hollow bony structure with a characteristic spiral shape. This complex morphology makes it challenging to study the sensory organ of hearing. Besides, it is difficult to work on cells from the basal turn due to the size of the cochlea of mice. Therefore, we developed a mathematical model that simulates the calcium spatiotemporal distribution patterns in Deiters' cells of the apical and middle turns and we compared it with the experimental results obtained previously, in order to get an accurate description of this complex system.

4 Materials and Methods

4.1 Experimental approach to the study of gap junctional coupling of Deiters' cells

All the experimental procedures and the animal care were in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The procedures were approved by the Animal Use Committee of Semmelweis University, Budapest. The experiment was conducted from 2016 to 2018 using BALB/c strain mice of both sexes aged 14 to 19 days. The majority of the physiological and structural bases of mice hearing are considered mature in mice older than 10-14 days [13]. The whole experiment protocol was based on previous studies carried out at Semmelweis University, Budapest [6, 13, 15]. The mice were first decapitated under isoflurane anesthesia and their cochleae were removed and placed in an ice-cold oxygenated solution that is similar to the perilymph (composition in mM : NaCl 22.5, KCl 3.5, CaCl₂ 1, MgCl₂ 1, Hepes 10, Na-gluconate 120, glucose 5.55 ; pH 7.4 ; 320 mOsm/L). The cochleae were placed into the cutting chamber of a vibratome (Vibratome Series 3000, Technical Products International Inc., St. Louis, Mo, USA) and halved along the midmodiolar plane while continuously bathed in the perilymph-like solution. Each hemicochlea obtained from the previous step was placed into an imaging chamber which was filled and perfused (speed : 3.5 mL/min) with the oxygenated experimental solution. The cells of interest were selected under a LUMPlanFl 40x/0.80w water immersion objective (Olympus, Japan) with oblique illumination, and were subsequently loaded by single-cell electroporation. For this procedure, the borosilicate pipettes (5-7 M Ω ; Harvard Apparatus, Holliston, MA, United States) were filled with lightexcitable Ca²⁺ indicator Oregon Green 488 BAPTA-1 hexapotassium salt (OGB-1) (ThermoFisher Scientific) dissolved in distilled water reaching a final concentration of 1 mM. The pipettes were mounted onto an electrode holder which was attached to a micromanipulator (Burleigh PCS-5000, Thorlabs, Munich, Germany). As the pipettes approached the cells of interest, a single square wave current impulse (10 ms, 10 μ A) was applied allowing the cells to be loaded with OGB-1. The current was generated by a pCLAMP10 software-guided stimulator system (Biostim STE-7c, Supertech Ltd., Pecs, Hungary ; MultiClamp 700B Amplifier and Digidata 1322A, Molecular Devices, Budapest, Hungary). The following calcium imaging procedure was performed at room temperature. In this method, an excitation light of 494 $\pm$ 5 nm (Polychrome II monochromator, TILL Photonics, Germany) was used to illuminate the cells and the light emitted was recorded after passing through a band-pass filter (523 $\pm$ 25 nm). An Olympus BX50WI fluorescence

microscope (Olympus, Japan) equipped with a Photometrics Quantix cooled CCD camera (Photometrics, USA) captured the fluorescent images. The system was controlled with the Imaging Workbench 6.0 software (INDEC BioSystems, USA). The image were taken at a frame rate of 1 Hz during the drug-evoked responses and, the rest of the time, the frame rate was 0.1 Hz to avoid phototoxicity and photobleaching. In the perfusion chamber, the volume of the buffer was about 1.9 mL. The cells were stimulated by injecting ATP (Sigma-Aldrich, St. Louis, MO., USA) with a concentration of 100 μ M in the perfusion chamber for 30 s. In order to properly analyse the cellular responses, the method comprised several steps and the fluorescence was recording during the whole process. Before stimulating the cells with ATP, a time period of minimum 3 minutes was registered. For the investigation of the gap junctional coupling mechanism, we treated the cells with CBX, which is a non-selective connexin blocker [23]. After the 1st ATP stimulus (for 30 s), CBX (Sigma-Aldrich, St. Louis, MO., USA) with a concentration of 100 μ M was added to the perfusion. After 15 minutes of continuous CBX administration, a 2nd ATP dose (for 30 s) was added to the perfusion solution to stimulate the cells a second time. Then, CBX was removed from the perfusion and normal solution was administered to the cells for another 15 minutes before exciting the cells with a 3rd ATP dose (for 30 s). The cochlea of mice has complex morphology and size in such a way that it was difficult to work on cells from the basal turn. Therefore, only Deiters' cells of the apical and middle turns of the cochlea were loaded in this study.

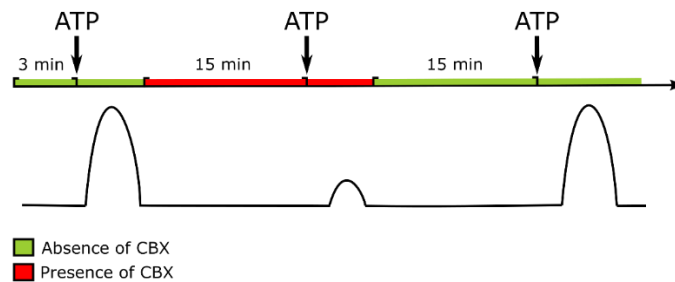


Figure 3 : Schematic representation of the experimental protocol. The 1st ATP stimulus is administered after 3 minutes of recording period. We observe a strong calcium response following this stimulus. Then, CBX is added to the perfusion solution and continuously perfused for 15 minutes before injection of the 2nd ATP dose. In presence of CBX, the gap junctions are blocked, and thus the calcium transient elicited is weaker. In a final step, CBX is washed out of the perfusion solution and the cells are perfused with normal solution for another 15 minutes before the last ATP stimulus is administered.

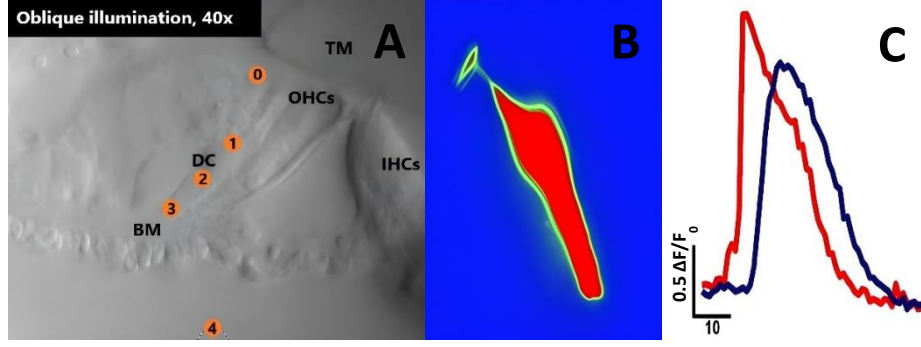


Figure 4 : Calcium imaging of the hemicochlea preparation. (A) The image shows the organ of Corti in the apical turn of the cochlea by oblique illumination. TM : Tectorial membrane, BM : basilar membrane, IHC : inner hair cell, OHC : outer hair cell, DC : Deiters' cell. The orange dots represent the five regions of interest. 0 : phalangeal process of DC ; 1 : apical part of the soma ; 2 : middle part of the soma ; 3 : basal part of the soma ; 4 : tympanic cavity. The calcium measurements were made at the process and in the three different areas of the soma of DC. We decided to use the middle part of the soma for the analysis of calcium signalling data. (B) A typical fluorescent image showing the elevation of cytosolic $[Ca^{2+}]$ in a Deiters' cell after ATP stimulation. We see that cytosolic $[Ca^{2+}]$ increases in both the phalangeal process and the soma of the cell. (C) ATP-evoked Ca^{2+} response in the phalangeal process (red) precedes the one in the soma (blue) (mean $\pm$ SEM : 5.28 ± 0.7 ; $p = 1.59 \cdot 10^{-9}$).

4.2 Mathematical model

Previous studies have highlighted possible similarities between auditory supporting cells, such as Deiters' cells, and glial cells in their roles and functions [3]. Therefore, we decided to base our model on several publications in which calcium dynamics in astrocytes were simulated [30, 31]. There are two main types of calcium activity in astrocytes : spontaneous and neurotransmitter-evoked Ca^{2+} excitability. The Taheri et al. [30] and De Pittà et al. [31] models account for Ca^{2+} dynamics evoked by neurotransmitters. In our experiment, calcium transients were elicited by ATP stimuli, making neurotransmitter-evoked Ca^{2+} dynamics models more suitable for simulating our experimental findings. A gap junctional flux of IP_3 was added to our model employing the Wu et al. [32] model.

Our model is an open-cell model in which the intracellular Ca^{2+} concentration can change due to exchange across the plasma membrane. We assumed that the spatial arrangement of calcium concentration is uniform in the cytosol and ER. Our model consists of a system of three differential equations. One equation models the changes in cytosolic Ca^{2+} concentration (1), the second equation accounts for the deactivation rate of the IP_3R (2) and the third equation represents the IP_3 dynamics within the cell (3), as follows :

$$(1) \frac{dc}{dt} = J_{P2X} - J_{PMCA} + J_{IP3R} - J_{SERCA} + J_{leak}$$

$$(2) \frac{dh}{dt} = \frac{h_{\infty} - h}{\tau}$$

$$(3) \frac{d[IP_3]}{dt} = v_{ATP} + v_{\delta} - v_{3K} - v_{5P} + 4 \times J_{GJC}$$

In these equations, the J_i represent the fluxes through the pumps and channels embedded in the plasma membrane and ER membrane of Deiters' cells (**fig. 2**). The expressions for the fluxes are given below.

P2X channels :

J_{P2X} represents the influx of Ca^{2+} to the cytosol through P2X channel when activated by ATP.

$$J_{P2X} = r_{P2X} (1 - c) [ATP]$$

PMCA and SERCA pumps :

J_{PMCA} is the outflux of Ca^{2+} across the plasma membrane through PMCA pump. J_{SERCA} is the flux of Ca^{2+} from the cytosol into the ER through SERCA pump. We assumed that PMCA and SERCA pumps have similar dynamics as for the astrocytes and can therefore be modelled with Hill function.

$$J_{PMCA} = v_{PCMA} \frac{c^2}{(c^2 + K_{PMCA}^2)}$$

$$J_{SERCA} = v_{SERCA} \frac{c^2}{(c^2 + K_{SERCA}^2)}$$

IP₃ receptors :

We used the Taheri et al. [30] model to reflect the J_{IP3R} (ie. the flux of Ca^{2+} from the ER to the cytosol through IP₃R channel). This model simplifies the opening probability of IP₃R by assuming that the gating kinetics is mainly driven by three binding sites on the receptor : an IP₃ binding site (m), a Ca^{2+} binding site for activation (n), and a Ca^{2+} binding site for deactivation (h). The dynamics of h are governed by the equation given previously.

$$J_{IP3R} = (r_{release} c_1 m_{\infty}^3 n_{\infty}^3 h^3)(c_{ER} - c)$$

where

$$c_{ER} = \frac{c_0 - c}{c_1}, m_{\infty} = \frac{[IP_3]}{[IP_3] + d_1}, n_{\infty} = \frac{c}{c + d_5}, h_{\infty} = \frac{Q_2}{Q_2 + c}, \tau = \frac{1}{a_2 (Q_2 + c)}, \text{ and } Q_2 = d_2 \frac{[IP_3] + d_1}{[IP_3] + d_3}$$

Additional Ca^{2+} fluxes :

J_{leak} is a nonspecific calcium leakage from the ER, it denotes additional Ca^{2+} fluxes through various pumps and channels not explicitly modelled.

$$J_{leak} = (r_{leak} c_1)(c_{ER} - c)$$

IP_3 dynamics :

IP_3 is produced from the hydrolysis of PIP_2 catalyzed by two PLC isoenzymes, PLC_{δ} and PLC_{β} . Binding of ATP to G-protein coupled P2Y receptors induces the activation of PLC_{β} releasing IP_3 (v_{ATP}). On the contrary, PLC_{δ} is mainly activated by intracellular Ca^{2+} elevation (v_{δ}). IP_3 concentration depends on its production and its degradation via two major pathways. The first degradation pathway is through dephosphorylation of IP_3 by the inositol polyphosphate 5-phosphatase (IP_5P) (v_{5P}). The second one is through Ca^{2+} -dependent phosphorylation of IP_3 by the IP_3 3-kinase (IP_3 -3K) (v_{3K}). Both reactions can be considered as of Michaelis–Menten type. However, IP_5P is not likely to be saturated by IP_3 so that the rate of IP_3 degradation can be linearly approximated.

$$v_{\delta} = \frac{\overline{v_{\delta}}}{1 + \frac{[IP_3]}{K_{\delta}}} \frac{c^2}{c^2 + K_{PLC\delta}}$$

$$v_{ATP} = \overline{v_{\beta}} \frac{[ATP]^{0.7}}{[ATP]^{0.7} + K_{\gamma}^{0.7}}$$

$$v_{3K} = \overline{v_{3K}} \frac{c^4}{c^4 + K_D^4} \frac{[IP_3]}{[IP_3] + K_3}$$

$$v_{5P} = \overline{r_{5P}} [IP_3]$$

Besides, we incorporated a J_{gjc} to our model, taking into consideration that IP_3 can diffuse through gap junctions. To this end, we used Wu et al. [32] model, in which a system of i cells is considered, generating a model with multiple equations to describe IP_3 and Ca^{2+} concentrations in each cell. To simplify this model, we assumed IP_3 concentration of the neighboring cells constant over time, at a concentration of 5 μM , and IP_3 diffusing only from the neighboring cells to the target Deiters' cell. Thus, the diffusion rate of IP_3 only depends

on its production and degradation over time in the experimental target cell. This simpler approach made it easier for us to investigate the gap junctional coupling mechanisms. In the equation (4), we multiplied J_{GJC} by 4 because Deiters' cells have usually four neighboring cells.

$$J_{GJC} = \gamma (5 - [IP_3])$$

ATP inputs :

We opted to treat ATP as inputs to our model (4). In our experiment, Deiters' cells were stimulated by injecting ATP for 30 seconds in the perfusion chamber. From the time of the stimulus until the end of the calcium wave, ATP increases while starting to activate both P2X and P2Y receptors, and it depletes after a while. In order to express this ATP wave, we assumed that ATP exponentially saturates to a certain level (s_∞), and then exponentially decays before becoming depleted.

$$(4) \quad ATP(t) \begin{cases} 0 & t < t^* \\ s_\infty (1 - e^{-r_{rise}(t-t^*)}) & t^* \leq t < t^* + d_{rise} \\ A e^{-r_{decay}(t-[t^*+d_{rise}])} & t^* + d_{rise} \leq t \end{cases}$$

where

$$s_\infty = \frac{A}{1 - e^{-r_{rise} d_{rise}}}, \text{ and } r_{decay} = -\frac{1}{d_{decay}} \log\left(\frac{0.005}{A}\right)$$

t^* represents the starting time of ATP stimulus, A is the maximum amplitude, r_{rise} and r_{decay} represent the rates of rise and decay respectively, and d_{rise} and d_{decay} are the durations of the rising and decaying phases of ATP concentration. We estimated the ATP concentration in the perfusion chamber based on the velocity of the solution in which ATP was injected (0.058 mL.s^{-1}), the volume of the chamber (2 mL), and the concentration of the injected ATP dose (100 μM). The estimated ATP values in the perfusion chamber were compared to the ATP model (4) and an estimation of the model parameter values was obtained using a non-linear least squares approach (function `nls` in R) (**fig. 5**).

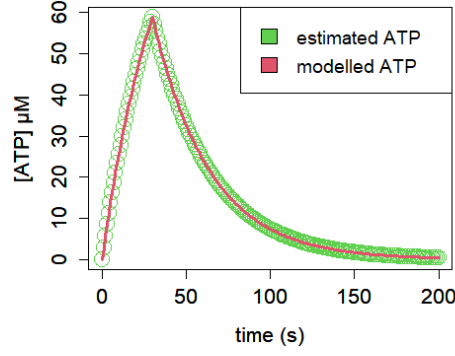


Figure 5 : ATP model fitted the ATP estimated concentration. Initial set of ATP model parameters : $A = 58.85 \mu M$; $d_{rise} = 30 s$. r was fitted first, and d_{decay} second, both using a non-linear least squares approach. The following results were obtained ($\pm SD$) : $r_{rise} = 0.0296 \pm 3.67e-12 s^{-1}$ ($***p < 0.001$), $d_{decay} = 316.7 \pm 3.238e-09 s$ ($***p < 0.001$).

We implemented our model in R 4.1.0 using forcing functions of the deSolve package with the aim of adjusting the parameter values to fit the model to our experimental data.

4.3 Data analysis

The experimental data were analysed subsequently. Fluorescence intensity responses were calculated as follow :

$$\frac{dF}{F_0} = \frac{F_t - F_0}{F_0}$$

where F_0 is the fluorescence intensity of the baseline, and F_t is the fluorescence intensity at time t . The duration, the amplitude and the AUC (area under the curve) of the Ca^{2+} transients were calculated in order to determine differences resulting from the treatments (presence or absence of CBX). The response durations were calculated as the width at half maximum. The amplitudes, defined as the maximal changes in intensity, were calculated as the height between 100% and 5% of the fluorescence intensity values. Data are presented as mean $\pm$ SEM (standard error of the mean). The number of experiments (n) indicates the number of cells. The results of the duration, amplitude and AUC were illustrated as boxplots (**fig. 7**).

Statistical analysis was performed in R 4.1.0. ANOVA and Tukey HSD tests were used to determine the statistical significance of the experimental results and analyse the differences among the treatments. Besides, T-tests and Wilcoxon signed-rank tests were used to compare experimental data of both turns, while T-tests were used to evaluate the fitness of our model and estimate the parameter values fitting with the experimental results. Differences were considered significant at $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$.

5 Results

5.1 Experimental comparison of ATP-evoked Ca^{2+} responses in Deiters' cells in presence or absence of carbenoxolone

As described previously, a single-cell electroporation technique was used to load the targeted Deiters' cells with OGB-1. Then, the cells were stimulated with ATP (100 μM for 30 seconds in the perfusion) and fluorescent images were recorded to visualize the intracellular Ca^{2+} transient responses [6]. For the investigation of the gap junctional coupling mechanism, the cells were treated with CBX at least 15 minutes, which is a non-selective connexin blocker [23]. ATP was administered three times in order to study the different calcium transient responses according to the treatments. The 1st ATP dose was administered in the absence of CBX, the 2nd in the presence of CBX, and the 3rd was injected after CBX has been washed out.

The results were illustrated as a graph of fluorescence intensity response (dF/F_0) versus time (in seconds) (**fig. 6**). The times were normalized so that all maximum intensity responses (100%) were at a time equal to 40s. The fluorescence intensity values at different percentages of the obtained calcium responses (5%, 10%, 50%, 90%, and 100%) were used to investigate the results.

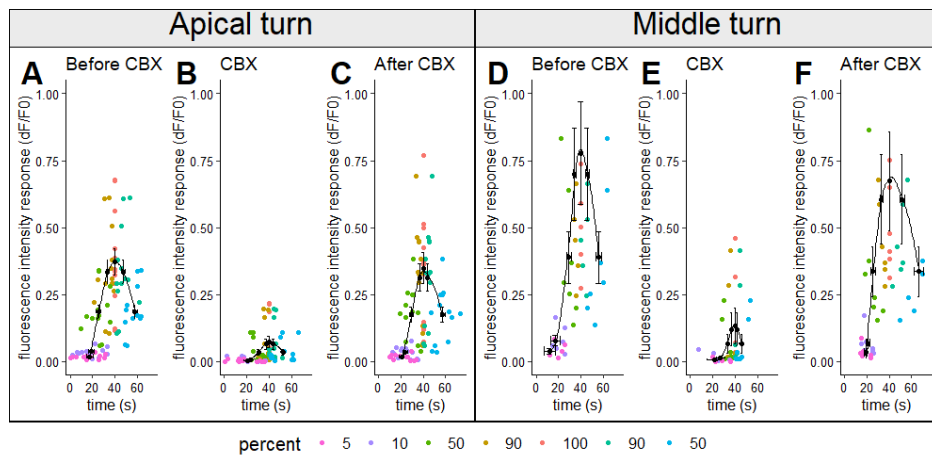


Figure 6 : ATP-evoked Ca^{2+} intracellular signalling in the soma of Deiters' cells of the apical and middle turns. The curves represent the fluorescence intensity response (dF/F_0) as a function of time (s). Each color stands for a certain percentage. The black curves show the extrapolated response calculated based on the mean of the intensity values at different percentages (black dots). The bar errors describe the mean $\pm$ SEM (apical turn : $n = 13$; middle turn : $n = 7$). (A, D) Before treatment with CBX. (B, E) In presence of CBX which blocks the connexins in gap junctions and hemichannels. (C, F) After CBX has been washed out.

In the apical turn, the cells treated with CBX showed weaker responses after being stimulated by ATP. The duration of the Ca^{2+} signals elicited by ATP stimuli did not differ in presence or absence of CBX. However, the amplitudes and the AUC were significantly weaker in presence of CBX (**fig. 7A, 7B, 7C**). In the middle turn, although the ATP-evoked calcium responses did not significantly differ in presence or absence of CBX based on TukeyHSD test, likely due to the smaller sample size and a greater variability of responses within the sample, the trend is clearly visible (**fig. 7D, 7E, 7F**). The fluorescence intensity of the calcium responses appears to be much greater in absence of CBX. A longer duration of response in absence of CBX is also suspected (**Table 1**).

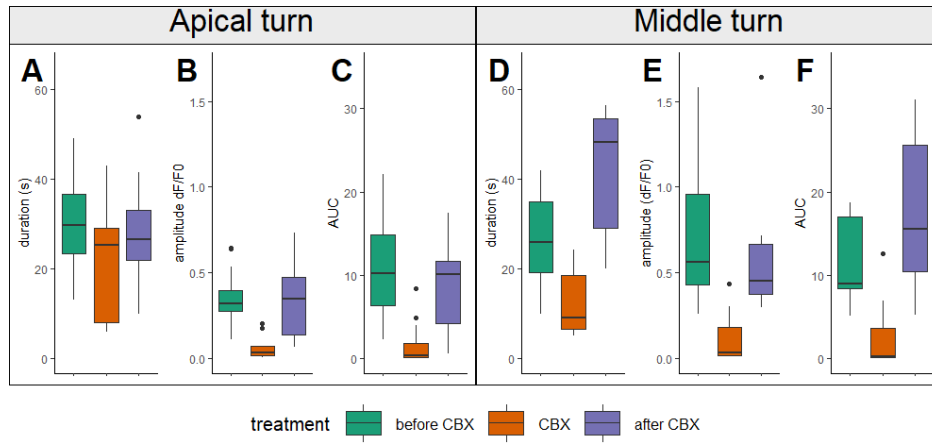


Figure 7 : Investigation of Ca^{2+} transient responses in the soma of Deiters' cells of the apical and middle turns that were stimulated with exogenous ATP, in presence or absence of CBX treatment. The boxplot shows the median, the 1st and 3rd quartiles and the outliers (dots). (A) The duration of the responses did not differ between treatments ($n = 13$; $p > 0.05$). (B) The amplitude in cells treated with CBX was significantly lower ($n = 13$; $***p < 0.001$). (C) The AUC in cells treated with CBX was significantly lower ($n = 13$; $*p < 0.05$). (D) The duration of the response was significantly longer once CBX has been washed out ($n = 7$; $***p < 0.001$). (E) The amplitude of the responses did not differ between treatments ($n = 7$; $p > 0.05$). (F) The AUC was not different between treatments ($n = 7$; $p > 0.05$).

Table 1 : Investigation of ATP-induced Ca^{2+} transient responses in the soma of Deiters' cells of the apical and middle turns of the cochlea. The table shows mean $\pm$ SEM (apical turn : $n = 13$; middle turn : $n = 7$) of duration, amplitude and extrapolated AUC of the fluorescence intensity responses according to the treatment.

Treatment	Apical turn			Middle turn		
	Duration (s)	Amplitude (dF/F ₀)	AUC	Duration (s)	Amplitude (dF/F ₀)	AUC
Before CBX	32.62 $\pm$ 4.08	0.35 $\pm$ 0.05	10.53 $\pm$ 1.73	26.57 $\pm$ 4.27	0.74 $\pm$ 0.18	22.36 $\pm$ 7.56
CBX	21.69 $\pm$ 3.56	0.07 $\pm$ 0.02	1.76 $\pm$ 0.7	12.62 $\pm$ 3.13	0.13 $\pm$ 0.06	2.94 $\pm$ 1.87
After CBX	28.13 $\pm$ 3.38	0.33 $\pm$ 0.05	8.74 $\pm$ 1.59	41.38 $\pm$ 5.63	0.64 $\pm$ 0.18	26.7 $\pm$ 9.85

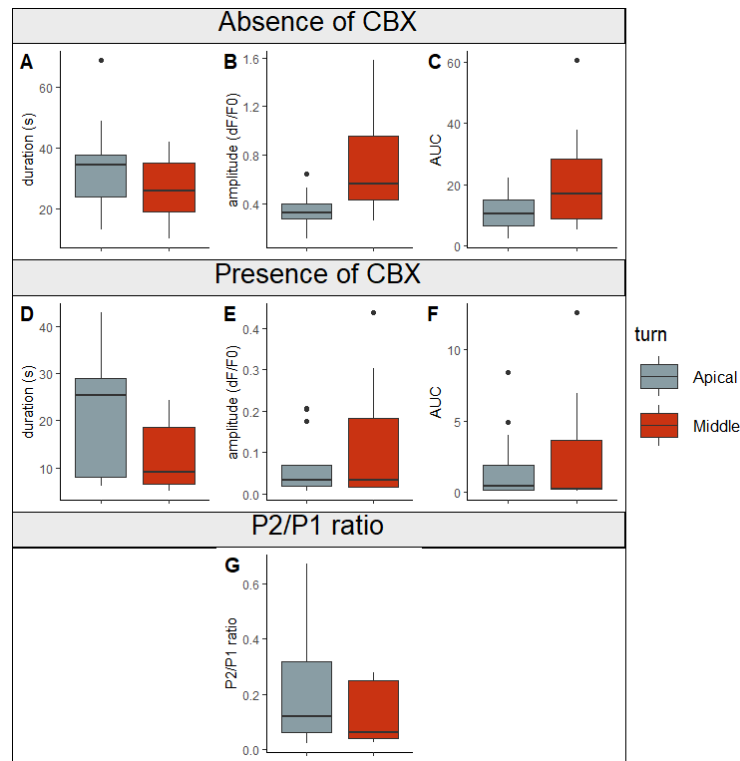


Figure 8 : ATP-evoked calcium signals in presence or absence of CBX, differences between apical and middle turn. The boxplots show median, 1st and 3rd quartiles and outliers (dots). In absence of CBX : (A) duration, (B) amplitude, and (C) AUC of calcium responses were not different between apical and middle turns (A : $n = 13$, $m = 7$, $p > 0.05$; B : $n = 13$, $m = 7$, $p > 0.05$; C : $n = 13$, $m = 7$, $p > 0.05$). In presence of CBX : (D) duration, (E) amplitude, and (F) AUC of calcium transients did not differ between both turns (D : $n = 13$, $m = 7$, $p > 0.05$; E : $n = 13$, $m = 7$, $p > 0.05$; F : $n = 13$, $m = 7$, $p > 0.05$). (G) Ratios of amplitude of fluorescence intensity responses in presence of CBX to amplitude in absence of CBX (P2/P1 ratio) were not different between apical turn and middle turn (G : $n = 13$, $m = 7$, $p > 0.05$).

T-tests and Wilcoxon signed-rank tests did not confirm any significant difference in calcium responses between apical and middle turn. However, we can notice that the duration of calcium transients seems generally longer in the apical turn both in presence or absence of

CBX, the amplitude seems higher in the middle turn in absence of CBX with a smaller P2/P1 ratio (**fig. 8**).

5.2 Modelling approach for investigating Ca^{2+} transients and the blocking effect of carbenoxolone

As mentioned earlier, our model was implemented in R 4.1.0 and forcing functions of the deSolve package were used to adjust the values for each model parameter to fit experimental data. A total of 2,329,148 parameter sets were tested. The fitness of the model was estimated based on t-tests performed to compare $[\text{Ca}^{2+}]$ and time values between model and experimental data at different percentages of the calcium transient peak (14 t-tests performed for each model parameter set and cochlear turn).

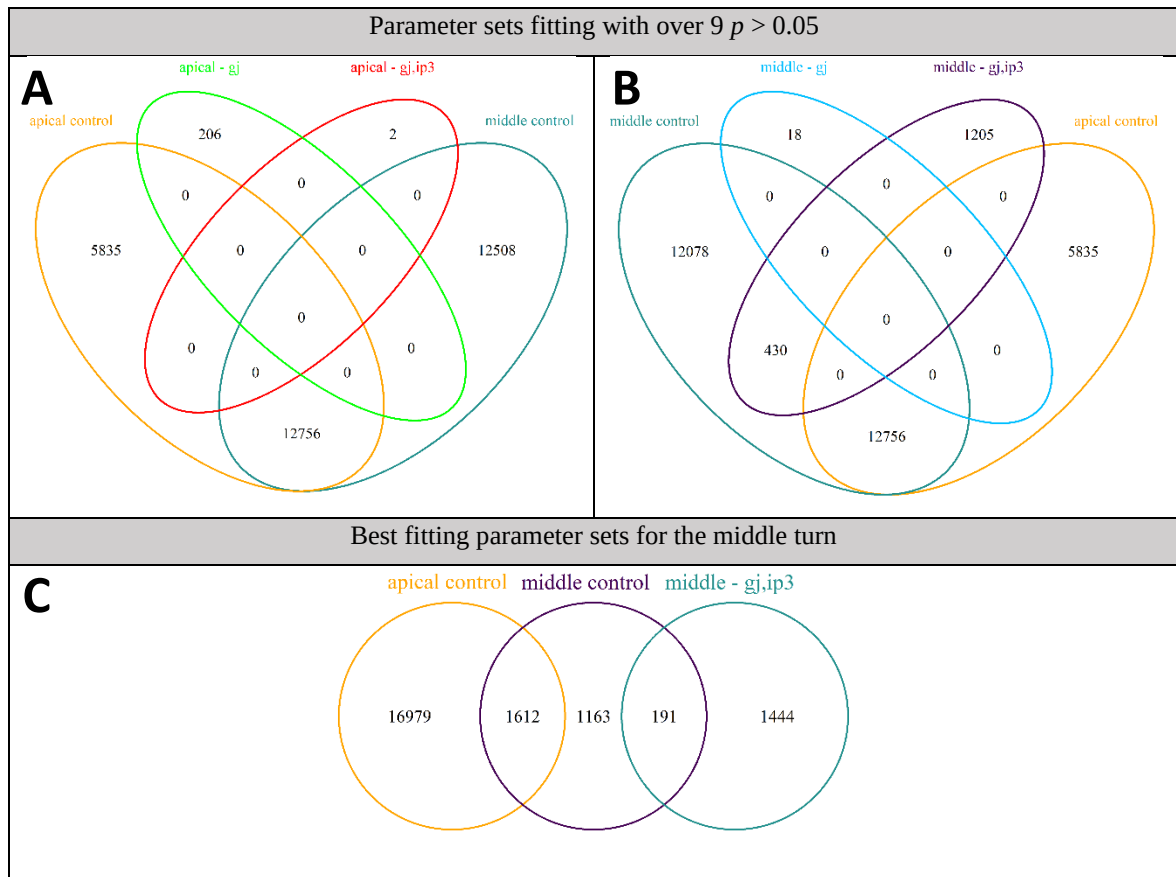


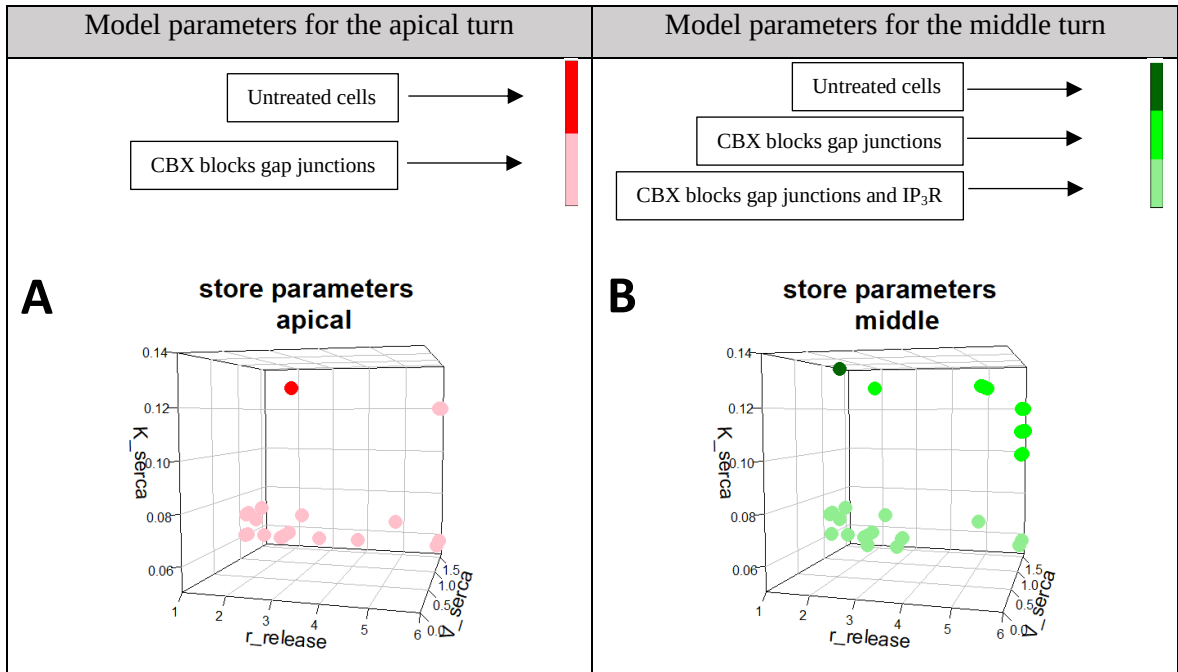
Figure 9 : Venn diagrams showing the fitting parameter sets that overlap different treatments (presence or absence of CBX) in different cochlear turns. ‘control’ : absence of CBX. ‘gj’ : presence of CBX, CBX blocking gap junctions only. ‘gj.ip3’ : presence of CBX, CBX blocking both gap junctions and IP_3R .

From the model parameter sets that were tested for this study, 18,591 parameter sets fitted well (with more than 9 $p > 0.05$) experimental data from untreated cells of the apical turn. However, none of them overlapped with experimental data from CBX-treated cells (**fig. 9A**). For the middle turn, 2,966 parameter sets fitted (with 14 $p > 0.05$) data from untreated cells. 191 of these parameter sets fitted (with more than 9 $p > 0.05$) data from treated cells of the middle turn and 1,612 fitted (with more than 9 $p > 0.05$) data from untreated cells of the apical turn (**fig. 9C**). The sets of model parameters that best fitted experimental data are summarized in **Table 2** below.

Table 2 : Model parameters. for the apical turn, the parameter sets fitted data from untreated cells with more than 9 $p > 0.05$, while it fitted with 14 $p > 0.05$ for the middle turn.

Parameter	Description	Parameter value/Unit		Initial condition	
		Fit data from apical turn	Fit data from middle turn	Fit data from apical turn	Fit data from middle turn
r_{P2X}	Rate of P2X channel flux	[0.015, 0.046] $\mu\text{M.s}^{-1}$	[0.025, 0.031] $\mu\text{M.s}^{-1}$	$c = [0.05, 0.09]$ μM	$c = [0.05, 0.06]$ μM
V_{PMCA}	Max PMCA flux	[8.07, 12.9] $\mu\text{M.s}^{-1}$	[8.11, 12.3] $\mu\text{M.s}^{-1}$	$h = [0.5, 0.9]$	$h = [0.61, 0.9]$
V_{SERCA}	Max SERCA flux	[1.1, 1.664] $\mu\text{M.s}^{-1}$	[0.11, 0.31] $\mu\text{M.s}^{-1}$	$[\text{IP}_3] = [0.1, 0.5]$ μM	$[\text{IP}_3] = [0.1, 60.4]$ μM
K_{PCMA}	Half-saturation for PMCA	2.49 μM	2.49 μM		
K_{SERCA}	Half-saturation for SERCA	[0.057, 0.13] μM	0.06 μM		
r_{release}	Maximal IP_3R channel permeability	1.6 s^{-1}	2 s^{-1}		
r_{leak}	Maximal rate of Ca^{2+} leak from the ER	0.11 s^{-1}	0.11 s^{-1}		
c_0	Total cell free $[\text{Ca}^{2+}]$ referred to the cytosol volume	1 μM	1 μM		
c_1	Ratio cytosol volume/ER volume	0.185 $\mu\text{M.s}^{-1}$	0.185 $\mu\text{M.s}^{-1}$		
d_1	IP_3 dissociation constant	0.13 μM	0.13 μM		
d_2	Ca^{2+} inactivation dissociation constant	1.049 μM	1.049 μM		
d_3	IP_3 dissociation constant	0.9434 μM	0.9434 μM		
d_5	Ca^{2+} activation dissociation constant	0.08234 μM	0.08234 μM		
a_2	IP_3R binding rate for Ca^{2+} inhibition	0.2 $\mu\text{M}^{-1}.\text{s}^{-1}$	0.2 $\mu\text{M}^{-1}.\text{s}^{-1}$		
r_{rise}	Rate of exponential growth	0.0296 s^{-1}	0.0296 s^{-1}		
d_{rise}	Duration of ATP rising phase	30 s	30 s		
d_{decay}	Duration of ATP decaying phase	316.7 s	316.7 s		

A	Max amplitude of ATP transient	58.85 μM	58.85 μM
γ	Gap junctional coupling strength	0.1	0.09
$\bar{v}_\delta$	Maximal rate of IP_3 production by PLC_δ	0.03 $\mu\text{M.s}^{-1}$	0.031 $\mu\text{M.s}^{-1}$
K_δ	Inhibition constant of PLC_δ activity	[1.5, 2.39] μM	1.5 μM
$K_{\text{PLC}\delta}$	Ca^{2+} affinity of PLC_δ	[0.085, 1.1939] μM	0.136 μM
$\bar{v}_\beta$	Maximal rate of IP_3 production by PLC_β	[0.0235, 0.4] $\mu\text{M.s}^{-1}$	0.1 $\mu\text{M.s}^{-1}$
K_γ	ATP affinity of PLC_β		
$\bar{v}_{3K}$	Maximal rate of degradation by $\text{IP}_3\text{-3K}$	[0.61, 3.8] $\mu\text{M.s}^{-1}$	1.3 $\mu\text{M.s}^{-1}$
K_D	Ca^{2+} affinity of $\text{IP}_3\text{-3K}$	[0.016, 0.86] μM	0.1 μM
K_3	IP_3 affinity of $\text{IP}_3\text{-3K}$	[0.3, 1.16] μM	0.9 μM
$\bar{\tau}_{5P}$	Maximal rate of degradation by IP-5P	[0.01, 0.0519] s^{-1}	0.024 s^{-1}



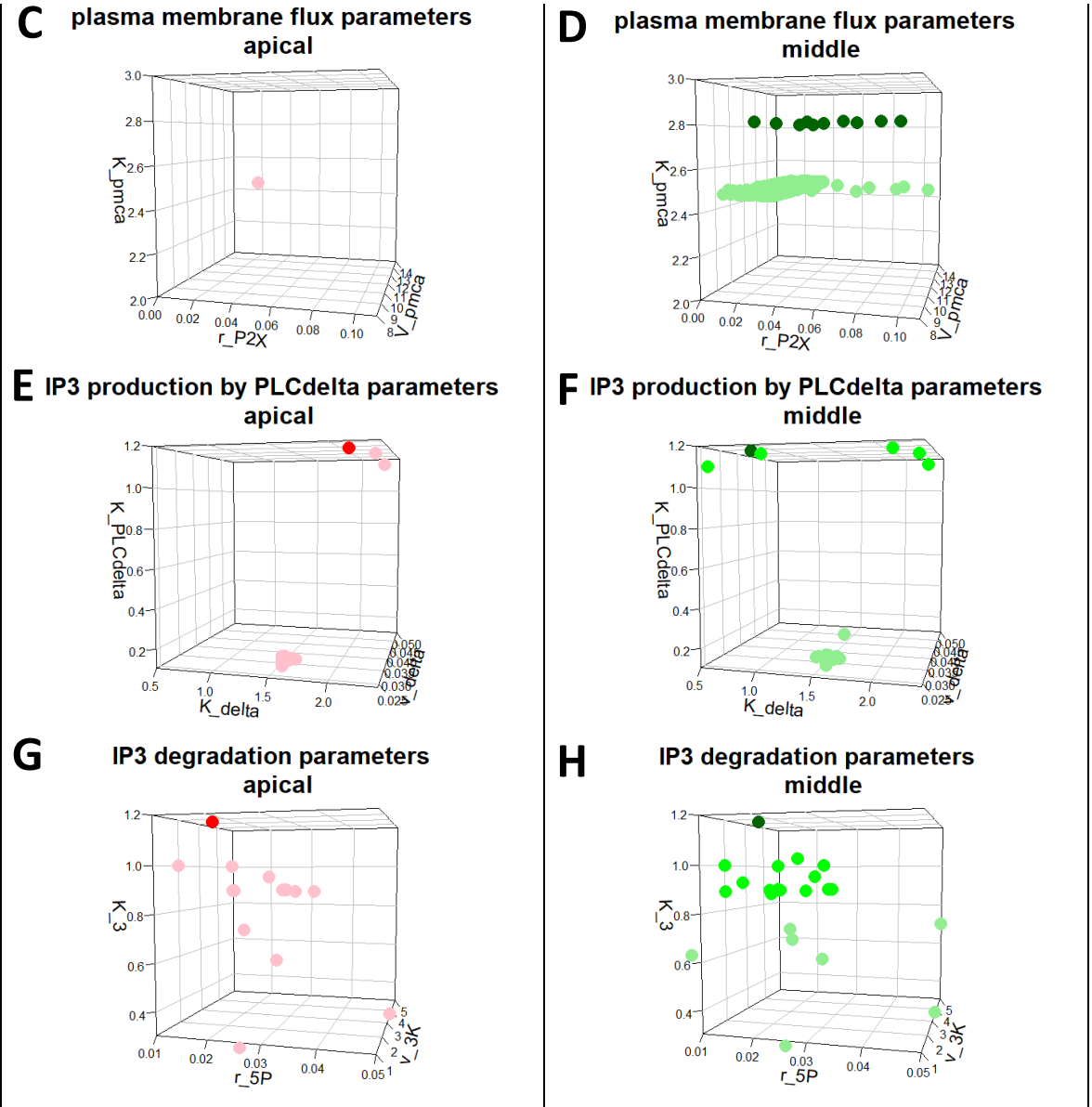


Figure 10 : 3D scatter plots illustrating differences in parameter space results between treatments and cochlear turns. Here, the parameter spaces represent the sets of model parameters fitting with $9 p > 0.05$ for both apical and middle turns. Model parameters for Ca^{2+} store in the apical turn (A) and middle turn (B). Model parameters for fluxes across the plasma membrane in the apical turn (C) and middle turn (D). Model parameters for PLC_δ activity in the apical turn (E) and middle turn (F). Model parameters for IP_3 degradation by $\text{IP}_3\text{-3K}$ and $\text{IP}_3\text{-5K}$ in the apical turn (G) and middle turn (H).

Each model parameter illustrated in **Figure 10** tends to stabilize at a certain value to fit the untreated cells data. Except r_{P2X} , the rate of P2X channel flux, and v_{PMCA} , the maximal PMCA flux, which greatly vary for the middle turn. Regarding the store parameters, SERCA pump parameters (K_{SERCA} , the half-saturation constant for SERCA, and v_{SERCA} , the maximal SERCA flux) are slightly lower while r_{release} , the maximal IP_3R permeability, is higher in the apical turn. In addition, K_δ , the inhibition constant of PLC_δ activity, is higher whereas $\bar{v}_\delta$, the maximal rate of IP_3 production by PLC_δ , and K_{PLC_δ} , the Ca^{2+} affinity of PLC_δ , are slightly

lower in this turn. $\overline{v_{3K}}$, K_3 and $\overline{r_{5P}}$ (the maximal rate of degradation by IP₃-3K, the IP₃ affinity of IP₃-3K and the maximal rate of degradation by IP-5P, respectively) have the same values in both turns (**fig. 10, untreated cells**).

In both turns, SERCA pump parameter values decrease in CBX-treated cells compared to untreated ones (**fig. 10**). Indeed, CBX treatment results in gap junctions block (and potentially IP₃R inhibition too) and thus reduces cytosolic Ca²⁺ concentration so that it may lower v_{SERCA} value (less Ca²⁺ will be taken up by the ER). In each turn, $\overline{v_{\delta}}$ does not vary between treated and untreated cells although $K_{PLC\delta}$ and K_{δ} vary. $K_{PLC\delta}$ is lower for CBX-treated cells in both turns while K_{δ} is higher for CBX-treated cells in the middle turn and lower for those in the apical turn. In both turns, K_3 decreases in CBX-treated cells whereas $\overline{r_{5P}}$ tends to increase.

In the middle turn, r_{P2X} seems to stabilize at a lower value for CBX-treated cells while values vary considerably for untreated cells. This parameter should not be affected by CBX treatment. K_{PCMA} , the half-saturation constant for PMCA, is clearly decreased for CBX-treated cells, although it should not change either. Only v_{PMCA} , however, may decrease due to the effect of CBX treatment on cytosolic Ca²⁺ concentration mentioned previously, but this is unclear here due to its high variability (**fig. 10B, 10D, 10F, 10H**).

Furthermore, no parameter sets fitting experimental data from CBX-treated cells in apical turn, assuming that CBX blocks both gap junctions and IP₃R, has been found (**fig. 10A, C, E, G**).

6 Discussion

6.1 Astrocyte models to simulate calcium dynamics in Deiters' cells.

Supporting cells play an important role in the inner ear. Some of its functions are very similar to those of glial cells, including astrocytes [3]. Here, we developed a mathematical model based on the emerging evidence that Deiters' cells function as a type of specialized glia. The Taheri et al. [30] and De Pittà et al. [31] models both simulate neurotransmitter-evoked Ca^{2+} dynamics in astrocytes, which seemed appropriate for our study as they consider calcium transients and not oscillations. However, they do not include gap junction diffusion of IP_3 which we added to the model employing the Wu et al. [32] model.

According to the results illustrated in **Figure 9**, Ca^{2+} transient and blocking effect of CBX were both described via our modelling approach. Astrocyte models seem suitable for the description of calcium dynamics in Deiters' cells, especially cells of the middle turn (**fig. 9C**). This relates to the hypothesis that Deiters' cells share similar functions and properties with astrocytes [3].

On the other hand, the Wu et al. [32] model simulates the effects of the gap junction coupling strength in hepatocytes, which limits the accuracy of our results on the investigation of the mechanism of action of CBX in Deiters' cells.

6.2 Tonotopic differences between Deiters' cells of apical and middle turns.

The tonotopic organization of the cochlea refers to characteristic structural and mechanical arrangements along its turns that are essential to enable sound discrimination [9]. This phenomenon has been mainly studied in hair cells but tonotopic differences in morphology and purinergic signalling in Deiters' cells have recently been described [15, 33]. The expectation in this study was to provide a better understanding of the tonotopic gradient of the functional properties of Deiters' cells in the organ of Corti.

The experimental results showed that the duration of Ca^{2+} responses is longer in Deiters' cells of the apical turn (**fig. 8A, 8D**). However, the amplitude of the calcium transients is higher in Deiters' cells of the middle turn (**fig. 8B**). These results put forward the question of tonotopic differences in Deiters' cells among the different turns. A higher amplitude would imply a stronger and faster response to the stimulus, perhaps due to a greater number of purinergic receptors, PLC or IP_3R , or a greater endoplasmic Ca^{2+} store. On the other hand,

a longer duration of the response would imply weaker and slower Ca^{2+} -removing mechanisms. This could be related to a reduced number of SERCA or PMCA pumps. An interesting observation has been made on the ability of OHCs to handle Ca^{2+} load along the tonotopic axis of the cochlea. Two main differences between hair cells of low- and high-frequency regions (apical and basal turns, respectively) have been demonstrated : larger amplitude of the MET current and fewer PMCA pumps in high-frequency OHCs. Both are likely to explain the Ca^{2+} overload in OHCs of the basal turn [11]. One would expect a similar tonotopic gradient in Deiters' cells.

We investigated the hypothesis of a tonotopic gradient in Deiters' cells properties by analyzing the modelling results. We found parameter sets overlapping untreated cells of the apical and middle turns which suggests some similarities between calcium responses in Deiters' cells from apical and middle turns (**fig. 9C**). Ca^{2+} imaging experiment was performed from hemicochlea preparation which has the advantages of preserving the cytoarchitecture of the organ of Corti and allowing investigation of cellular and molecular tonotopic differences in older animals [6]. Hemicochlea preparations were obtained by placing the cochleae into the cutting chamber of a vibratome (Vibratome Series 3000, Technical Products International Inc., St. Louis, Mo, USA) to half them along the midmodiolar plane. As the cochleae are not always cut in the exact same place, some apical cells selected for the experiment may be located closer or further from the middle turn and middle cells closer or further from the apical turn. The terms 'base', 'middle' and 'apex' are used to simplify the complex morphology of the cochlea, although the morphology and properties of cochlear cells change gradually along the cochlear turns. This is indeed referred to as the 'tonotopic gradient' [11]. Therefore, such similarities between apical and middle Deiters' cells suggested in **Figure 9C** may be linked to the likelihood that our cells of study were not selected in exactly the same location in each turn.

However, several differences in the parameter values were noticed (**fig. 10**) from which it can be hypothesized that, in the apical turn, there is less endoplasmic Ca^{2+} storage and production of IP_3 by PLC_δ isoenzyme while there are similar IP_3 -5P and IP_3 -3K activities to that of the middle turn. This is indeed correlated with the experimental results shown in **Figure 8**.

This study suggests that the tonotopic organization of the cochlea also applies to Deiters' cells. However, investigating the other model parameters would provide better insight into additional tonotopic differences in Deiters' cells. In future research, it could be interesting to investigate whether the calcium-binding proteins known to have specific spatial expression patterns in hair cells also follow the same pattern in Deiters' cells [34, 35]. Further study on cytoplasmic calcium-binding proteins and mitochondria could also be performed to estimate their importance in buffering Ca^{2+} , as is the case in hair cells [11].

6.3 Mechanism of action of CBX.

In the organ of Corti, supporting cells are coupled with gap junction channels that allow the propagation of intercellular Ca^{2+} signals via the diffusion of Ca^{2+} -mobilizing second messengers [23]. In order to study the gap junctional communication between Deiters' cells, CBX can be used, which is one of the most widely used gap junction blockers [25].

To our knowledge, no research has ever been performed on connexin tonotopic gradient. However, our results show that CBX has a stronger effect on the middle turn, suggesting that more connexins are present in the middle part of the cochlea than the apical one. Indeed, in absence of CBX, the amplitude of the average calcium responses was higher in the middle turn with a P2/P1 ratio being smaller. P2/P1 ratio emphasizes how CBX treatment influenced Ca^{2+} responses in each cochlear turn (**fig. 8B, 8G**).

A recent study raised the question of the blocking effect of CBX on IP_3R , in addition to acting as a connexin blocker, though this new area of research still needs to be investigated [26]. In this study, well-fitting model parameter sets for the middle turn overlapped untreated and CBX-treated cells, while assuming that CBX inhibits both gap junctions and IP_3R (**fig. 9**). However, making the same assumption, no parameter sets has been found for the CBX-treated cells in the apical turn (**fig. 10**). Our modelling results correlate with the experimental results shown in **Figure 8** and would support the hypothesis that CBX blocks both gap junctions and IP_3R in Deiters' cells of the middle turn whereas it only blocks gap junctions in Deiters' cells of the apical turn.

As mentioned above, CBX acts by blocking connexin channels, and potentially IP_3R too [23, 26]. Thus, it should neither affect the parameters for PLC_δ activity nor the parameters for IP_3 degradation by $\text{IP}_3\text{-3K}$ or $\text{IP}_3\text{-5P}$. Our modelling approach made it possible to investigate IP_3

production mechanisms in Deiters' cells, which had never been done before. In the results illustrated in **Figure 10**, $\overline{v_\delta}$ did not vary between treated and untreated cells of both turns, but a lower $K_{PLC\delta}$ for CBX-treated cells was noticed and a higher K_δ for CBX-treated cells in the middle turn while it was lower in the apical turn. Besides, both turns had a decreased K_3 in CBX-treated cells whereas $\overline{r_{5P}}$ tended to increase. Although, CBX should not influence the values of these parameters, unless it does not act directly on IP₃R but rather on the number of active IP₃. The present study suggests that CBX potentially decreases PLC_δ activity in the middle turn and thus reduces the amount of active IP₃ in the cytosol. Therefore, IP₃R activity would be reduced, and even indirectly blocked by CBX. In addition, a higher $\overline{r_{5P}}$ would suggest an elevated IP-5P activity in CBX-treated cells, which in turn also reduces the amount of active IP₃ in the cytosol. This also supports the assumption that CBX indirectly blocks IP₃R by amplifying IP-5P activity. However, a smaller K_3 value is not expected. This would imply that CBX decreases IP₃ degradation by IP₃-3K and positively maintains IP₃R activity.

All together, our study questions support the hypothesis that CBX inhibits IP₃-mediated Ca²⁺ release by acting on pathways unrelated to gap junctions and thus questions the usefulness of CBX as a gap junction channel blocker [26]. However, further studies would be needed in order to investigate its effect on gap junctions compared to other connexin blockers. Additional research could also be done to compare the effect of CBX with those of PLC or IP-5P blockers on intracellular Ca²⁺ transients.

7 Abstracts

7.1 English abstract

Deiters' cells are important supporting cells of the sensory receptor cells in the organ of Corti. They provide a physical and metabolic support to the outer hair cells, modulate their electromotility and contribute to the cochlear amplification of the sound. However, their role and their mechanism of action are not completely understood. The purpose of developing a deeper understanding of the Deiters' cells' functions and properties is believed to have an interest in pharmacological and therapeutical treatments. It could help improve hearing and develop a possible treatment for hearing impairment. To this context, Ca^{2+} signalling pathways in Deiters' cells are studied to provide insight into the regulation of their intracellular processes and intercellular communication.

The cochlea, in which the organ of Corti lies, has a characteristic snail shape. It can be divided into three cochlear turns : apical, middle and basal turns. In mice, the most commonly used animal models in hearing research, the cochlea has a complex morphology and size making its cells difficult to study. Thus, mathematical models would be a valuable tool for the study of Deiters' cells of the different cochlear turns.

This study aimed at developing a mathematical model to simulate ATP-evoked Ca^{2+} signalling and Ca^{2+} -induced gap junctional opening and closing mechanisms in Deiters' cells. The model was compared with data obtained from a Ca^{2+} imaging experiment performed previously to demonstrate Deiters' cells' Ca^{2+} activity. The model was based on Taheri et al. (2017) and De Pittà et al. (2009) models. ATP stimuli were treated as inputs to the model. Besides, Wu et al. (2004) model was used to describe gap junctional IP_3 diffusion from neighboring cells. Indeed, carbenoxolone was experimentally used to investigate gap junctional coupling mechanisms in Deiters' cells. Although it was considered to block the connexin channels non-selectively, it is also thought to block other pathways such as the IP_3R pathway. Both hypotheses were tested with our model.

Over 300,000 model parameter values were tested in order to successfully model ATP-induced Ca^{2+} transients in the apical and middle turns of the organ of Corti. Our model fits with the experimental results. Furthermore, it may provide a better understanding of the mechanism of action of carbenoxolone and the importance of gap junctions in Deiters' cells.

7.2 Hungarian abstract

A Deiters sejtek a Corti-féle szervben helyet foglaló szőrsejtek fontos támasztósejtjei. A fizikai és metabolikus támogatás mellett, feltehetően befolyásolják azok eletromotilitását, ezzel részt vesznek a cochleáris amplifikáció erősítő mechanizmusában. Szerepük azonban korántsem teljesen tisztázott. A Deiters sejtek fejlődésének és funkcióinak feltárása, jobb megértése újabb farmakológia támadáspontokat nyithat meg a halláskárosodások gyógyításában. A sejtek Ca^{2+} szignalizációs pályáinak tanulmányozása hozzájárul a sejten belüli mechanizmusok és a sejtek közötti kommunikációs folyamatok megértéséhez.

A belfül csigája, a cochlea, amelyben a hallószerv is található egy jellegzetesen feltekeredett struktúra az emlősökben. Egérben három kanyarulatot ír le, melyeket a csúcstól indulóan apikális, középső és bazális jelzőkkel illetünk és fentről lefelé az egyre magasabb frekvenciájú hang rezgések felfogásáért felelősek. A bonyolult anatómia struktúra és a szerv érzékenysége miatt nehezen tanulmányozhatóak a sejtek, így érdemes matematikai modellekkel alkotott predikciókkal csökkentenünk a bonyolult és költséges kísérletek számát.

A dolgozat célja egy matematikai modell kifejlesztése volt, amely jól prediktálja az ATP-, mint parakrin szignalizációs molekula, által kiváltott intracelluláris Ca^{2+} szint növekedést a Deiters sejtekben, a Corti-szervben leírt, sejtek közötti gap junction kapcsoltság figyelembe vételével. Az elkészült modellt Ca^{2+} imaging vizsgálatokból származó kísérletes eredményekkel hasonlítottuk össze. Alapját Taheri és munkatársai 2017-es és De Pittà és munkatársai 2009-es gliasejt modelljei képezték. A gap junction kapcsoltság leírása Wu és munkatársai 2004-es modelljét alapul véve került a modellbe és az intracelluláris jelátvivő, IP_3 molekula diffúzióját tette lehetővé. A külső stimulus a kísérletnek megfelelő koncentrációjú ATP volt. A kísérletekben gap junction blokkolóként szereplő carbenoxolonról feltételezik, hogy a gap junction blokkoláson kívül más módon is (IP_3 receptor gátlás) beleszól az intracelluláris Ca^{2+} szignalizációba. Modellünk alkalmas volt ennek a feltételezésnek a tesztelésére is.

Több mint 300 000 paraméter csoport tesztelése során sikeresen felállítottunk egy modellt, ami alkalmas a Deiters sejtek ATP indukálta Ca^{2+} szint emelkedésének modellezésére és a sejtek közötti gap junction kapcsoltság részletesebb vizsgálatára.

8 **References**

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