Physiol. Res. 52: 615-628, 2003

Changes in Neuronal Activity of the Inferior Colliculus in Rat after Temporal Inactivation of the Auditory Cortex

J. POPELÁŘ, F. C. NWABUEZE-OGBO, J. SYKA

Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Received July 1, 2002
Accepted November 1, 2002

Summary

The role of cortico-tectal pathways in auditory signal processing was studied in anesthetized rats by comparing the extracellular single unit activity in the inferior colliculus (IC) before and after functional ablation of the auditory cortex (AC) by tetrodotoxin (TTX). The responses of several IC neurons to sound stimuli were simultaneously recorded with a 16-channel electrode probe introduced into the IC. Click-evoked middle latency responses (MLR) recorded from the AC were suppressed for several hours after TTX injection. During AC inactivation the firing rate of IC neurons increased (40 % of neurons), decreased (44 %) or did not change (16 %) in comparison with control conditions. In several IC neurons, TTX injection resulted in alterations in the shape of the rate-level functions. Response thresholds, tuning properties and the type of discharge pattern of IC neurons were not altered during AC inactivation. However, in one-third of the neurons, the initial part of the response was less altered than the later, sustained part. In two-thirds of neuronal pairs, functional decortication resulted in a change in the cross-correlation coefficient. The results reveal the complex changes that appear in IC neuronal activity after functional ablation of the ipsilateral auditory cortex.

Key words
Inferior colliculus • Auditory cortex • Functional ablation • Descending pathway • Neurons

Introduction

Efferent neuronal pathways have been documented in all sensory systems. In the auditory system, many descending fibers from the auditory cortex (AC) (arising mainly from pyramidal cells of layers V and VI) innervate the inferior colliculus (IC) and the medial geniculate body (MGB). In the rat, as in other animal species, the principal targets of descending cortical fibers in the IC were found to be the dorsal cortex (DCIC) and, to a lesser extent, the external cortex (ECIC) of the IC (Beyerl l978, Druga and Syka 1984a, 1984b, Faye-Lund 1985, Syka *et al.* 1988, Druga *et al.* 1997, Winer *et al.* 1998).

In previous experiments, Syka *et al*. (1988) studied the physiological manifestations of cortico-tectal innervation in the IC by investigating the depth profile of gross electrical responses in the IC evoked either by acoustic or electrical stimulation of the AC. Whereas acoustically evoked responses dominated in the central part of the IC, electrical stimulation of the AC produced responses mainly in the peripheral parts of the IC. Click-evoked responses recorded in the peripheral parts of the IC were significantly suppressed by a preceding electrical

Fax +420 241 062 164
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

pulse to the AC. This finding is consistent with the projection of afferent fibers to the central IC nucleus and descending cortical fibers to the external and dorsal cortices of the IC.

Whereas the morphology of corticofugal pathways is well documented, their role in the processing of auditory signals has not been resolved. Studies carried out in the last 40 years using activation (electrical stimulation) or inactivation (cooling, drug application) of the AC demonstrated either inhibitory, excitatory or a combination of both influences on the neuronal activity in the tectum and thalamus (Burešová *et al.* 1964, Watanabe *et al.* 1966, Amato *et al.* 1969, Andersen *et al.* 1972, Orman and Humphrey 1981, Syka and Popelář 1984, Sun *et al.* 1989, Villa *et al.* 1991, Jen *et al.* 1998, Torterolo *et al.* 1998). Several experiments were performed in the bat studying the role of corticofugal fibers on subcortical specialized neurons related to the processing of biosonar signals such as frequency modulation-sensitive (FM) neurons (Yan and Suga 1996) and Doppler-shifted constant-frequency (DSCF) neurons (Zhang *et al.* 1997, Zhang and Suga 2000). These studies revealed that focal electrical stimulation of the AC facilitates the auditory responses of matched FM-FM IC neurons and sharpens their delay-tuning curves, whereas the responses of unmatched collicular FM-FM neurons are depressed and their delay-tuning curves are shifted away from the best delays of the electrically stimulated cortical FM-FM neurons. Such plasticity manifested by a shift in midbrain frequency tunings toward the characteristic frequencies of the electrically stimulated cortical neurons was also observed in mice (Yan and Ehret 2001, 2002). The results mentioned above suggest that cortical neurons selectively extract, adjust and improve auditory information encoded in the incoming signals to subcortical nuclei *via* the corticofugal system. Since descending fibers from the IC innervate the superior olivary complex and cochlear nucleus (Syka *et al.* 1988, Schofield and Cant 1999), corticofugal modulation ultimately influences the function of the outer hair cells in the cochlea *via* the olivocochlear bundle (Warr *et al.* 1986, Liberman and Brown 1986, Robertson and Gummer 1988, Warren and Liberman 1989).

In the present study, we explored the role of the cortico-tectal pathways in rats using the method of comparing a single unit activity in the IC recorded before and after functional ablation of the auditory cortex induced by a voltage-gated sodium channel blocker, tetrodotoxin (TTX). Previously, we described the effects of intracortical TTX application on click-evoked responses (IC-ER) and multiple unit activity recorded with implanted electrodes in the IC in rats (Nwabueze-Ogbo *et al.* 2002). After inactivation of the AC, the IC-ER amplitude either increased or decreased and IC-ER wave latencies were prolonged, but the discharge pattern and response thresholds of multiple unit activity were not altered. However, we did not find any systematic changes in evoked responses or multiple unit activity related to CF or the place of recording within the IC. The aim of the present study was to investigate the effects of functional ablation of the AC on individual IC neurons recorded with a 16-channel electrode probe in anesthetized rats. This method enabled us to evaluate the changes in the activity of a small population of individual neurons recorded simultaneously from a restricted part of the IC and to analyze the mutual relationship between individual neurons by using a cross-correlation technique.

Methods

Extracellular single unit activity in the IC was recorded in eleven adult rats (Long Evans strain) each weighing between 250 and 450 g.

Surgery

In anesthetized animals (37.5 mg/kg of ketamine and 5 mg/kg of xylazine i.m.), two small holes (diameter 5 mm) were made in the skull above the IC and AC, respectively. The stereotaxic coordinates of the center of the trephine openings were bregma –9 mm and 3 mm medio-lateral for the IC, and bregma –4.5 mm and 5 mm dorso-ventral for the AC (Paxinos and Watson 1996). The animal's head was rigidly held in a stereotaxic apparatus by a U-shaped metallic holder, the base of which was fixed to the skull by two screws and acrylic resin. The holder enabled keeping the auditory meati open for free-field acoustical stimulation. A rectal temperature of 37-38 ºC was maintained by a DC-powered electric heating pad.

Recording of single unit activity

Electrophysiological measurements were performed in a sound-attenuated anechoic room. Extracellular IC unit activity was recorded with a multichannel 16-electrode probe (obtained from the Center for Neural Communications Technology, University of Michigan). A silicon substrate (15 μm thickness) supported an array of 16 conductive tips inlayed with gold and iridium oxide for interfacing to the tissue, with a distance of 50 μm between the tips. Detailed information on the probe is available at www.engin.umich.edu/facility/cnct.

The signals from up to four electrode tips were simultaneously amplified using a custom-made four channel differential amplifier (gain 60 dB, filters 300 Hz - 3 kHz). The neuronal activity was processed with a CED 1401plus interface connected to a PC using CED Spike2 software (sampling frequency 20 kHz). The isolation of spikes of individual neurons from the signal by a template-matching method and the statistical processing of data were made off-line. The CED algorithm uses an adaptive system of template generation. Template shapes are computed from the signal averages of matching spikes, with a match deemed to occur if a sufficient number of a spike´s sample points differ from the corresponding template samples by less than a predefined amount. In our case the maximum percent amplitude change for match was set at 10 %, and the minimum percentage of points in a template was 60 %. One or two of the most prominent spike templates in each channel were further processed statistically. To ensure that identical neurons were evaluated before and after TTX injection, the templates generated from recordings before TTX injection were used for spike sorting in all consecutive recordings.

Rats were stimulated under free-field conditions from loudspeakers (Tesla ARN 5614 and Motorola KSN-1005) placed 70 cm in front of the animal's head. The acoustic system was calibrated with a B&K 4133 microphone, placed in the position of the animal's head and facing the speakers. Pure tone pips at the neuronal CF and broad-band noise (BBN) bursts (duration 100 ms, 3 ms rise/fall times) generated by a Hewlett-Packard sound-wave generator (HP 33120A) and shaped with an electronic switch were used for stimulation at a repetition rate of 2 Hz.

Physiological inactivation of the AC was performed by injecting TTX into the auditory cortex. To cover the whole extent of the AC, three doses of 1 μl of TTX (10 ng/ μl each) were injected using a Hamilton syringe at three points forming an equilateral triangle (spacing: 1-2 mm apart) 1 mm below the pial surface of the AC.

Experimental protocol

After inserting the electrode probe into the IC, the signal at individual electrode tips was checked, and the four electrodes displaying the largest spike amplitudes were connected to four individual inputs of the amplifier for further recording. To ensure the maximal stability for long-term recording, the electrode probe was fixed to the skull by acrylic resin.

At first, the characteristic frequency (CF) of neurons in each channel was determined (CF represents the tonal frequency at which the excitatory threshold of responses was lowest). In three experiments, the tuning properties of twelve single neurons were evaluated in more detail by constructing their tuning curves. During this procedure the intensity of the tonal stimulus was decreased in 5 or 1 dB steps, and the minimal threshold of a response at individual frequencies was determined. The responses of individual neurons to CF or BBN stimulation were recorded at several intensities in 10 dB steps. Each recording session lasted 15-20 min. To check signal variability, one control recording was repeated immediately before TTX injection. Neuronal activity was recorded before and at different periods after the injection of TTX into the AC, and post-stimulus time histograms (PSTHs) were constructed.

Statistical analysis

The firing rate of IC neurons for a given stimulus intensity and frequency was defined as the total number of spikes in relation to the stimulus-on time (100 ms) minus the spontaneous activity (computed from a prestimulus period of 300 ms). A significant change in the firing rate was defined as either an increase or decrease in the discharge rate of more than 20 % from the pre-TTX injection level. The same criterion of a 20 % change was used to evaluate changes in the PSTH.

The correlation between two neurons was considered significant when the peak in a cross-correlogram (CCG) exceeded 2 standard deviations (S.D.) of the correlation coefficient r above zero (i.e. $r > 0.012$, which is equivalent to $p<0.01$) or 4 SD (i.e. $r > 0.024$, which is equivalent to $p<0.0001$) (Eggermont 1992). (1-ms bins in a 30-s-long record results in 30 000 bins in the record and a S.D. equal to 0.006.) Two CCGs were considered significantly different when their peak values differed by more than 20 %.

Control of the functional status of the AC

The functional status of the AC before and after TTX injection was monitored by recording the click-evoked middle latency responses (MLR) with a ball electrode (teflon-coated platinum-iridium wire) fixed in the central part of the AC. The signal from the electrode was amplified by a DAM differential amplifier (gain 60 dB, filters 10 Hz-1 kHz) and processed with an A/D converter of the TDT system using BioSig software.

Histological control of the electrode localization

At the end of each experimental session, the electrode was advanced more deeply into the IC (to mark

more clearly the electrode track) and removed. The rat was sacrificed with an i.p. overdose of 100-200 mg/kg of pentobarbital (Pentobarbital, Spofa, 50 mg/ml) and perfused intracardially with 10 % formaldehyde. The brain was sectioned on a freezing MICROM microtome (slice thickness: 40 μm) and stained with cresyl violet. The positions of the IC electrodes were reconstructed from histological sections under a light microscope with the aid of a stereotaxic atlas of the rat brain (Paxinos and Watson 1996). Microscopic observation revealed that all the evaluated neurons were localized in the ECIC or DCIC.

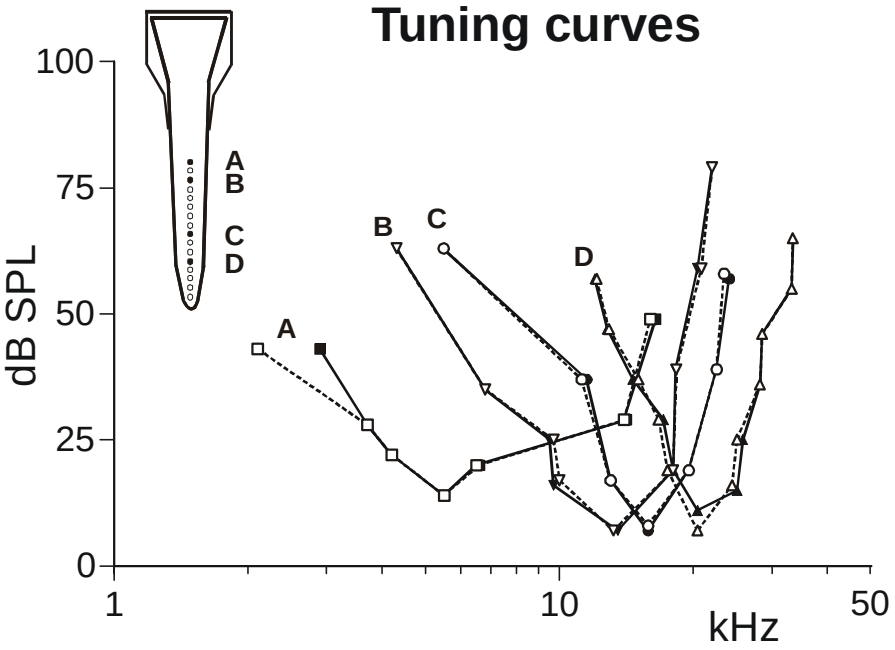

Fig. 1. *Examples of tuning curves of four IC neurons recorded in three animals before (filled symbols, full lines) and 30 minutes after (open symbols, interrupted lines) AC inactivation by TTX injection. Inserted schema represents the positions of the recording electrode tips.*

Results

Effect of TTX application on MLR recorded from the auditory cortex

Two to ten minutes after TTX injection into the AC, the MLR waves almost entirely disappeared, whereas short-latency brainstem-evoked responses were evidently not altered after TTX injection. MLR recording performed at the end of the experimental session revealed that the MLR waves were still abolished in all cases.

IC extracellular unit activity

In total, the activity of 50 neurons were recorded long enough to evaluate their activity before and 1-2 hours after AC inactivation.

Tuning curves

The tuning properties of twelve neurons recorded in three rats were evaluated before and after TTX injection into the AC. Figure 1 demonstrates examples of the tuning curves of four of these neurons and the position of the recording tips on the electrode array. After TTX injection into the AC, the shape of the tuning curves of all tested neurons was almost identical with that observed before AC inactivation. The small variations in response thresholds found at several isolated frequencies did not occur systematically.

The firing rate and rate-level functions

The firing rate of IC neurons was evaluated from neuronal responses evoked by CF or BBN bursts. The rate-level functions (RLF) were constructed from firing rates evaluated at individual stimulus intensities before and after TTX injection into the AC. Twenty to thirty minutes after TTX injection, many IC neurons decreased or increased their spiking during stimulus presentation without changing the threshold of their responses. In neurons with a monotonic RLF (the number of spikes in the response increased monotonically with increasing stimulus intensity), the change in firing rate usually resulted in a change in the RLF slope preserving the monotonic character of the RLF curve (panels a and b in Fig. 2). The effect of AC inactivation on IC neurons with a saturated RLF appears to be represented by a shift of the saturation level while keeping the saturated character unchanged (Fig 2c, 34-6), although three of the IC neurons changed their RLF from the saturated to the monotonic type (Fig. 2d, 37-12). The case with the most

evident changes after TTX injection is demonstrated in Figure 2e (34-1) in the neuron with a non-monotonic RLF (the number of spikes reaches a maximal value, and the further increase of sound intensity reduces the level of activity by more than 50 % of the maximal value). In this neuron the functional ablation of the AC resulted in a change from a non-monotonic to a monotonic RLF. In both IC neurons with an inhibitory response to tonal stimulation, the character of the response was preserved after AC inactivation even though the spontaneous firing rate level slightly decreased (Fig. 2f, 38-6).

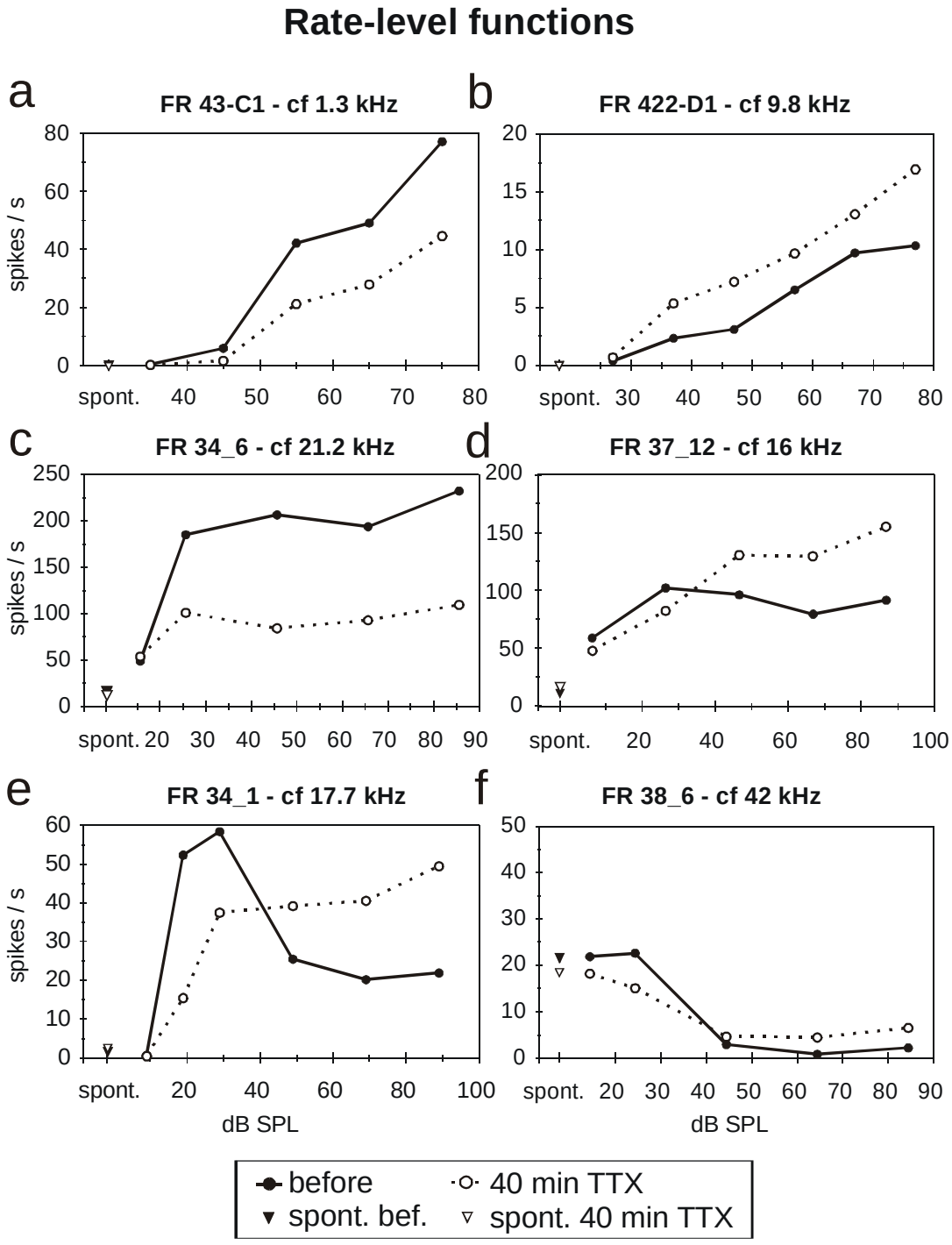

Fig. 2. *Examples of rate-level functions (RLF) evaluated in five animals before (filled symbols, full lines) and after (open symbols, dotted lines) AC inactivation by TTX injection. (a) and (b) monotonic RLFs, (c) and (d) saturated RLFs, (e) non-monotonic RLF and (f) RLF of a neuron showing inhibition of spontaneous activity.*

The scatter diagrams in Figure 3 represent the relative responses of individual IC neurons to CF (left) or BBN (right) stimulation as a function of their CF. The relative response was calculated for each neuron as an average of the ratios of postinjection and preinjection firing rates computed at individual stimulus intensities. During AC inactivation, the firing rate of IC neurons in response to either CF or BBN stimulation either increased (40 % of neurons), decreased (44 %), or did not change (16 %) by more than 20 % of the original firing rate. The data also demonstrate that low-frequency neurons have a tendency to depress their activity after AC inactivation, whereas high-frequency neurons tend to increase their firing rate post-injection. The effect of TTX injection into the AC on the firing rate of individual IC neurons was similar for CF and BBN stimulation, and the linear regression lines in both panels significantly deviate from the horizontal level ($p<0.013$ for CF and $p<0.007$ for BBN stimulation). However, in many cases, even neighboring IC neurons with the same CF displayed an opposite change in their firing rate. The firing rates of most IC neurons at least partly recovered toward preinjection levels during the period of 1-2 hours after TTX injection.

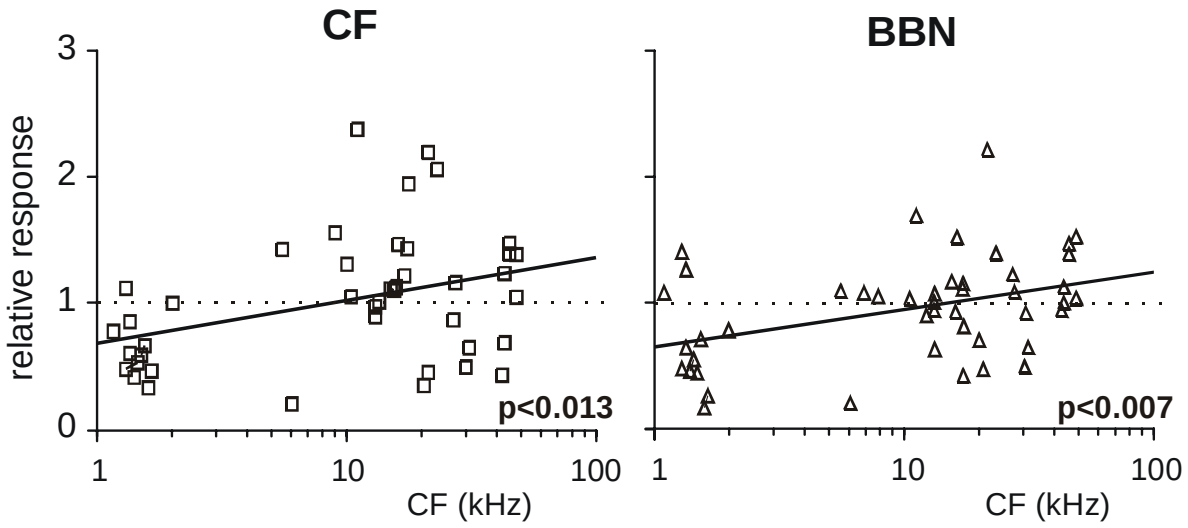

Fig. 3. *Scatter diagrams demonstrating the changes in the relative response to CF (squares) and BBN (triangles) stimulation after AC inactivation. "p" indicates the statistical significance value of the divergence of the linear regression line from the horizontal level.*

Spontaneous firing rate

In 46 IC neurons (92 %), AC functional ablation resulted in the same change (i.e. a decrease or an increase) in spontaneous activity as in evoked activity. In the remaining four IC units, the change in the spontaneous firing rate was different from that in the driven firing rate.

Post-stimulus time histograms

The time pattern of neuronal activity during stimulus presentation was evaluated by constructing post-stimulus time histograms (PSTHs). Even though the number of spikes in the responses of the majority of neurons changed after AC inactivation, the basic shape of the PSTHs was not significantly altered (example in Fig. 4, left panel). However, in 16 neurons out of 46 (35 %), a more pronounced change in the firing rate occurred mainly in the later, sustained part of the PSTH (more than 25 ms after the stimulus onset), whereas the onset part of the PSTH was either not affected at all or changed insignificantly. Neurons with unequal changes in the onset and sustained parts of the PSTH were found in both peripheral IC subnuclei. In 14 neurons (out of 46) the sustained part of the PSTH was significantly reduced (middle panel in Fig. 4), whereas in two neurons, localized in the DCIC, the sustained part of the response was enhanced without changing the value of the onset peak (example in the right panel in Fig. 4). The opposite effect, i.e. changing the onset part of the PSTH without affecting the later part of the response, was not observed in any of the recorded neurons.

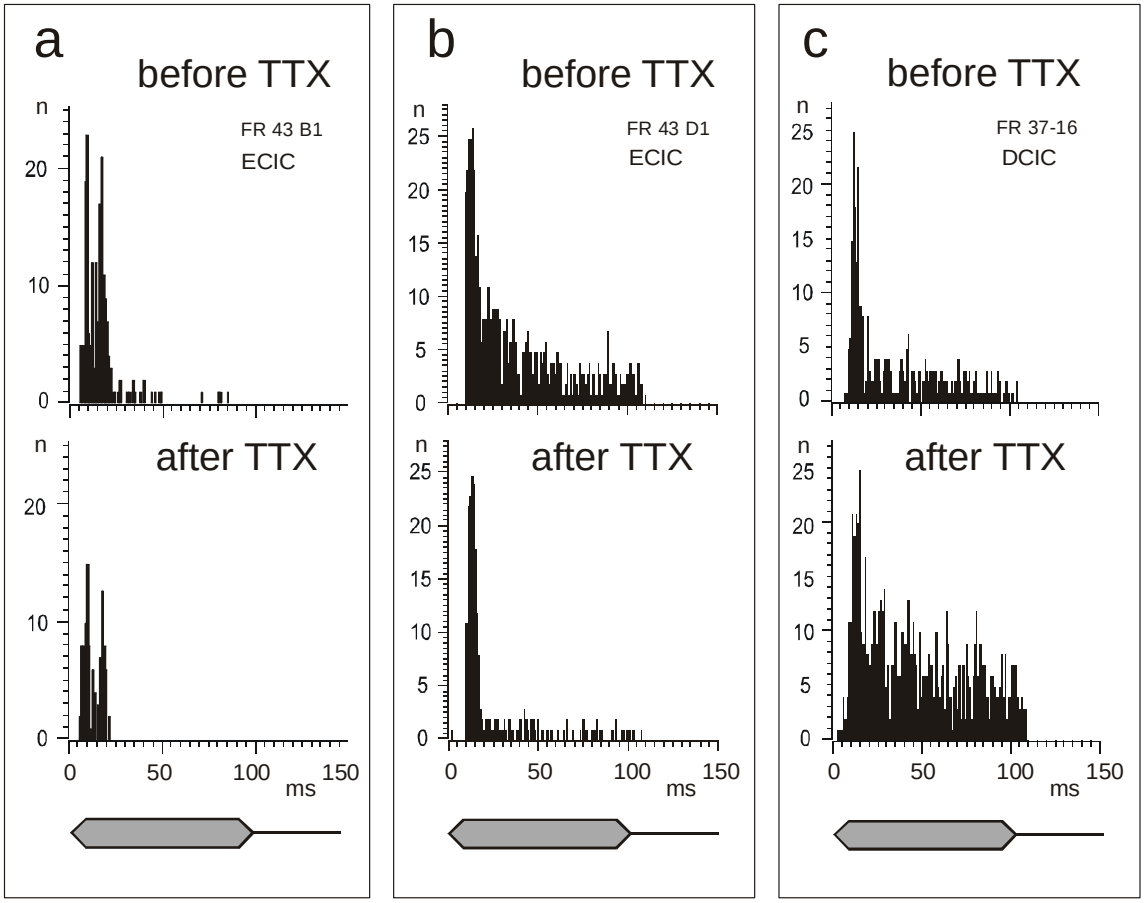

Fig. 4. *Examples of PSTHs of responses in three neurons recorded before and after AC inactivation by TTX injection. Bottom – schema of the acoustical stimulus.*

Mutual relationship of IC neurons

In four rats, the responses to CF or BBN bursts of up to eight neurons recorded simultaneously from four electrode tips were distinguished, and their mutual relationship was evaluated by calculating cross-correlograms (CCG). The CCG is defined as the time-distributed number of spikes from neuron B related to the spikes of neuron A, placed at time zero. For more details see Perkel *et al.* (1967), Eggermont (1992), Kvašňák *et al.* (2000).

In the whole group of 48 IC neuronal pairs recorded from separate electrode tips, 80 % of them displayed a synchronization of their activity, which was manifested by a peak occurring a few milliseconds around zero time. The synchronization in 65 % of these neuronal pairs was weak (correlation coefficient r ranging from 0.012 to 0.024), in the remaining 35 % of pairs, the correlation was stronger ($r > 0.024$). Usually a high degree of synchronization was observed between neurons recorded from neighboring electrode tips (distance between them up to 150 μm) or between neurons with a similar CF. The most frequent types of CCG were characterized by a single narrow peak located 4-6 ms around zero time (47 % of neuronal pairs) or a sharp peak superposed on a wider pedestal (24 %). Such CCG types are thought to represent a common shared input of neurons. In eleven neuronal pairs out of 38 (i.e. 29 %), several peaks occurred in the CCG, indicating more complex connection assemblies.

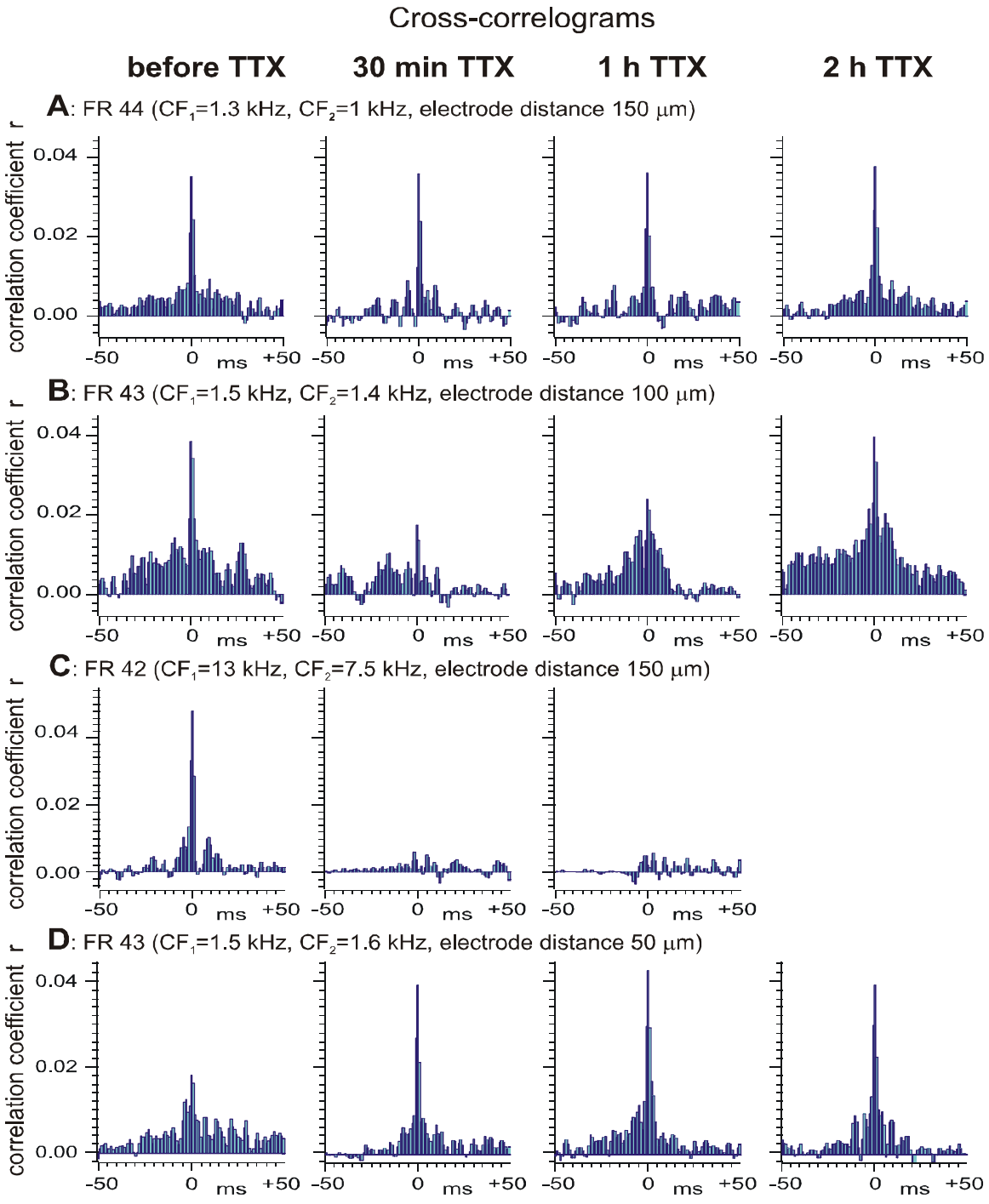

Fig. 5. *Examples of cross-correlograms (CCGs) calculated from recordings in four neuronal pairs (A – D) before and after AC inactivation by TTX injection.*

Examples of individual CCGs calculated from recordings before and after temporal decortication are shown in Fig. 5. The strength of the correlation was not changed in 34 % of the evaluated neuronal pairs (Fig. 5, neuronal pair A). A significant decrease in the cross-correlation value was present in 45 % of neuronal pairs. (Fig. 5, neuronal pairs B and C). In contrast, an originally small correlation became stronger after TTX injection in 21 % of cases (Fig. 5, neuronal pair D). The type of changes in the CCG after TTX injection were not related

to the CF, neuronal position within the IC or type of firing rate changes (i.e. increase or decrease) of either or both neurons in a pair.

Discussion

The present data document complex changes in the single unit activity recorded in the rat IC after functional ablation of the AC by TTX. In many neurons functional decortication resulted in a decrease or increase in their firing rate, changes in the shape of rate-intensity functions or changes in the strength of their mutual relationship without significantly altering their tuning properties. Even though these changes were small, they were related to the actual functional status of the AC and not to incidental fluctuation of neuronal activity within the IC. In our previous paper (Nwabueze-Ogbo *et al.* 2002), we demonstrated that repeated TTX injections into the AC of the same animal with implanted electrodes resulted in almost identical, reproducible IC-ER amplitude changes.

TTX, which was used for reversible temporal inactivation of the AC in the present study, is a powerful sodium channel blocker. Zhuravin and Bureš (1991) have previously demonstrated that impulse transmission and conduction induced by TTX injection are blocked in a spherical volume of brain tissue about 3 mm in diameter and that this effect lasts at least 2 h and slowly decays over the subsequent 20 h. The effect of TTX injection on the function of the AC was confirmed by the disappearance of MLR, whereas short-latency responses originating from the brainstem were not significantly altered after TTX injection.

Physiological data about corticofugal effects on subcortical auditory neurons reported in the literature are not consistent and are, in many cases, controversial: the studies have demonstrated either excitation of the responses, inhibition or both. The depression of auditory responses in the inferior colliculus after cortical inactivation with muscimol (an agonist of GABA) was demonstrated in the bat by Zhang and Suga (1997). The results of Jen *et al.* (2001) obtained in the big brown bat, *Eptesicus fuscus*, demonstrate that the AC sends excitatory projections to the external nucleus of the IC. However, in the present study, both types of response changes (i.e. increase or decrease) were found in the ECIC after AC inactivation. Similar proportions of response changes in the rat IC during AC inactivation by spreading depression were demonstrated by Burešová *et al.* (1964). Out of 118 IC units, 40 % of the responses remained unchanged, 42 % were decreased, 15 % increased and in 3 % an inversion of the response type was found. Both effects, i.e. suppression and enhancement of neuronal activity after AC cooling, were found in the medial geniculate body in cats (Orman and Humphrey 1981, Villa *et al.* 1991), and analogous results were demonstrated in the visual system (Singer 1977).

The role of corticofugal fibers has been studied in experiments evaluating the effects of electrical stimulation of the AC on individual IC neurons (Syka and Popelář 1984, Sun *et al.* 1989, Torterolo *et al.* 1998). Syka and Popelář (1984) found that the majority of IC neurons (73 %) respond to electrical stimulation of the AC with a short excitatory burst with a latency range of 3 to 15 ms, and 25 % of neurons react to AC electrical stimulation by inhibition of spontaneous or sound-evoked activity occurring 3 to 15 ms after the stimulus onset and lasting 30-300 ms. Both effects, i.e. excitation or inhibition of IC activity after cortical electrical stimulation, were demonstrated in the guinea pig by Torterolo *et al.* (1998). These authors found that contralateral cortical stimulation elicited mostly a decrease in the responses of IC neurons (in 23 %), whereas ipsilateral AC stimulation had mostly excitatory effect (34.8 %).

Changes in response magnitude can be related to the plasticity of frequency maps in mammalian auditory brain centers. Yan and Ehret (2001, 2002), using focal electrical stimulation of the AC in mice, demonstrated that collicular best frequencies are shifted toward the stimulated cortical best frequencies when the cortical and collicular frequencies are different. In addition, cortical stimulation elevates the collicular minimum thresholds and reduces both dynamic ranges and response magnitudes. If the cortical and collicular best frequencies are similar but the minimum thresholds are different, the collicular minimum thresholds are shifted toward the stimulated cortical thresholds, the dynamic ranges and response magnitudes may either increase or decrease in this scenario. These results suggest that the corticofugal adjustment has a centre-surround organization with regard to both cortical best frequencies and cortical minimum thresholds. The midbrain processing of sound components in the center of cortical feedback is largely enhanced while processing in the surround is suppressed. In our experiments, the entire AC was inactivated, and therefore the resulting changes in IC activity depended on the complex network in which the recorded neuron participates.

In the present study, the more pronounced changes in the firing rate (increase or decrease) occurred mainly in the later part of the response (as reflected in the sustained part of the PSTH), whereas the onset part of the PSTH (duration of 15 ms) was either not affected or changed only slightly. More pronounced changes in the late phases of the response of IC neurons were also reported by Burešová *et al.* (1964) during cortical spreading depression. It is thought that the onset part of the PSTH represents incoming afferent activity from the subcollicular structures whereas the later, sustained part of the PSTH arises from the processing of neuronal activity within the IC. The IC neurons with unequal changes in the onset and sustained parts of the PSTH may represent those units that are directly influenced by descending pathways from the AC, whereas in the neurons with a decreased or increased firing rate during the whole period of stimulus presentation, the tonic modulatory cortical influence may prevail.

The complexity of the reaction of IC neurons to AC inactivation is in accordance with the complex biochemical profile of mediators within the IC. The morphological data of Fonnum *et al.* (1981) and Feliciano and Potashner (1995) have shown that cortico-thalamic and cortico-tectal fibers are excitatory and glutamatergic. In the IC a large proportion of neurons are immunopositive for γ-aminobutyric acid (GABA) or its synthesizing enzyme, glutamic acid decarboxylase (GAD) (Roberts and Ribak 1987, Moore and Moore 1987, Oliver *et al.* 1994, Fubara *et al.* 1996). Thus, the complex excitatory-inhibitory interactions in the IC can determine the type of RLF of individual IC neurons. In the present study all monotonic RLFs remained monotonic after AC inactivation with only an increased or decreased slope of the RLF curve. In one-third of the neurons with a saturated or non-monotonic RLF, AC inactivation resulted in an increase in the saturation level or in an RLF change from a saturated or non-monotonic type to a monotonic one. Several previous authors demonstrated that GABA plays an important role in determining the neuronal firing rate and thus shaping the RLFs. In experiments in which GABAergic system was blocked with bicuculline, an antagonist specific for $GABA_A$ receptors, the spontaneous and evoked activity in many units increased, the discharge pattern in many cases changed from a phasic to a tonic type and a pre-drug non-monotonic RLF changed to a monotonic one (Yang *et al.* 1992, Pollak and Park 1993, Le Beau *et al.* 1996, Jen and Feng 1999, Chen and Jen 2000, Wang *et al.* 2000). The occurrence of similar changes observed in the present study indicates that relatively short-lasting functional ablation of the AC may alter the distribution of GABA mediators in the IC. Yang *et al.* (1992) demonstrated in the IC of the mustached bat that iontophoretic application of bicuculline also broadened the tuning curves of 42 % of units sharply tuned to 60 kHz. The extent of change in most units varied with the sound level: tuning curves were least affected, or not affected at all, within 10 dB of threshold and showed progressively greater changes at higher sound levels. A broadening of tuning curves was not observed in the small sample of twelve units in our study, but we found changes in firing rate at higher sound intensities, resulting in a transformation of non-monotonic rate-level functions into weakly non-monotonic or monotonic ones. However, in addition to GABA, several other excitatory, inhibitory and neuroactive substances have been detected in the IC, such as glycine, acetylcholine, cholecystokinin, substance P, nitric oxide and others (Sanes *et al.* 1987, Glendenning and Baker 1988, Druga and Syka 1993, Wynne *et al.* 1995). Thus, the resulting effect of AC inactivation depends on many factors, e.g. the distribution of individual receptors in a particular IC neuron, the distribution of synapses and transmitters, etc.

The corticofugal efferent system also consists of fibers descending to the medial olivary complex, either directly or *via* the inferior colliculus (Weedman and Ryugo 1996, Druga *et al.* 1997, Schofield and Cant 1999). Previous physiological studies demonstrated that activation of the peripheral efferent system by electrical stimulation or contralateral acoustical stimulation suppresses neuronal activity in the auditory nerve and lower auditory nuclei (Desmedt 1962, Klinke *et al.* 1969). Based on these data we may tentatively assume that the suppression of neuronal activity in the IC after cortical ablation results from decreased neuronal afferent activity entering the IC from lower auditory nuclei.

The simultaneous recording of neural activity from up to four electrode tips of a 16-channel electrode array enabled us to monitor the effect of AC inactivation on the mutual relationship between adjacent IC neurons evaluated by the cross-correlation technique. To the best of our knowledge, no data are available in the literature showing the modification of the functional connection of individual neurons in the IC by AC inactivation. In our sample, 80 % of neuronal pairs displayed a synchronization of their activity before AC inactivation, which was manifested by a peak occurring a few milliseconds around zero time. Such a high degree of synchronization of neuronal activity in the IC is

comparable with results obtained in the medial geniculate body (Allon and Yeshurun 1985, Heierli *et al.* 1987, Villa *et al.* 1999, Kvašňák *et al.* 2000). Most frequently, CCGs characterized by a single narrow peak located 4-6 ms around zero time or a sharp peak superposed on a wider pedestal were found. Such types of CCG are thought to represent a common shared input of neurons (Gerstein and Perkel 1972). Two-thirds (66 %) of neuronal pairs changed their correlation strength after TTX injection into the AC, either decreasing (45 %) or increasing (21 %) the correlation coefficient. Similar results, i.e., a change in the synchronization of neuronal pairs after temporal inactivation of the AC by cooling, were observed in the spontaneous activity of neurons recorded in the MGB in rats, guinea pigs and cats (Villa *et al.* 1999). In the present study, the changes in the mutual relationship of adjacent neurons in the absence of cortical activity can be considered as an example of fast temporal plasticity of the tectal circuitry. Such modulation of synchronization in neuronal pairs with a shared input during cortical inactivation can be caused by affecting a common source, such as local inhibitory interneurons or an extracollicular excitatory or inhibitory neuron.

The physiological role of descending cortico-tectal pathways in signal processing in the auditory system has also been studied in behavioral experiments. However, studies performed in different animal species have yielded contradictory results. In the original studies, ablation of the AC in the cat, ferret and squirrel monkey resulted in severe sound localization inability, hearing loss, impairment of frequency discrimination and other hearing deficits (Neff 1968, Heffner and Heffner 1989, Jenkins and Merzenich 1984). In contrast to these data, studies in the rat revealed little impairment in sound localization (Kelly 1980) or temporal resolution indicated by gap detection threshold (Rybalko *et al.* 2001) and no obvious abnormality in conditioned autonomic responses to auditory stimuli following bilateral ablation of the AC (LeDoux *et al.* 1984). The relative lack of permanent behavioral deficits after lesions of the AC in the rat raises the question of the role of the auditory cortex for hearing function in this species. This problem should be resolved in future experiments.

Acknowledgements

This work was supported by the ASCR grant (AV0Z5039906), grant NK/6454-3 of the Ministry of Health of the Czech Republic and grant 309/01/1063 of the Grant Agency of the Czech Republic. Multichannel silicon probes were provided by the University of Michigan Center for Neural Communication Technology sponsored by NIH NCRR grant P41-RR09754.

References

ALLON N, YESHURUN Y: Functional organization of the medial geniculate body's subdivisions of the awake squirrel monkey. *Brain Res* **360**: 75-82, 1985.

AMATO G, LA GRUTTA V, ENIA F: The control exerted by the auditory cortex on the activity of the medial geniculate body and inferior colliculus. *Arch Sci Biol (Bologna)* **53:** 291-313, 1969.

ANDERSEN P, JUNGE K, SVEEN O: Corticofugal facilitation of thalamic transmission. *Brain Behav Evol* **6:** 170-184, 1972.

BEYERL BD: Afferent projections to the central nucleus of the inferior colliculus in the rat. *Brain Res* **145**: 209-223, 1978.

BUREŠOVÁ O, MARUSYEVA AM, BUREŠ J, FIFKOVÁ E: The influence of cortical spreading depression on unit activity in the colliculus inferior of the rat. *Physiol Bohemoslov* 1**3**: 227-235, 1964.

CHEN QC, JEN PH: Bicuculline application affects discharge patterns, rate-intensity functions, and frequency tuning characteristics of bat auditory cortical neurons. *Hear Res* **150**: 161-174, 2000.

DESMEDT JE: Auditory evoked potentials from cochlea to cortex as influenced by activation of the efferent olivo-cochlea bundle. *J Acoust Soc Am* **34**: 1478-1496, 1962.

DRUGA R, SYKA J: Ascending and descending projections to the inferior colliculus in the rat. *Physiol Bohemoslov* **33**: 31-42, 1984a.

DRUGA R, SYKA J: Neocortical projections to the inferior colliculus in the rat. (An experimental study using anterograde degeneration techniques). *Physiol Bohemoslov* **33**: 251-253, 1984b.

DRUGA R, SYKA J: NADPH-diaphorase activity in the central auditory structures of the rat. *NeuroReport* **4**: 999-1002, 1993.

DRUGA R, SYKA J, RAJKOWSKA G: Projections of auditory cortex onto the inferior colliculus in the rat. *Physiol Res* **46**: 215-222, 1997.

EGGERMONT JJ: Neural interaction in cat primary auditory cortex. Dependence on recording depth, electrode separation, and age. *J Neurophysiol* **68**: 1216-1228, 1992.

FAYE-LUND H: The neocortical projection to the inferior colliculus in the albino rat. *Anat Embryol (Berl)* **173**: 53-70, 1985.

FELICIANO M, POTASHNER SJ: Evidence for a glutamatergic pathway from the guinea pig auditory cortex to the inferior colliculus. *J Neurochem* **65**: 1348-1357, 1995.

FONNUM F, STORM-MATHISEN J, DIVAC I: Biochemical evidence for glutamate as neurotransmitter in corticostriatal and corticothalamic fibers in rat brain. *Neuroscience* **6**: 863-873, 1981.

FUBARA BM, CASSEDAY JH, COVEY E, SCHWARTZ-BLOOM RD: Distribution of $GABA_A$, $GABA_B$, and glycine receptors in the central auditory system of the big brown bat, Eptesicus fuscus. *J Comp Neurol* **369**: 83-92, 1996.

GERSTEIN GL, PERKEL DH: Mutual temporal relationships among neuronal spike trains. Statistical techniques for display and analysis. *Biophys J* **12**: 453-473, 1972.

GLENDENNING KK, BAKER BN: Neuroanatomical distribution of receptors for three potential inhibitory neurotransmitters in the brainstem auditory nuclei of the cat. *J Comp Neurol* **275**: 288-308, 1988.

HEFFNER HE, HEFFNER RS: Unilateral auditory cortex ablation in macaques results in a contralateral hearing loss. *J Neurophysiol* **62**: 789-801, 1989.

HEIERLI P, DE RIBAUPIERRE F, DE RIBAUPIERRE Y: Functional properties and interactions of neuron pairs simultaneously recorded in the medial geniculate body of the cat. *Hear Res* **25**:209-225, 1987.

JEN PH, FENG RB: Bicuculline application affects discharge pattern and pulse-duration tuning characteristics of bat inferior collicular neurons. *J Comp Physiol [A]* **184**: 185-194, 1999.

JEN PH, CHEN QC, SUN XD: Corticofugal regulation of auditory sensitivity in the bat inferior colliculus. *J Comp Physiol [A]* **183**: 683-697, 1998.

JEN PH, SUN X, CHEN QC: An electrophysiological study of neural pathways for corticofugally inhibited neurons in the central nucleus of the inferior colliculus of the big brown bat, Eptesicus fuscus. *Exp Brain Res* **137**: 292-302, 2001.

JENKINS WM, MERZENICH MM: Role of cat primary auditory cortex for sound-localization behavior. *J Neurophysiol* **52**: 819-847, 1984.

KELLY JB: Effects of auditory cortical lesions on sound localization by the rat. *J Neurophysiol* **44**: 1161-1174, 1980.

KLINKE R, BOERGER G, GRUBER J: Alteration of afferent, tone-evoked activity of neurons of the cochlear nucleus following acoustic stimulation of the contralateral ear. *J Acoust Soc Am* **45**: 788-789, 1969.

KVAŠŇÁK E, ŠUTA D, POPELÁŘ J, SYKA J: Neuronal connections in the medial geniculate body of the guinea pig. *Exp Brain Res* **132**: 87-102, 2000.

LIBERMAN MC, BROWN MC: Physiology and anatomy of single olivocochlear neurons in the cat. *Hear Res* **24**: 17-36, 1986.

LE BEAU FE, REES A, MALMIERCA MS: Contribution of GABA- and glycine-mediated inhibition to the monaural temporal response properties of neurons in the inferior colliculus. *J Neurophysiol* **75**: 902-919, 1996.

LEDOUX JE, SAKAGUCHI A, REIS DJ: Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned to acoustic startle. *J Neurosci* **4**: 683-698, 1984.

MOORE JK, MOORE RY: Glutamic acid decarboxylase-like immunoreactivity in brainstem auditory nuclei of the rat. *J Comp Neurol* **260**: 157-174, 1987.

NEFF WD: Behavioral studies of discrimination: localization of sound in space. In: *Hearing Mechanisms in Vertebrates*. AVS DE REUCK, J KNIGHT (eds), Churchill Press, London, 1968, pp 207-231.

NWABUEZE-OGBO FC, POPELÁŘ J, SYKA J: Changes in the acoustically evoked activity in the inferior colliculus of the rat after functional ablation of the auditory cortex. *Physiol Res* **51**: S95-S104, 2002.

ORMAN SS, HUMPHREY GL: Effects of changes in cortical arousal and of auditory cortex cooling on neuronal activity in the medial geniculate body. *Exp Brain Res* **42**: 475-482, 1981.

OLIVER DL, WINER JA, BECKIUS GE, SAINT MARIE RL: Morphology of GABAergic neurons in the inferior colliculus of the cat. *J Comp Neurol* **340**: 27-42, 1994.

PAXINOS G, WATSON C: *The Rat Brain in Stereotaxic Coordinates*. Academic Press, Sydney 1996.

PERKEL DH, GERSTEIN GL, MOORE GP: Neuronal spike trains and stochastic point processes. II. Simultaneous spike trains. *Biophys J* **7**: 419-440, 1967.

POLLAK GD, PARK TJ: The effects of GABAergic inhibition on monaural response properties of neurons in the mustache bat's inferior colliculus. *Hear Res* **65**: 99-117, 1993.

ROBERTS RC, RIBAK CE: An electron microscopic study of GABAergic neurons and terminals in the central nucleus of the inferior colliculus of the rat. *J Neurocytol* **16**: 333-345, 1987.

ROBERTSON R, GUMMER M: Physiology of cochlear efferents in the mammal. In: *Auditory Pathway - Structure and Function*. J SYKA, RB MASTERTON (eds), Plenum Press, New York, 1988, pp 269-278.

RYBALKO N, MAZELOVÁ, SYKA J: Influence of auditory cortex ablation and noise exposure on gap detection thresholds in rats. *Abstract of the Twenty-fourth ARO Meeting*, St. Petersburg Beach, 4-8. 2. 2001, No. 305, p 86, 2001.

SANES DH, GEARY WA, WOOTEN GF, RUBEL EW: Quantitative distribution of the glycine receptor in the auditory brain stem of the gerbil. *J Neurosci* **7**: 3793-3802, 1987.

SCHOFIELD BR, CANT NB: Descending auditory pathways: projections from the inferior colliculus contact superior olivary cells that project bilaterally to the cochlear nuclei. *J Comp Neurol* **409**: 210-223, 1999.

SINGER W: Control of thalamic transmission by corticofugal and ascending reticular pathways in the visual system. *Physiol Rev* **57**: 386-420, 1977.

SUN X, JEN PH, SUN D, ZHANG S: Corticofugal influences on the responses of bat inferior colliculus to sound stimulation. *Brain Res* **495**: 1-8, 1989.

SYKA J, POPELÁŘ J: Inferior colliculus in the rat: neuronal responses to stimulation of the auditory cortex. *Neurosci Lett* **51**: 235-240, 1984.

SYKA J, POPELÁŘ J, DRUGA R, VLKOVÁ A: Descending central auditory pathway – structure and function. In: *Auditory Pathway – Structure and Function*. J SYKA, RB MASTERTON (eds), Plenum Press, New York, 1988, pp 279-292.

TORTEROLO P, ZURITA P, PEDEMONTE M, VELLUTI RA: Auditory cortical efferent actions upon inferior colliculus unitary activity in the guinea pig. *Neurosci Lett* **249**: 172-176, 1998.

VILLA AE, ROUILLER EM, SIMM GM, ZURITA P, DE RIBAUPIERRE Y, DE RIBAUPIERRE F: Corticofugal modulation of the information processing in the auditory thalamus of the cat. *Exp Brain Res* **86**: 506-517, 1991.

VILLA AE, TETKO IV, DUTOIT P, DE RIBAUPIERRE Y, DE RIBAUPIERRE F: Corticofugal modulation of functional connectivity within the auditory thalamus of rat, guinea pig and cat revealed by cooling deactivation. *J Neurosci Methods* **86**: 161-178, 1999.

WANG J, CASPARY D, SALVI RJ: GABA-A antagonist causes dramatic expansion of tuning in primary auditory cortex. *Neuroreport* **11**: 1137-1140, 2000.

WARR WB, GUINAN JJ JR, WHITE JS: Organization of the efferent fibers: the lateral and medial olivocochlear systems. In: *Neurobiology of Hearing: The Cochlea*. RA ALTSCHULER, DW HOFFMAN, RP BOBBIN (eds), Raven Press, New York, 1986, pp 333-348.

WARREN EH, LIBERMAN MC: Effects of contralateral sound on auditory-nerve responses. 1. Contributions of cochlear efferents. *Hear Res*. **37**: 89-104, 1989.

WATANABE T, YANAGISAWA K, KANZAKI Y, KATSUKI Y: Cortical efferent flow influencing unit responses of medial geniculate body to sound stimulation. *Exp Brain Res* **2**: 302-317, 1966.

WEEDMAN DL, RYUGO DK: Pyramidal cells in primary auditory cortex project to cochlear nucleus in rat. *Brain Res* **706**: 97-102, 1996.

WINER JA, LARUE DT, DIEHL JJ, HEFTI BJ: Auditory cortical projections to the cat inferior colliculus. *J Comp Neurol* **400**: 147-174, 1998.

WYNNE B, HARVEY AR, ROBERTSON D, SIRINATHSINGHJI DJ: Neurotransmitter and neuromodulator systems of the rat inferior colliculus and auditory brainstem studied by in situ hybridization. *J Chem Neuroanat* **9**: 289-300, 1995.

YAN J, EHRET G: Corticofugal reorganization of the midbrain tonotopic map in mice. *Neuroreport* **12**: 3313-3316, 2001.

YAN J, EHRET G: Corticofugal modulation of midbrain sound processing in the house mouse. *Eur J Neurosci* **16**: 119-128, 2002.

YAN J, SUGA A: Corticofugal modulation of time-domain processing of biosonar information in bats. *Science* **273**: 1100-1103, 1996.

YANG L, POLLAK GD, RESLER C: GABAergic circuits sharpen tuning curves and modify response properties in the mustache bat inferior colliculus. *J Neurophysiol* **68**: 1760-1774, 1992.

ZHANG Y, SUGA N: Corticofugal amplification of subcortical responses to single tone stimuli in the mustached bat. *J Neurophysiol* **78**: 3489-3492, 1997.

ZHANG Y, SUGA N: Modulation of responses and frequency tuning of thalamic and collicular neurons by cortical activation in mustached bats. *J Neurophysiol* **84**: 325-333, 2000.

ZHANG Y, SUGA N, YAN J: Corticofugal modulation of frequency processing in bat auditory system. *Nature* **387**: 900-903, 1997.

ZHURAVIN IA, BUREŠ J: Extent of the tetrodotoxin induced blockade examined by pupillary paralysis elicited by intracerebral injection of the drug. *Exp Brain Res* **83**: 687-690, 1991.

Reprint requests

Dr. Jiří Popelář, Institute of Experimental Medicine , Academy of Sciences of the Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic, e-mail: jpopelar@biomed.cas.cz