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**ABSTRACT:**

Auditory cortex (AC) is the ultimate target of afferent auditory pathways and plays a crucial role in the perception and localization of complex sounds. In the thesis were investigated and discussed three aspects of the AC function: i) diversity of the intrinsic passive and active electrical properties of core and belt AC neurons, ii) the modulatory function of the descending connections from the auditory cortex to the inferior colluculus and iii) the effect of a complex acoustical environment applied during the critical period on the responsiveness of auditory cortex neurons in rats.

Within the AC can be distinguished two fundamental areas: centrally located core area and peripheral belt area. Recordings of sound-evoked AC activity revealed striking differences between response patterns of neurons from the core and belt areas. Most of core neurons displayed short latency, phasic responses, unlike the prevalence of longer latency tonic responses in the belt area. It has been hypothesized that incoming signals could be processed differently by neurons in these areas. In the thesis we have investigated the intrinsic electrical properties of layer V pyramidal neurons by patch-clamp recording in acute rat AC slices. Results showed that the pyramidal neurons from the core AC are more excitable at rest with increased time membrane constants and input resistances and generated action potentials with shorter latencies and lower rheobases compared to neurons from the belt region. The different excitability resulted from an elevated constitutive shunting conductance activated in belt neurons at the resting membrane potential. The hyperpolarization activated/cyclic nucleotide-gated cation (HCN) channels were identified as the mediator for this elevated conductance in the belt pyramidal neurons. Analysis of voltage-dependence and gating kinetics of HCN-mediated currents (I<sub>h</sub>), single-cell RT-qPCR and immunohistochemistry revealed the HCN1 and HCN2 as the major subtypes expressed in both auditory areas. Experimental blockade of I<sub>h</sub> confirmed its important role in the regulation of spiking ability of pyramidal neurons in the belt regions.

The existence of massive projections descending from the auditory cortex (AC) to the inferior colliculus (IC) is well documented, yet their function is not fully understood. The modulatory effect of corticofugal projection on the responses of neurons in the rat IC was examined using a reversible cortical inactivation, achieved by cooling of the AC. The frequency tuning and thresholds of the IC neurons did not show any significant changes

during the cooling period. But at the same time, cooling of the AC produced an increase in spontaneous activity as well as in magnitude of the sound-evoked response in 47% of the IC neurons. The final segments of the sustained responses and the off responses were more affected than the onset segments. Changes in the neuronal activity were observed in the dorsal cortex as well as in the central nucleus of the IC. Inactivation of the AC resulted also in a suppression of the post-excitatory inhibition and neuronal adaptation, which was reflected in a pronounced enhancement of synchronized responses to a series of fast repeated clicks. The response parameters recovered, to the pre-cooling levels within 1 h after the cooling cessation. The results demonstrate that AC cooling inactivates excitatory corticofugal pathways and results in a less activated intrinsic inhibitory network in the IC.

Acoustical environment plays an important role during the maturation of the auditory system. It has been shown that the sensory inputs to the developing centres influence the development of the structure of projections, neuronal responsiveness, excitatory-inhibitory balance, or tonotopical arrangement, throughout the auditory pathway. The current work provides evidence that rats reared in a complex acoustic environment (spectrally and temporally modulated sound reinforced by an active behavioral paradigm with a positive feedback) exhibit permanently improved response characteristics of the AC neurons. In particular, the enriched animals had lower excitatory thresholds, sharper frequency selectivity, and a lower proportion of non-monotonic rate-intensity functions. In addition to this, the enrichment changed the AC responsiveness to frequency-modulated and also amplitude-modulated stimuli. For a repetitive stimulus, the neurons exhibited a lower spike count variance, indicating a more stable rate coding. At the level of individual spikes, the discharge patterns showed a higher degree of similarity across stimulus repetitions. Furthermore, the neurons followed more precisely the temporal course of the stimulus, as manifested by improved phase-locking to temporally modulated sounds. These acoustical enrichment-induced changes developed during system maturation were permanent and detectable in adulthood. The findings indicate that an acoustically enriched environment during the critical period of postnatal development influences basic properties of neuronal receptive fields in the auditory cortex, which may have implications for the ability to detect and discriminate sounds and also affects the stochasticity, reproducibility, and fine structure of neuronal spiking patterns.

Results of the thesis contributing to knowledge about neuronal implication of signal processing within the AC circuits, cortical control of the subcortical processing of acoustical stimuli and a role of acoustical stimulation for formation of the auditory system during maturation.

## **ABSTRAKT:**

Sluchová kůra (AC) je konečným cílem aferentních sluchových drah a hraje klíčovou roli ve vnímání a lokalizaci složitých zvuků. V předložené práci jsou zkoumány a diskutovány tři aspekty funkce sluchové kůry na modelu potkana: i) diverzita vnitřních pasivních a aktivních elektrických vlastností korových neuronů v centrální oblasti sluchové

kůry (označované jako core) a v oblasti periferního pásu (označované jako belt), ii) modulační funkce sestupných drah ze sluchové kůry do colliculu inferior (IC) a iii) vliv komplexní akustické stimulace v průběhu kritického období dozrávání sluchového systému na aktivitu sluchových neuronů.

V rámci AC lze rozlišit dvě základní oblasti: centrální oblast (v literatuře označovaná jako core) a oblast periferního pásu (v literatuře označovaná jako belt). Záznamy neuronové aktivity vyvolané zvukovou stimulací ukázaly výrazné rozdíly mezi odpovědi neuronů v centrální oblasti a neuronů v oblasti periferního pásu. Většina neuronů centrální oblasti reaguje s krátkou latencí a fázičkým charakterem odpovědi, na rozdíl od tonických odpovědí s delšími latencemi v oblasti periferního pásu. Obecně se předpokládá, že zpracování příchozích signálů neurony sluchové kůry se může v jednotlivých oblastech AC významně lišit. V práci jsme analyzovali vnitřní elektrické vlastnosti pyramidových neuronů páté vrstvy v obou částech AC snímané s využitím techniky terčíkového zámku (patch-clamp) v korových řezech u potkana. Výsledky ukázaly, že pyramidové neurony v centrální oblasti AC mají ve srovnání s neurony v oblasti periferního pásu vyšší excitabilitu v klidovém stavu, delší časovou konstantu membrány, větší vstupní odpor a generují akční potenciály s kratšími latencemi při nižší reobázi. Různá excitabilita byla důsledkem zvýšené nespecifické (tzv. shunting) vodivosti v neuronech periferního pásu při klidovém membránovém potenciálu. Jako prostředník pro tuto zvýšenou vodivost byly v pyramidových buňkách identifikovány neselektivní HCN (hyperpolarization activated/cyclic nucleotide-gated; hyperpolarizací aktivované, cyklickými nukleotidy řízené) kanály. Analýza napěťové charakteristiky a kinetiky proudů zprostředkovaných HCN kanály (I<sub>h</sub>), RT-qPCR jednotlivých buněk a imunohistochemie identifikovala HCN1 a HCN2 jako dva hlavní podtypy přítomné v obou oblastech sluchové kůry. Experimenty s blokováním I<sub>h</sub> proudů potvrdily jeho důležitost pro regulaci generování akčních potenciálů pyramidovými neurony v periferních páslech AC.

Existence masivních projekcí sestupujících od sluchové kůry ke colliculu inferior (IC) byla již dobře zdokumentována, avšak jejich funkce ještě stále není plně vysvětlena. Modulační účinek sestupné dráhy ze sluchové kůry na odpovědi neuronů v IC byl u potkana studován s použitím dočasně inaktivace AC, která byla dosažena jejím ochlazením. Vyřazení sluchové kůry neovlivnilo frekvenční ladění ani hodnoty prahů u neuronů v IC, chlazení AC však vedlo k významnému zvýšení jak spontánní aktivity tak i odpovědi vyvolaných zvukovou stimulací u přibližně poloviny neuronů v IC. V rámci časového průběhu evokovaných odpovědí byla aktivita neuronů více ovlivněna v pozdější ustálené (tzv. sustained) fázi a na konci odpovědi (tzv. off reakce) než v počáteční (tzv. onset) části. Změny neuronové aktivity byly pozorovány jak v oblasti dorsální kůry IC, tak v centrálním jádru IC. Vyřazení AC vedlo také k potlačení post-excitační inhibice a k menší adaptaci neuronů, což se projevilo ve výrazném zvýšení synchronizovaných odpovědí na zvukovou stimulaci řadou rychle se opakujících impulsů. Po ukončení chlazení se parametry neuronové aktivity vrátily na původní úroveň před chlazením v průběhu cca 1 hodiny. Výsledky dokazují, že vyřazení sluchové kůry deaktivuje excitační sestupnou dráhu do IC a tím dochází k menší aktivaci vnitřní inhibice v neuronových obvodech IC.

Akustická stimulace hraje důležitou roli při zrání sluchového systému. Bylo prokázáno, že senzorická stimulace v průběhu dozrávání sluchového systému významně ovlivňuje utváření neuronové konektivity, receptivní pole neuronů, rovnováhu mezi excitací a inhibicí nebo tonotopické uspořádání v celé sluchové dráze. Předložená práce ukazuje, že potkani chovaní v prostředí s komplexní akustickou stimulací (tzv. obohacené prostředí, v našem případě šlo o zvukovou stimulaci obsahující frekvenční a amplitudovou modulaci a doplněnou o operantní podmiňování s pozitivní zpětnou vazbou) vykazují zlepšené charakteristiky odpovědi neuronů sluchové kůry, které přetrvávají do dospělosti. Komplexní akustická stimulace se projevila v nižších sluchových prazích, větší frekvenční selektivitě a v menším zastoupení neuronů s nemonotonní intenzitní funkcí. Komplexní akustická stimulace ovlivnila i reakci neuronů v AC na frekvenčně i amplitudově modulované zvukové podněty. Neurony těchto zvířat vykazovaly menší rozptyl počtu akčních potenciálů (AP) v odpovědích na opakující se stimulus, což dokumentuje spolehlivější reprezentaci podnětu pomocí tzv. rate-kódu, tedy kódu založeného na četnosti AP. I v případě srovnání časových průběhů posloupností AP měly neurony zvířat chovaných v prostředí s komplexní akustickou stimulací vyšší podobnost mezi jednotlivými odpověďmi na opakovaný podnět. Neurony byly také schopny přesnějšiho sledování časového průběhu zvukového podnětu, což bylo patrné ve vyšší synchronizaci neuronové aktivity s modulací zvukového stimulu. Popsané změny vyvolané komplexní akustickou stimulací v průběhu zrání systému měly dlouhodobý charakter a přetrvávaly do dospělosti. Výsledky jasně ukazují, že akusticky obohacené prostředí během raného období postnatálního vývoje ovlivňuje jak základní vlastnosti receptivních polí neuronů sluchové kůry, tak náhodnost, reprodukovatelnost a strukturu posloupností AP, což může významně ovlivnit schopnost detekovat a rozlišovat zvuky.

Předložená práce přináší nové poznatky, které přispívají k pochopení principů zpracování sluchové informace neurony sluchové kůry, ukazují roli sluchové kůry při řízení zpracování akustických podnětů v podkorových centrech sluchové dráhy a ukazují důležitost akustické stimulace pro formování sluchového systému během jeho dozrávání.

## **INTRODUCTION:**

The AC is the ultimate target of afferent auditory information and has several subdivisions (Purves, 2008). The two most distinct in their responsiveness to auditory stimulus and connections AC fields are: centrally located primary auditory cortex (core region) and the secondary belt auditory area, located around the core (Hackett, 2011). A number of anterograde and retrograde tracing studies have shown that core auditory cortex receives its largest input from the ventral division of the medial geniculate nucleus, with minor inputs from the medial and dorsal divisions, while the major inputs to the fields of the belt regions are from the dorsal nucleus (Roger and Arnault, 1989) and multisensory nuclei in the posterior thalamus (de la Mothe, et al., 2006, Hackett, 2011). In contrast with primary auditory cortical neurons, which readily respond to relatively simple acoustic elements, such as pure tones, neurons in the belt cortex area prefer complex stimuli including broad-band noise (BBN) and vocalizations (Profant, et al., 2013, Rauschecker, et al., 1997). It has been

hypothesized that incoming signals are processed differently by neurons of the core and the belt area (Bai, et al., 2004, de la Mothe, et al., 2006, Herbert, et al., 1991).

In the auditory system the descending pathways are represented by complex, multisynaptic and glutamatergic projections originating in the AC, passing through several subcortical and brainstem auditory structures, ultimately terminating in receptors of the organ of Corti in the inner ear via the olivocochlear bundle (for review see (Malmierca, 2003). An important component of the descending auditory pathway is the cortico-collicular projection, which connects pyramidal cells of layers V and VI of the AC with the IC. The cortico-collicular system has been previously described as being predominantly ipsilateral pathways which mainly target the dorsal (DCIC) and external (ECIC) cortices of the IC (Diamond, et al., 1969, Druga and Syka, 1984, Druga, et al., 1997, Saldana, et al., 1996) demonstrated that auditory cortico-collicular projections also innervate the central nucleus of the IC (CIC). Thus, the entire IC appears to be a target of corticofugal input even though peripheral regions receive the densest projections. In contrast to many detailed histological observations there is little known about the functional role of the cortico-collicular projections. The parameters of sound are first encoded in the cochlea and further processed in the nuclei of the central auditory system. Descending projections from the AC may serve as one source among others for modulating neuronal activity in subcortical auditory centers. Several previous studies have used focal electrical stimulation of the AC in bats (Yan and Suga, 1996), cats (Mitani, et al., 1983), rats (Syka and Popelar, 1984), mice (Yan and Ehret, 2001), and guinea pigs (Tortorolo, et al., 1998) to activate the descending cortico-tectal pathways. Another study used different inactivation methods of the AC with the aim to investigate the functional role of the AC in the descending auditory pathway (Bajo, et al., 2010, Popelar, et al., 2003, Villa, et al., 1991). Among the various inactivation methods, cooling of the AC was found to effectively and reversibly influence the functional state of the AC without producing any damaging effects, even when cooling the AC repeatedly (Coomber, et al., 2011, Lomber, et al., 1999, Nakamoto, et al., 2008, Nakamoto, et al., 2010, Yan and Zhang, 2005, Zhou and Jen, 2005).

For the auditory system of rats, the early postnatal developmental period (the so called critical period, CP) represents the epoch of increased plasticity and susceptibility (de Villers-Sidani, et al., 2007, Insanally, et al., 2009, Rybalko and Syka, 2001). Progressive refinements taking place during this time substantially modulate the structure and function of the auditory centers, often leaving a lifelong trace. An important message brought by recent research is that the final maturation of the system and development of its proper response characteristics are heavily influenced by ongoing auditory stimulation (Bures, et al., 2014, Zhang, et al., 2001, Zhou, et al., 2008). Interventions such as sensory deprivation, monotonous stimulation, or excessive sound exposure result in impairments of neuronal responsiveness both in the AC and in subcortical structures such as IC (Bures, et al., 2010, Bures, et al., 2017, Chang and Merzenich, 2003, Grecova, et al., 2009, Zhou, et al., 2008). On the other hand, a richer and stimulating environment may improve the response properties of the system by, for example, more favorable shaping of the neuronal receptive fields

(Bures, et al., 2014, Engineer, et al., 2004, Oliver, et al., 2011). In the simplest case, when the augmented stimulation comprises an elementary sound like pure tone or click train, the outcome is usually characterized by selective enhancement of responses related to that specific stimulus (de Villers-Sidani, et al., 2007, Miyakawa, et al., 2013, Zhang, et al., 2001). A more beneficial situation, however, is when the responses of the auditory system are amended globally in a non-specific manner. To achieve this, the enriched environment has to be more complex and possibly contain a positive feedback to motivate the animals to attend to the stimulation (Bures, et al., 2014, Percaccio, et al., 2007, Polley, et al., 2006). Our previous results have shown that application of a complex acoustically enriched environment (AEE) during the period of development non-selectively and permanently improves the representations of tonal frequency and intensity in IC and AC neurons by increasing their sensitivity and frequency selectivity, and by lowering the number of non-monotonic rate-intensity functions (Bures, et al., 2014, Pysanenko, et al., 2018). Advancing one step further from the basic evaluation of neuronal receptive fields performed in our previous work, the aim of the current study is to address the ability of neurons to precisely encode a given stimulus using either the rate code or the temporal code. Employing mostly complex temporally-modulated stimuli, we focus on assessment of the reliability, stability and temporal acuity of individual responses. By providing more stable and repeatable response patterns that could be more easily detected in a background spiking noise, the acoustical enrichment during the developmental period, complemented with an active stimulus-reward paradigm, may ultimately enhance the stimulus discrimination ability of the animals.

## **AIMS AND HYPOTHESES:**

### **Intrinsic electrical properties of pyramidal neurons in the core and belt areas of the auditory cortex in rat**

**Hypothesis:** The centrally located core region and surrounding belt area are morphologically and functionally distinct parts of the AC in their responsiveness to acoustical stimuli and also in their connections to other cortical and subcortical structures. We hypothesize that the differences in sound-evoked responses could be partially explained by different distribution and activity of ion channels and distinctive electrical properties of principal neurons in these two AC areas.

Experimental aims:

- To characterize intrinsic electrical properties of layer V pyramidal neurons recording in acute rat AC slices using the patch-clamp technique.
- To identify ion channels which determine resting excitability of layer V neurons in the core and belt areas of the AC.
- To characterize functional properties of hyperpolarization-activated channels in layer V AC neurons.

### **Corticofugal modulation of neuronal responses in the inferior colliculus in rat**

**Hypothesis:** The cortico-collicular projection, an important component of the descending auditory pathway, was suggested to play a role in modulation of both inhibitory circuits in the IC and sound-evoked responses of IC neurons. We hypothesize that AC inactivation by a cooling procedure that silence corticofugal pathways will result in a lower activation of intrinsic inhibitory network within the IC and altered neuronal responses to sound stimuli.

Experimental aims:

- To study the changes in the spontaneous and sound-evoked neuronal activity in the IC after the inactivation of the AC by the cooling procedure.
- The reveal changes in the frequency tuning properties of IC neurons during the cooling period.
- To investigate the effects of the AC inactivation on synchronized responses of IC neurons to a series clicks repeated at high frequency.

### **Acoustical enrichment-induced plasticity in rat auditory cortex**

**Hypothesis:** Acoustical environment plays an important role in the maturation of the auditory system. We hypothesize that acoustical enrichment during the developmental period, which is complemented with an active stimulus-reward paradigm, influences the development of the structure of projections, neuronal responsiveness, excitatory-inhibitory balance, or tonotopical arrangement and may enhance the stimulus discrimination ability of the animals by providing more reliable neuronal response patterns.

Experimental aims:

- To study the influence of the acoustically enriched environment (AEE) on excitatory thresholds, frequency selectivity and rate-intensity functions in AC neurons.
- To reveal whether the enrichment changed the AC responsiveness to frequency-modulated and also to amplitude-modulated stimuli.
- To investigate the effects of the AEE on the neurons spike count variance for a repetitive stimulus and a degree of similarity across stimulus repetitions, to assess the influence of the AEE on the reliability, stability and temporal acuity of individual responses of the AC neurons.

## **MATERIALS AND METHODS:**

### **Animals**

All experiments were performed on rats. Rats were housed under standard conditions on a 12 h/12 h light/dark cycle, with food and water available ad libitum. The specific treatment during acoustical enrichment is described bellow in details. The care and use of animals and all experimental procedures were performed in compliance with the guidelines of the Ethical Committee of the Institute of Experimental Medicine, Czech Academy of

Sciences, and followed the European Community Directive 2010/63EU.

The first study of the electrical properties of the layer V pyramidal AC neurons was made on the 30-40 days old Wistar rats. The other two studies, Long Evans rats aged 3-6 months, weighing  $328\pm 80$  g were used.

#### **Acoustically enriched environment (AEE)**

The *enriched* group of rats ( $n=27$ ) was exposed to an AEE reinforced with active behavioral feedback between P14 and P28. The AEE stimulus represented by a broad-band amplitude-modulated rippled noise with temporally variable sinusoidal spectral envelope 55 dB SPL and it was supplemented with several types of target sounds at 60 dB SPL with 500ms duration. The target signals appeared randomly in time and the frequency-modulated tone triggered the release of a reward – a drop of sweet syrup. A group of age-matched *control* rats ( $n=24$ ) were raised in standard housing conditions with no acoustical enrichment. The AEE was presented during the night active period. The activity of AC neurons was recorded in adulthood at the age of 3 to 6 months.

#### **Anaesthesia**

All hearing measurements, surgical procedures and extracellular measurements of the neuronal activities were performed on anaesthetized animals. The rats were anaesthetized with an intramuscular injection of a mixture of 35 mg/kg ketamine (Narkamon 5%; Spofa, Prague, Czech Rep. or Calypsol 50 mg/ml; Gedeon Richter, Budapest, Hungary) and 6 mg/kg xylazine (Sedazine 2%; Fort Dodge, Animal Health, Fort Dodge, Iowa or Xylapan 20 mg/ml; Vetoquinol SA., Lure Cedex, France).

#### **Recording of auditory brainstem responses (ABR)**

ABRs were recorded to assess the hearing thresholds of rats. ABRs were evoked using short tone bursts (3 ms duration, 1 ms rise/fall times, frequencies of 2, 4, 8, 16, 32 and 40 kHz) delivered with decreasing stimulus level (from 100 to 0 dB SPL) with 5dB step.

#### **Extracellular recording of the neuronal activity in the IC and AC**

Single or multiple extracellular unit activity was recorded in a sound-proof anechoic room. A craniotomy with diameter about 5 mm was made into the skull above the dorsal part of the right IC and another hole was made above the right AC. Location of primary AC field was determined using the custom-made low-impedance 15-channel electrode array which was placed on the AC surface in several positions and middle latency responses (MLRs) were recorded in each location. MLR was elicited in a free field condition by a series of pure tones of 60 ms duration in the frequency range of 1 to 45 kHz with 0.5 octave step at 50 dB SPL. For recording the extracellular unit activity the 16-channel electrode array (NeuroNexus Technologies, single shank probe, 50  $\mu$ m or 100  $\mu$ m distance between electrode spots) was introduced into the IC or AC using a three-axis electronic driver. Responses evoked by single tones, broad band noise, frequency or amplitude modulated and click trains were recorded. Using custom made scripts for Matlab software obtained results were analyzed and following parameters were calculated: excitatory response threshold, characteristic frequency (CF), level of spontaneous activity, quality factor, rate-intensity functions, vector strength and Van Rossum distance.

### **Inactivation of the AC using a cooling probe**

The cooling device was based on a combination of a Peltier element, supplied by the regulated DC voltage, and a cold water cooling system. The cooling part of the Peltier element was attached to a round-shaped probe and covered the whole extent of the AC surface. Measurement of the temperature profile within the AC by the micro thermocouple demonstrated that within one minute after starting the cooling procedure the temperature on the surface of the AC reached 3-5°C.

### **Patch clamp recordings from neurons in acute brain slices**

To reveal intrinsic electrical properties of layer 5 pyramidal neurons of core and belt region, acute coronal AC slices (350-400  $\mu\text{m}$  thick) were isolated from young adult rats. Whole-cell patch-clamp recordings were made from somata of pyramidal neurons and following passive and active neuronal electrical properties were measured: resting membrane potential, membrane time-constant, cell resistance, stimulus threshold (rheobase), action potential threshold, steady state firing rate and the latency of the first AP. Voltage traces during subthreshold current injection were used for the sag detection and its time constant was determined by fitting with a single-exponential function. Responses to voltage ramp stimulation with constantly changing holding potential from -140 up to -50 mV were recorded before and after bath application of 1 mM  $\text{BaCl}_2$  solution and used for construction of the current-voltage of  $\text{Ba}^+$ -sensitive current.  $I_h$  magnitude was recorded in voltage-clamp mode as amplitude of the tail currents measured in the various sets of the hyperpolarization voltage steps. Stimulation were repeated after the bath application of the blocker of  $I_h$  ZD 7288 and ZD 7288-sensitive tail currents were calculated by subtraction of their amplitude measured before and after blocker application. Trains of spikes induced by the application of 100 short (30 ms) just suprathreshold (detected for each particular neurons) current pulses with 100 and 150 ms interstimulus intervals were used for spike latency measurement and calculation of the standard deviation or “jitter” in the timing of these individual spikes averaged over all spikes in trains.

### **Histology and morphometric analysis**

Intracellular labelling of the layer V pyramidal AC neurons with biocytin hydrochloride was made for their three-dimensional reconstruction with NeuroLucida computerized reconstruction system (MicroBrightField, Williston, VT).

RT-qPCR and immunohistochemistry were used to identify and localize HCN channel subtypes in core regions and belt area layer V pyramidal neurons.

### **Statistical analysis**

Statistical analyses were performed using Clampfit 9.2 (Axon Instruments), Excel 2007 (Microsoft office), GraphPad Prism version 6.00 for Windows (GraphPad Software, San Diego, California, USA) and Matlab R2007b (MathWork, Inc.). Data are presented either as the mean  $\pm$  standard deviation (SD) or standard error of the mean (SEM). To assess differences in the mean values between groups Student's t-test with Welch's correction, two-way analysis of variance (ANOVA) with Bonferroni post-hoc test was computed. A Chi-square test was employed to compare the percentage of phase-locking neurons. To evaluate

relationships between two trends, a linear regression with a subsequent F-test was used. In all cases, the alpha level was set to 0.05.

## RESULTS:

### **Intrinsic electrical properties of pyramidal neurons in the core and belt areas of the auditory cortex in rat**

We compared properties of neurons of two AC areas that strikingly differ in their sound-evoked responses, the core and the belt. These AC areas were preidentified in each animal using extracellular recording of their characteristic neuronal responses to sound stimuli. Electrical properties of layer V pyramidal neurons were studied by the patch-clamp technique. The membrane time constant ( $\tau_m$ ) (core:  $27.4 \pm 10.4$  ms; belt:  $18.0 \pm 5.7$  ms,  $p < 0.001$ ) and input resistance ( $R_{in}$ ) (core:  $191.0 \pm 64.2$  M $\Omega$ ; belt:  $122.2 \pm 41.2$  M $\Omega$ ,  $p < 0.001$ ) of core neurons were significantly increased compared to those found at neurons from belt; additionally we found significantly lower values of rheobase for core neurons than for neurons from the belt. The threshold of action potentials evoked was not significantly different between these two groups. On the other hand, the latency of action potentials induced by weakly depolarizing current injections was significantly shorter in core neurons than in neurons from belt (core:  $28.5 \pm 18.5$  ms; belt:  $56.3 \pm 28.8$  ms,  $p < 0.001$ ). The data therefore indicate a higher basal excitability of core neurons. Slope of linear regression of relationship between rheobase and  $R_{in}$  was significantly steeper for belt neurons than for those from core, indicating that the shunting conductance and its effect on basal excitability were significantly stronger in neurons from the belt.

Basal excitability of layer V cortical neurons was previously shown to be controlled by constitutively active low voltage activated K<sup>+</sup> channels (LVAK), inwardly rectifying Kir2.2/2.3 K<sup>+</sup> channels (KIR), K<sup>+</sup>-selective leak ( $K_{leak}$ ) channels and hyperpolarization/cyclic nucleotide gated (HCN) cation channels (Bekkers and Delaney, 2001, Day, et al., 2005, Huggenberger, et al., 2009). Application of the 4-AP (inhibitor of LVAK) and BaCl (inhibitor of  $K_{leak}$  and KIR channels) revealed no differences in activity of these channels between core and belt neurons. We next determined membrane conductance induced by activity of HCN channels in neurons from core and belt. We obtained significantly increased conductance density of HCN channels in neurons of belt (core:  $2.4 \pm 1.6$  nS; belt:  $3.4 \pm 1.5$  nS,  $p = 0.012$ ). This suggested that  $I_h$  was not uniformly distributed among AC areas and could thus contribute to differences in excitability of neurons from core and belt.

To investigate effects of  $I_h$  on basal excitability of core and belt neurons, we compared electrical properties of these neurons in the absence and presence of ZD7288. We found that ZD7288 significantly hyperpolarized neurons and increased  $R_{in}$  and  $\tau_m$ , lowered rheobase and shortened latency of action potentials induced by weak depolarizing stimuli. ZD7288-induced effects on the electrical properties appeared to be stronger in belt neurons than in neurons of core.

Our next hypothesis was that depolarizing sag (hallmark of neurons expressing  $I_h$ ) would accelerate the decay of spike afterhyperpolarization (AHP). Neurons of belt responded to trains of 100 current steps by steady state firing rates that were actually significantly increased compared to those produced by neurons of core. The increase was observed for action potentials stimulated at 150 but not at 100 ms intervals, finding which was consistent with the slow activation kinetics of  $I_h$ . Thus the data suggest that at certain stimulation frequencies layer V AC neurons could generate either the onset type or the sustained type of response, mostly depending on their  $I_h$  density.

### **Corticofugal modulation of the neuronal responses in the inferior colliculus of rat**

In adult rat (Long Evans strain), the ipsilateral AC was inactivated using a cooling probe and single- or multiple-unit activity in the IC in response to acoustical stimulation was recorded before, during and after the AC cooling. In total, 13 electrode probe insertions representing 208 electrode spots were analyzed.

Mapping of the neuronal excitatory response areas in individual IC neurons was performed under pre-cooling conditions, during the AC cooling and after the cooling. AC cooling did not change significantly any parameters of the frequency tuning curves (neuronal CF, CF threshold, shape of the response area) in any of the recorded IC neuron.

During AC cooling the spike rate in response to 60-ms BBN stimulus presentation significantly increased in comparison with the pre-cooling state in 47% of neurons ( $p < 0.05$ ) located through the whole IC. In the remaining neurons, the spike rate either did not change (in 39% of units,  $p > 0.05$ ) or decreased (in 14% of units,  $p < 0.05$ ). The maximal increase of responses to BBN stimulation during AC inactivation was detected in the DCIC, reaching average values of  $177.1 \pm 25.2\%$  and  $216.5 \pm 40.2\%$ . At deeper depths in the IC the AC cooling produced only minimal and non-significant changes in the response magnitude ( $119.9 \pm 10.7\%$ , and  $117.2 \pm 9.0\%$ , respectively). This area can be considered as a transition zone between the DCIC and CIC, neurons recorded in this area show minimal changes not only in evoked responses, but also in spontaneous activity. In the central part of the IC the response magnitude again increased, ranging between 119% and 159% of the pre-cooling.

Individual time segments of the response to BBN stimulation were influenced by AC cooling in a different extent; changes in the onset part of the responses were generally much smaller than the changes in the end segments of the responses. A pronounced effect of the AC cooling was observed on excitatory off-responses, which usually followed 0-10 ms after the end of the normal response (i.e. 10-20 ms after the stimulus offset). During the cooling procedure, the off response in many neurons increased enormously and recovered 60 min after the end of the cooling procedure.

The changes in the evoked activity were also accompanied by changes in the spontaneous firing rate. Spontaneous firing rate was extracted from the last 100 ms of the recorded activity to stimulation using low-level BBN stimuli. Relative changes in the spontaneous firing rate at individual electrodes were very large, much larger than changes in the evoked responses, reaching sometimes more than 500% of pre-cooling values.

In many IC neurons the response to sound stimuli under control conditions was followed by a period of post-excitatory suppression of spontaneous activity lasting 50-200 ms. Of the 80 IC neurons expressing a sufficient level of spontaneous activity, 30 neurons demonstrated significantly reduced post-excitatory inhibition during AC cooling: spontaneous activity appeared immediately after the end of the evoked response.

To test the adaptability of individual IC neurons, five animals from the whole group of tested rats were stimulated with a train of clicks with linearly decreasing and then increasing interstimulus intervals. During AC cooling, the responses of IC neurons to the click trains were different. Most of the IC neurons, including neurons localized in the DCIC, changed their response patterns: they responded with spikes synchronized to click train even at a high click repetition rate and also responded well to the later part of the click train with increasing click inter-stimulus intervals. The average relative response magnitudes during AC cooling ranged between 120% and 260% of pre-cooling values. However, the increase in the number of spikes in a response was not a dominant effect; the main effect of AC cooling was the improved synchronization of spikes with individual clicks up to high repetition rates. During AC cooling the response percentage almost in all IC neurons enormously increased and recovered to pre-cooling level 30-60 min after the AC cooling.

### **Acoustical enrichment-induced plasticity in rat auditory cortex**

Long Evans rat pups (n=27) with mother spent two weeks in an AEE consisting of amplitude-modulated rippled noise presented at 55 dB SPL. To attract the animals' attention, the noise background was supplemented with four types of embedded target sounds presented randomly in time at 60 dB SPL (frequency-modulated tone, pure tone, 1/3-octave noise, sawtooth signal); the FM tone triggered release of sweep syrup. The AEE was applied only during active night periods. Controls (n=24) were raised in standard housing conditions. The hearing of animals was tested 3-4 months after the enrichment with recording of the auditory brainstem responses and multiunit neuronal activity in the AI area of the AC.

Recording the responses from the auditory brain stem, evoked by the tones bursts of different frequencies, showed lower auditory thresholds in the enriched animals compared to the controls ( $p=0.0006$ ).

The receptive fields were analyzed in 975 units from the control animals and 966 units from the enriched animals. The enriched animals show significantly lower values of the excitatory thresholds compared to the controls (enriched:  $7.980 \pm 0.254$  dB SPL; controls:  $11.62 \pm 0.261$  dB SPL;  $p < 0.0001$ ). The sharpness of the frequency tuning was analyzed using the quality factor  $Q_{30}$ . The enriched animals have a significantly higher quality factor than the control animals (enriched:  $0.7621 \pm 0.0303$ ; controls:  $0.674 \pm 0.0320$ ;  $p < 0.05$ ), indicating that the V-shaped units have a higher frequency selectivity in the enriched rats.

The responses to white noise bursts of varying intensity were analyzed in 1714 units from the control animals and 2051 units from the enriched animals. Firstly, the magnitudes of responses to the stimulus at 70 dB SPL were compared. The enriched animals have slightly,

but significantly lower evoked magnitudes (enriched:  $74.51 \pm 1.14$  spikes/sec; controls:  $79.83 \pm 1.4$  spikes/sec;  $p < 0.01$ ).

Subsequently, the shapes of the rate-intensity functions were classified. The enriched animals exhibit a lower proportion of non-monotonic rate-intensity functions (enriched: 25.1%; controls: 33.4%;  $p < 0.0001$ , Chi-square test).

The stability of the rate code, in which information is carried by the number of discharges during a time window, was measured in 2051 units of enriched animals and 1714 units of control animals, by computing the variances of spike counts for responses evoked by broad-band noise pulses. The enriched animals exhibit significantly lower variances, indicating a higher stability of responses ( $p < 0.001$ ). When the spike count variances are compared at individual stimulus levels, significant differences occur at 50, 60, and 70 dB SPL ( $p < 0.001$  in all three cases). Furthermore, the reliability of responses improves with the increasing sound level in the enriched animals.

To see whether the exposure to an acoustic environment containing modulated sounds affected the responses to amplitude-modulated (AM) and frequency-modulated (FM) stimuli, for AM-noises measurements were made in 593 units of enriched animals and 640 units of control animals; in 288 units of enriched animals and 259 units of control animals for FM tones. The shapes of the AM-noise modulation-transfer functions (MTFs) in the enriched and control animals have a nearly identical shape; only slightly lower relative response magnitudes in the enriched rats were observed. The vector strength (VS) quantifies how well the individual spikes are synchronized (phase-locked) with a periodic signal. The enriched animals show a significantly higher degree of synchronization to AM-noises both when analyzed in a frequency-dependent manner ( $p < 0.001$ ) and when analyzing the pooled data (enriched:  $0.28 \pm 0.0024$ , control:  $0.26 \pm 0.0021$ ,  $p < 0.0001$ ). In the case of the FM tones, the enriched and control groups the MTFs differ again significantly ( $p < 0.0001$ ). As in the case of the AM noise, the enriched animals exhibit significantly higher VS values of responses to the FM tones ( $p < 0.001$ ; pooled data enriched:  $0.19 \pm 0.0009$ , control:  $0.17 \pm 0.0007$ ,  $p < 0.0001$ ).

Phase-locking ability to a series of clicks with different repetition rates was tested in 290 units of enriched animals and 255 units of controls. In this case, the repetition-rate dependent curves are not significantly different as a whole ( $p = 0.06$ ), nevertheless, the post-hoc tests show higher VS values at low repetition rates ( $p < 0.001$  at 2 Hz,  $p < 0.01$  at 3.5 Hz). In addition, the pooled values also indicate an improved synchronization in the enriched group (enriched:  $0.19 \pm 0.004$ , control:  $0.17 \pm 0.003$ ,  $p = 0.0014$ ).

In order to see whether the individual neurons give response patterns that are similar to each other, we computed van Rossum distances of different responses to the same stimulus. Clearly, the enriched animals express a higher degree of similarity of responses across multiple stimulus repetitions both for the FM tones (enriched:  $10.5 \pm 0.029$ , control:  $11.66 \pm 0.034$ ,  $p < 0.0001$ ) and for the series of clicks (enriched:  $13.7 \pm 0.15$ , control:  $15.47 \pm 0.17$ ,  $p < 0.0001$ ).

## DISCUSSION:

### **Intrinsic electrical properties of pyramidal neurons in the core and belt areas of the auditory cortex in rat**

#### ***Differential excitability of layer V neurons from core and belt***

We have identified  $I_h$  as a strong regulator of excitability of layer V AC neurons. The distribution of  $I_h$  density in neurons which belong to distinct AC areas appeared to be non-uniform, reaching the highest levels in those of the auditory belt. Accordingly, the belt neurons exhibited strikingly low excitability when compared with neurons of the core area. Inhibition of  $I_h$  by ZD7288, HCN channel blocker (Harris and Constanti, 1995), hyperpolarized layer V AC neurons, increased their  $\tau_m$  and  $R_{in}$ , decreased their rheobase and shortened latency of action potentials induced by weak depolarizing stimuli. The effects of ZD7288 were significantly stronger in neurons of belt, consistent with  $I_h$  as main determinant of the differential excitability of core and belt neurons. This interpretation though relies on specificity of the drug for HCN channels. ZD7288 is the most widely used experimental blocker of  $I_h$  (Biel, et al., 2009). Previously reported evidence has indicated, however, that ZD7288 at higher concentrations may also interact with other ion channels such as low voltage activated  $Ca^{2+}$  channels or  $Na^+$  channels (Sanchez-Alonso, et al., 2008, Wu, et al., 2012). In our experiments, voltage-gated  $K^+$  or  $Na^+$  channels are unlikely target of ZD7288 because the drug did not block amplitudes of action potentials and hyperpolarized layer V AC neurons, and other  $K^+$  channel blocker, 4-AP, similarly reduced excitability in core and belt neurons. Furthermore, T-type  $Ca^{2+}$  channels were not found in layer V pyramidal neurons (Almog and Korngreen, 2009). We therefore suggest that ZD7288 exerted its effects in layer V AC neurons via specific inhibition of  $I_h$ .

#### ***$I_h$ -mediated modulation of spiking of layer V AC neurons***

Our results indicated a complex role of  $I_h$  in mechanisms that determine spiking properties of layer V AC neurons. We found that  $I_h$  1) shunts small depolarizing stimuli and thus increases rheobase and the spike latency, 2) stabilizes the steady state firing rates, and 3) changes the time course of action potentials. The shortening of spike latency in the presence of ZD7288 suggests that weak depolarizing stimuli can effectively inactivate HCN channels in layer V AC neurons. The channels, in turn, cease their shunting action and allow the membrane potential to reach the spike threshold. It also suggests that the tonic excitatory function of  $I_h$ , to depolarize the membrane potential near the resting membrane potential (Kase, et al., 2012), is not affecting spiking properties of layer V AC neurons significantly. In agreement with this, belt neurons that exhibit higher density of  $I_h$  also show significantly tighter correlation between rheobase and  $R_{in}$  than between rheobase and  $V_{rest}$ .

The depolarizing effect of  $I_h$  seems to play a role in mechanisms that elevate probability of action potentials repeatedly triggered with high frequency. We found that layer V AC neurons responded to hyperpolarizing stimuli by a ZD7288-sensitive depolarization sag (Robinson, 2003) that accelerated the decay of spike AHP. The shortening of the AHP led to faster recovery of the membrane potential, which increased the probability of a subsequent spike in the train and facilitated sustained activities of the belt neurons. Our

results are consistent with previously published observations of relationship between  $I_h$ , AHP and neuronal excitability (Aponte, et al., 2006, Momin, et al., 2008, Nolan, et al., 2007, Shah, 2014). It is well established that properly timed neuronal firing improves the representation of the stimulus by a sensory spike train (Bialek, et al., 1991, Billimoria, et al., 2006). In neurons integrating multiple inputs, e.g. in the belt AC neurons, the modulation of spike timing is very important for temporal integration of information and could significantly affect the reliability of the neuronal response for sensory neurons (Billimoria, et al., 2006).

The effect of  $I_h$  on the shape of action potentials would be consistent with the view that HCN channels create a shunt that partially prevents the charging of the membrane in response to depolarizing inputs and affects activities of  $Na^+$  channels in layer V AC neurons. The first derivative of the action potential in these neurons shows two components that correspond to the axonal and somatic action potentials (Colbert and Johnston, 1996, Kress, et al., 2008). The delay in the second component originates as the action potential needs to back-propagate from the axonal initial segment to the soma. ZD7288 facilitated both components indicating that somatodendritic  $I_h$  indeed controlled both the generation and spreading of spikes in these neuronal compartments. We identified HCN1 and HCN2 as prevalent subtypes expressed by layer V AC neurons. We did not find any obvious difference in expression levels or localization of both subtypes in core and belt neurons. It is therefore possible that higher density of  $I_h$  in belt neurons reflected increased activity of HCN channels opening with higher probabilities due to e.g. elevated intracellular concentration of cAMP in these neurons (Zong, et al., 2012).

Overall, our results suggested that differential distribution of  $I_h$  in layer V neurons of distinct AC areas could contribute to variability of sound-evoked responses of these neurons observed in vivo. Besides the AC (Huggenberger, et al., 2009), the role of  $I_h$  in the modulation of neuronal excitability was already established in hippocampus (Aponte, et al., 2006, Magee, 1998), subiculum (van Welie, et al., 2006), inferior colliculus (Koch and Grothe, 2003), sensorimotor cortex (Strauss, et al., 2004), frontal cortex (Day, et al., 2005) or entorhinal cortex (Nolan, et al., 2007). In neocortical layer V pyramidal neurons, the density of  $I_h$  linearly increases with the distance from the cell body along the apical dendrites (Williams and Stuart, 2000). The highest density of  $I_h$  in the apical dendrite enables decreasing the likelihood of summation of dendritic synaptic inputs and increasing integration of the excitatory postsynaptic potentials at the cell soma and axon (Desjardins, et al., 2003, Pavlov, et al., 2011, Williams and Stuart, 2000).

### **Corticofugal modulation of the neuronal responses in the inferior colliculus of rat**

For the revealing of the corticofugal influences on the responsiveness of rat inferior colliculus we used the reversible inactivation of the AC by cooling. In our experiments we used a modified cooling probe of Lomber et al. (1999) based on Peltier elements and cold water cooling system. In these studies the neuronal activity was analyzed in the whole IC.

#### ***Effects of AC inactivation on individual time segments of the response***

AC inactivation influenced individual time segments of the response to BBN stimulation differently. The changes in the onset part of the responses (10-20 ms after stimulus onset) were much smaller than the changes in the last 10 ms of the responses (60-70 ms after stimulus onset). Similar results, i.e., more pronounced changes in the firing rate (increase or decrease) in the later part of the responses in comparison with the onset parts were demonstrated by Popelař et al. (2003) during AC inactivation by tetrodotoxin. On the contrary, Anderson and Malmierca (2013) found mostly changes in magnitude of the onset responses; however, these authors recorded and evaluated preferentially the onset-responding neurons due to their strong SSA, which might have influenced the overall result. In the present study, we observed changes of both the onset and the offset parts of the responses, but the onset changes were much smaller than changes of the offset parts. We assume that the onset part of the response represents the incoming afferent activity from the subcollicular structures whereas the later, sustained part of the response arises from the processing of neuronal activity within the IC that can be modulated by cortical descending fibers.

#### ***Effect of AC inactivation on the DCIC and CIC neurons***

In the present study the inactivation of the AC in rats resulted in an increase of the spontaneous and evoked activity in the majority of IC neurons. The results indicate that AC cooling produces an excitatory-inhibitory dis-balance on the level of the IC in favor of a more expressed excitation and suppressed inhibition. In guinea pigs, Nakamoto et al. (2008) also observed increased or decreased neuronal activity in the IC during AC inactivation, but in a larger portion of neurons the responses were suppressed rather than enhanced (82 vs 50 neurons). However, the authors found no difference between the effects of AC cooling in the CIC and ECIC.

The effects of AC cooling on neurons recorded in the area located between the DCIC and CIC at a depth of 300-500  $\mu\text{m}$  in the IC was minimal even though CFs of neurons recorded in this area by electrode spots 4 and 5 are not beyond the dorso-ventral gradient of neuronal CFs. We suppose that the neurons recorded in the depth 300-500  $\mu\text{m}$  in the IC probably belonged to functionally unspecific transition zone between the DCIC and CIC.

#### ***Effects of AC inactivation on neuronal adaptation***

AC cooling resulted not only in an increase in the spontaneous and evoked activity in the majority of IC neurons, but also in the suppression of post-excitatory inhibition and changes in neuronal adaptation resulting in an increase in the ability to respond to individual clicks in a series of clicks with a fast repetition rate. The most prominent effect of the AC cooling was a significantly larger response accompanied by a higher degree of synchronization to high click repetition rates, occurred in 90 % of IC neurons studied.

Neuronal adaptation to fast repeated stimuli is a characteristic of the standard adaptation phenomenon resulting from intrinsic properties of the neuron. The effects of AC cooling on higher level adaptation, stimulus-specific adaptation (SSA), which is dependent on the history of stimulation, was studied by Anderson and Malmierca (2013) and reviewed by Malmierca et al. (2015).

### ***The function of the efferent system***

Other studies focused on the function of the corticofugal efferent system used several approaches based typically on either activation or inactivation of the cortico-collicular projections. Both situations represent different experimental situations. Whereas electrical stimulation of the AC stimulates nerve fibers that target individual cells in the IC, AC inactivation changes the excitatory-inhibitory balance influencing lower auditory structures. In the IC it has been demonstrated that the AC cooling altered the ability of IC neurons in guinea pigs to localize the sound source in space based on interaural level differences (Nakamoto, et al., 2008), altered the temporal and/or the spike response to the concurrent, monaural harmonic complexes (Nakamoto, et al., 2010) and modified the stimulus-specific adaptation in rats (Anderson and Malmierca, 2013). Nakamoto et al. (2008) observed increased or decreased neuronal activity in the IC during AC inactivation in guinea pigs, but in a larger portion of neurons the responses were suppressed rather than enhanced (82 vs. 50 neurons).

### **Acoustical enrichment-induced plasticity in rat auditory cortex**

#### ***Effects of the enriched acoustic environment (AEE) on the neuronal representation of intensity and frequency***

The current study extends our investigations of the inferior colliculus (IC) neurons (Bures, et al., 2014). Similarly as in the IC neurons, the cortical neurons of enriched animals had lower excitatory thresholds, indicating an increased sensitivity to faint sounds. This interpretation is also supported by the significantly lower hearing thresholds examined using the auditory brainstem responses. The result agrees with the finding of (Engineer, et al., 2004); (Jiang, et al., 2015), on the other hand, observed no significant impact of an enrichment on the minimal thresholds of the A1 units.

#### ***Improved stability of the rate-based stimulus representation***

Stimuli of different sound levels will result in different evoked spike counts; however, for the difference to be detectable, it must be large enough not to be masked by the spiking variability (Colburn, et al., 2003, Silver, 2010). The reduced spike count variance observed in the enriched animals thus suggests an improved discrimination ability of sound level changes. Furthermore, the variance decreases as the stimulus intensity increases further from the threshold, and reaches a shallow minimum of around 60 dB SPL. This may relate to the average SPL of the exposure stimulation which was 55 dB SPL for the background modulated noise and 60 dB SPL for the target stimuli. As the animals were motivated to discriminate between different types of stimuli to obtain a reward, it may be that the minimum observed variance corresponds to the level of the behaviorally most important sounds.

#### ***Influence of the AEE on the selectivity of the AC neurons responses to modulated stimuli***

There are only a few studies that deal with the influence of the AEE on the selectivity of the AC neurons to frequency and amplitude modulation. Furthermore, the reported results

are relatively ambiguous, showing a high dependence on the precise setting of the environmental conditions. In our present work, the perception of the AM noise was similar in the enriched and control groups, possibly due to the prevalence of AM sounds in the natural environment, so that the control group wasn't completely deprived of the AM stimulation. Periodically frequency-modulated tones, on the other hand, are rarer and even rat vocalizations are minimally modulated in frequency (Brudzynski, 2009, Ouda, et al., 2016, Portfors, 2007). Despite the fact that only one particular FM tone was associated with the reward; FM-MTFs differed in a wide range of modulation frequencies, probably thanks to the complex modulated character of the background rippled noise.

Temporal coding employs precise positioning of individual spikes in time, which may potentially carry larger information content than the rate code, and furthermore the information is conveyed almost immediately (Tiesinga, et al., 2008). Considering also the neuronal populations, if a group of neurons projecting to another neuron produces spike patterns that are well-aligned in time, the higher synchrony will improve the detection of the signal. We studied the temporal acuity using two measures: the vector strength quantifying the synchronization of spikes with sound period, and van Rossum distance quantifying the similarity of individual evoked patterns. The neurons in the enriched animals exhibited an improved synchronization with temporally structured stimuli – amplitude-modulated noise, frequency-modulated tones, and click trains. The observed higher vector strength values indicate that the evoked spikes are positioned more precisely within the period of the signal modulation (in the case of amplitude-modulated noise and frequency-modulated tones) or repetition (in the case of click trains). Results suggest that the enriched animals might have a better ability to detect and discriminate temporally varying stimuli. The proportion of significantly phase-locking neurons is not different in the two groups.

Reproducibility and self-consistency of firing patterns as a response to a given stimulus may play an important role in stimulus detection and discrimination tasks, particularly in complicated listening conditions such as a noisy environment. The smaller van Rossum distances of firing patterns to a repetitive stimulus in the enriched group indicate that the individual neuronal responses are more similar to each other, thus encoding more precisely or reliably the respective stimulus. This fact may relate to the experimental paradigm in which the enriched animals were motivated to discriminate several similar stimuli to obtain a reward. Smaller van Rossum distances after an acoustic training have also been observed by Cheng et al. (2017).

### ***Role of the AEE in the human early postnatal development***

The finding that an acoustic environment during early development may have profound and permanent effects on the state of the auditory system is also relevant for human neonatal and infant medicine. For children, the early auditory surrounding plays a crucial role in the language acquisition process (Friederici, 2012, Kuhl, 2010, Newport, 2003). Experimental and clinical experiences with cochlear implantation show that the optimal outcome for auditory performance results only from implantation during the first 1-3 years of life (Geers and Sedey, 2011, Kral and O'Donoghue 2010, Niparko, et al., 2010). Language

learning is the easiest in early life and even regular passive exposure to a foreign language, without its active usage during early childhood, results in a more native-like accent than when the subject learns the language in adulthood (Au, et al., 2002). The connection between congenital hearing loss, severe otitis media in early childhood and the development of speech disorders, is also well established (Gravel and Wallace, 1998, Keogh, et al., 2010, Laws and Hall, 2014, Samelli, et al., 2017, Wertzner, et al., 2012).

## **CONCLUSIONS:**

### **Intrinsic electrical properties of layer V pyramidal neurons in the core and belt areas of the auditory cortex in rat**

- Layer V pyramidal neurons in the core and belt areas of the AC significantly differ in their intrinsic electrical properties, in particular the membrane time constant and input resistance.
- Pyramidal neurons in the belt area of the AC are less excitable than the core neurons due to an elevated shunting conductance active at resting membrane potential.
- Constitutively active HCN1 and HNC2 channels contribute to the excitability of layer V pyramidal neurons in the core as well as belt areas of the AC.
- Increased density of  $I_h$  in layer V pyramidal neurons from the AC belt area affects their firing properties: prolongs the latency of single action potentials and decreases jitter of high frequency spikes.
- *Diversity of intrinsic electrical properties of layer V AC pyramidal neurons could be an important factor contributing to different sound-evoked responses.*

### **Corticofugal modulation of the neuronal responses in the inferior colliculus of rat**

- Inactivation of the AC using a cooling procedure did not alter frequency tuning and thresholds of the inferior colliculus neurons, but significantly modulated their spontaneous and sound-evoked neuronal activity.
- AC inactivation results in a significant increase of spontaneous and sound-evoked neuronal activity in almost half of the IC neurons. Modulatory effect of AC cooling was apparent also in lower post-excitatory suppression, emphasized off-responses and synchronization to click train stimulation.
- Effects of cortical inactivation were observed in the dorsal cortex of the IC as well as in the central part of the IC.
- Characteristics of the neuronal activity in the IC recovered within one hour after the cooling cessation.
- *Observed effects point to a weaker inhibition as a consequence of a reduced activation of the intrinsic GABAergic network within the IC after AC inactivation. It suggests that corticofugal pathways can provide an effective control of the neuronal activity in the dorsal cortex and the central part of the IC.*

### **Acoustical enrichment-induced plasticity in rat auditory cortex**

- Acoustically enriched environment during the critical period of postnatal development influences basic properties of neuronal receptive fields in the auditory cortex. In particular, the AEE lowers the excitatory thresholds, sharpens the frequency selectivity, reduces the maximum response strength and lowers the proportion of non-monotonic RLFs.
- The AEE also affects the processing of temporally structured stimuli (amplitude and frequency modulated sounds).
- Enriched environment presented shortly after the onset of hearing has the power to improve the stability of evoked cortical responses and to lower the variance of spike rates in response to a repetitive stimulus.
- Temporal acuity of individual spikes expressed by synchronization and similarity indexes (vector strength and van Rossum distance, respectively) was significantly amended in the enriched animals compared to the controls.
- Changes in neuronal responsiveness are retained to adulthood, suggesting that they had their origin in developmental processes, presumably shaping the properties of excitatory and inhibitory synaptic transmission.
- *The observed changes suggest that complex acoustic environment may have positive implications for the individual's ability to detect and discriminate sounds, especially in complex listening environments.*

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## LIST OF AUTHOR'S PUBLICATIONS:

### Publications related to the thesis:

#### With impact factor:

- Popelář J., Šuta D., Lindovský J., Bureš Z., **Pysanenko K.**, Chumak T. and Syka J. Cooling of the auditory cortex modifies neuronal activity in the inferior colliculus in rats. *Hearing Research*, February 2016, Vol: 332, pp: 7–16. ISSN: 0378-5955. **IF: 2.906**
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- Hruskova B., Trojanova J., Kulik A., Kralikova M., **Pysanenko K.**, Bures Z., Syka J., O. Trussel L., and Turecek R. Differential distribution of glycine receptor subtypes at the rat calyx of Held synapse. *The Journal of Neuroscience*, November 2012, Vol. 32, Issue 47, pp: 17012-17024. ISSN: 0270-6474. **IF: 8.38**

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## LIST OF ABSTRACTS:

1. **Pysanenko K.**, Profant O., Syka J., Turecek R. Comparison of the electrophysiological membrane properties of layer V pyramidal neurons in the primary auditory cortex and belt area of the rat, 47th Inner Ear Biology Workshop, p. 140, Prague, Czech Republic, August 29-September 1, 2010. Poster presentation.
2. **Pysanenko K.**, Profant O., Syka J., Turecek R. Electrophysiological diversity of layer V pyramidal neurons in the primary auditory cortex and the belt area of the rat, 34th ARO MidWinter Meeting (Association for Research in Otolaryngology), p. 58, Baltimore, Maryland, USA, February 19 – 23, 2011. Poster presentation.
3. **Pysanenko K.**, Profant O., Syka J., Turecek R. Electrophysiological diversity of layer V pyramidal neurons in the primary auditory cortex and the belt area of the rat, Scientific Conference of 2<sup>nd</sup> Medical Faculty Motol, Charles University, April 13-14, 2011, Prague, Czech Republic. Poster presentation.
4. **Pysanenko K.**, Suta D., Syka J. Neuronal responses to frequency-modulated tones in the right and left auditory cortices in the rat, Joint conference of the Czech and Slovak neuroscience societies, p. 84, Smolenice, Slovakia, May 18-21, 2011. Poster presentation.

5. **Pysanencko K.**, Profant O., Králíková M., Chumak T., Valihrach L., Anderova M., Syka J., Tureček R. Study of intrinsic electrical properties of L5 pyramidal neurons from the rat auditory cortex, 10th Conference of the Czech Neuroscience Society, Prague, Czech Republic, November 18-19, 2015. Poster presentation.
6. **Pysanencko K.**, Lindovský J., Bureš Z., Rybalko N., Syka J. The effects of an acoustically enriched environment applied during early ontogenesis on the neuronal activity in rats' primary auditory cortex, 10th FENS Forum of Neuroscience, July 2-6, 2016, Copenhagen, Denmark. Poster presentation.
7. **Pysanencko K.**, Lindovský J., Bureš Z., Syka J. Effect of acoustically enriched environment applied during early ontogenesis on the neuronal activity in the primary auditory cortex in rat, Scientific Conference of 2<sup>nd</sup> Medical Faculty Motol, Charles University, April 26-27, 2017, Prague, Czech Republic. Poster presentation.
8. **Pysanencko K.**, Mácová I., Chumak T., Dvořákova M., Bohuslavová R., Pavlínková G., Syka J., Fritzsck B. Dysfunction of the auditory system in mice with conditionally deleted transcription factor Neurod1, 11th Conference of the Czech Neuroscience Society, Prague, Czech Republic, November 28-29, 2017. Oral presentation.
9. **Pysanencko K.**, Lindovský J., Bureš Z., Syka J. The effect of complex acoustic environment during early development on the responses of auditory cortex neurons in rats, 57th Otological day, December 7. 2017, Pardubice, Czech Republic. Poster presentation.