Hearing Research 314 (2014) 42–50

Contents lists available at ScienceDirect

Hearing Research

journal homepage: www.elsevier.com/locate/heares

Research paper

Prolonged sound exposure has different effects on increasing neuronal size in the auditory cortex and brainstem

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ARTICLE INFO

Article history:
Received 7 May 2014
Accepted 25 May 2014
Available online 6 June 2014

ABSTRACT

Tone at moderate levels presented to young rats at a stage (postnatal week-4) presumably that has passed the cortical critical period still can enlarge neurons in the auditory cortex. It remains unclear whether this delayed plastic change occurs only in the cortex, or reflects a change taking place in the auditory brainstem. Here we compared sound-exposure effects on neuronal size in the auditory cortex and the midbrain. Starting from postnatal day 22, young rats were exposed to a low-frequency tone (4 kHz at 65 dB SPL) for a period of 3 (postnatal day 22–25) or 7 (postnatal day 22–29) days before sacrifice. Neurons were analyzed morphometrically from 7 μm-thick histological sections. A marked increase in neuronal size (32%) was found at the cortex in the high-frequency region distant from the exposing tone. The increase in the midbrain was even larger (67%) and was found in both the low and high frequency regions. While cell enlargements were clear at day 29, only in the high frequency region of the cortex a slight enlargement was found at day 22, suggesting that the cortical and subcortical changes are synchronized, if not slightly preceded by the cortex. In contrast, no changes in neuronal size were found in the cochlear nucleus or the visual midbrain. Such differential effects of sound-exposure at the auditory centers across cortical and subcortical levels cannot be explained by a simple activity-driven change occurring earlier in the brainstem, and might involve function of other structures as for example the descending auditory system.

1. Introduction

Augmented acoustic environment produces plastic changes in the brain (Syka, 2002). In young rats, prolonged sound stimulation remodels tonotopic maps in the auditory cortex (Zhang et al., 2002; Zhou and Merzenich, 2009) as well as in the midbrain (Chiu et al., 2003; Poon and Chen, 1992; Yu et al., 2007; Grecová et al., 2009). The frequency area containing neurons that respond to the exposing tone typically expands in an activity-dependent manner. Furthermore, cytomorphology of the sound-driven neurons is also altered. Cortical neurons from young rats reared in an enriched environment develop more synapses and longer dendrites (Markham and Greenough, 2004; Bose et al., 2010). The converse, sensory deprivation, also changes cytomorphology. For example, in deaf animals, brainstem auditory neurons shrink in size and show fewer dendritic spines (Niparko and Finger, 1997; Saada et al., 1996). Similar changes also appear in the visual cortex after sensory deprivation (Argandona and Lafuente, 2000). In post-mortem brains of dementia and schizophrenia patients, the neuronal size, dendritic field and synaptic density are all reduced compared with normal (Bundgaard et al., 2001; Kolluri et al., 2005; Pierri et al., 2001; Stark et al., 2005). In songbirds, the song-control neurons show seasonal fluctuation in size, a process that is regulated by hormones (Meitzen and Thompson, 2008; Nixdorf-Bergweiler, 1998). In neurosecretory and glandular cells, the variation in nuclear size is metabolism-driven (Berendes, 1965; Rensing et al., 1965; Schmidt and Schibler, 1995; Sempoux et al., 1998; Wang et al., 1996). In tumors, actively dividing cells are larger (Montironi et al., 1994; Stege et al., 2000) and larger cells tend to have more nucleic acids and higher transcriptional activity (Schmidt and Schibler, 1995; Sims et al., 2009). In summary, findings all support a strong link between activity and cytomorphology,

Abbreviations: A1, primary auditory cortex; BF, best frequency; CN, cochlear nucleus; dB SPL, decibel sound pressure level; IC, inferior colliculus; PBS, phosphate buffer solution; PBSX, phosphate buffer solution with triton

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http://dx.doi.org/10.1016/j.heares.2014.05.005
0378-5955/

in particular, the nuclear size. This simple approach of cell size measurements taken at different time points could therefore reflect the history of over-activity occurring in central neurons, and could provide useful insights in the study of neural plasticity.

Our earlier study on juvenile rats (Lu et al., 2009b) showed that neurons in the auditory cortex enlarge after exposure to sound at a moderate level. Since the sound is presented during postnatal week 4, a stage by which time the cortical critical-period for tonal stimuli has presumably passed (de Villers-Sidani et al., 2007; Zhang et al., 2002), the unexpected finding of cortical changes suggested a complex picture of events could have taken place, leaving open some interesting questions unanswered. First, does this change occur only in the cortex, or reflect an activity-driven change that has taken place in the auditory brainstem (Oliver et al., 2011)? Second, during this early postnatal period, the brain is still developing. Therefore does the sound effect interact with the normal development of neuronal size and would it mask or enhance the sound exposure effects (Ptacek and Fagan-Dubin, 1974; Rubel and Fritzsch, 2002; Stark et al., 2007)? Answers to these questions are important for understanding auditory plasticity especially in the early postnatal days. In this follow-up study, the effects of sound exposure on neuronal size were compared in the auditory cortex and brainstem after two different durations of the sound exposure. Furthermore, neuronal sizes at the low and high frequency regions of the auditory cortex and brainstem were compared to determine whether changes are simply activity-driven or reflect other unknown underlying mechanisms.

2. Materials and methods

2.1. Animals and sound exposure

In the first part of experiment, we used 26 post-weaned young rats (Sprague–Dawley, 40–50 g) which were randomly divided into 4 groups: 2 control groups, and 2 exposed groups that received sound exposure of 2 durations (details see below and in Supplementary Table 1). The experimental procedures were approved by the Animal Ethics Committee of NCKU Medical College. Animals were reared inside a sound-proof room. The exposing monotone was 4 kHz and 65 dB SPL (measured at the animals), delivered continuously in free-field at night (22:00–07:00) starting on postnatal day 22. Rats exposed to this moderate level of sound showed no behavioral signs of stress (Zhang et al., 2001). Sound exposure lasted for either 3 days or 7 days. Control animals were reared without the acoustic stimulation.

2.2. Brain tissue preparation

After sound exposure (on postnatal day 25 or 29), rats were euthanized (pentobarbital, 50 mg/kg, i.p.) and perfused with phosphate-buffered saline (PBS), followed by 4% paraformaldehyde. Detailed histological procedures have been reported earlier (Lu et al., 2009b). In brief, brains were removed and post-fixed before paraffin embedding and sectioning at 7 μm in the coronal plane. Sections were deparaffinized, hydrated, and stained with toluidine blue (1%, 5 min). After rinsing, dehydration and xylene treatment, sections were mounted on glass slides for histological study (see below). Control brains were similarly processed.

2.3. Immunohistochemistry

In the second part of experiment, we used another 6 control rats to confirm the location of the low frequency region in the auditory cortex and brainstem using the immunohistochemical staining of an activity marker (c-Fos). Detailed histological procedures have been reported (Lu et al., 2009a). In brief, animals, 3 in a group, were exposed to either a 4- or 9-kHz continuous tone (~65 dB SPL) for 90 min before euthanization (supplementary Table 1). Brains were perfused with PBS, followed by 4% paraformaldehyde in 0.1 M PBS. The removed brains were post-fixed overnight and cryoprotected in 30% sucrose PBS. Frozen sections (40 μm) were cut in the coronal plane and incubated in the following solutions, interspersed with PBS rinses: (a) 1:4000 dilution of Fos antibody (rabbit polyclonal IgG, SC52) with normal goat serum (1:250) and 0.02% PBSX (0.02% Triton in PBS) for 2 days at 4 °C; (b) biotinylated anti-rabbit IgG (1:1000) in PBSX (1.3%) for 4 h; (c) avidin–biotin complex solution (1:400) in PBSX for 2 h. After rinses in PBS and 0.1 M acetate buffer, sections were reacted with the glucose oxidase-nickel-diaminobenzidine solution, and washed in 0.1 M acetate buffer before being fixed on glass slides for microscopic study.

2.4. Determining the brain areas for cell analysis

2.4.1. Auditory midbrain

To determine where to sample neurons in the auditory midbrain (inferior colliculus, IC), we followed the scheme of IC subdivisions provided by Loftus (Loftus et al., 2008), in conjunction with our own results on localizing the area activated by 4 kHz in the c-Fos experiment (Fig. 1A), as well as the results from our earlier report on c-Fos staining after 9 kHz stimulation (see Fig. 3 in Lu et al., 2009a). The middle of the IC was first identified using landmarks according to the rat brain atlas (Paxinos and Watson, 1998, Fig. 2A). To distinguish the area driven by the exposing tone, we divided the tonotopically-organized central IC into 2 regions for separate sampling (Fig. 2B–C): (a) a dorso-lateral portion, representing the low-frequency region (or the region <10 kHz, including the exposing tone of 4 kHz); and (b) a ventro-medial portion, representing the high-frequency region (>10 kHz) (Rubel and Fritzsch, 2002; Ryan et al., 1988). The sampling areas in the IC appeared comparable across animals under gross histological examination (Supplementary Fig. 1).

2.4.2. Auditory cortex

To determine where to sample in the primary auditory cortex (A1), we first identified A1 using landmarks from the brain atlas. The high- and low-frequency regions were further identified with respect to bregma based on the single-unit electrophysiological findings (Doron et al., 2002; Polley et al., 2007; Profant et al., 2013), in conjunction with our own results in the c-Fos experiment (after 4 kHz tone stimulation; Fig. 1B). Again, separate samples were taken from the high- and low-frequency regions, similar to the sampling in the IC. Only neurons in cortical layers III to V were analyzed since they respond strongly to sound (Hromádka et al., 2008; Profant et al., 2013). For details of the sampled areas, please refer to our earlier report (Fig. 1 in Lu et al., 2009a).

2.4.3. Cochlear nucleus

To determine the sampling areas in the cochlear nucleus (CN; Fig. 3A–B), we used landmarks from the brain atlas (Paxinos and Watson, 1998, Fig. 3C) in conjunction with our results in the c-Fos experiment (using a 4 or 9 kHz tone as the stimulus; Fig. 1C). The separate labeling of the 4 and 9 kHz allowed a better delineation of the frequency subdivisions in the three-dimensionally complex CN. Separate samples were taken in each of the three cochlear subdivisions from its low- (~4 kHz) and high-frequency regions (compare Figs. 1C and 3C). The sampling areas in the CN appeared comparable across animals under gross histological examination (Supplementary Figs. 2 and 3).

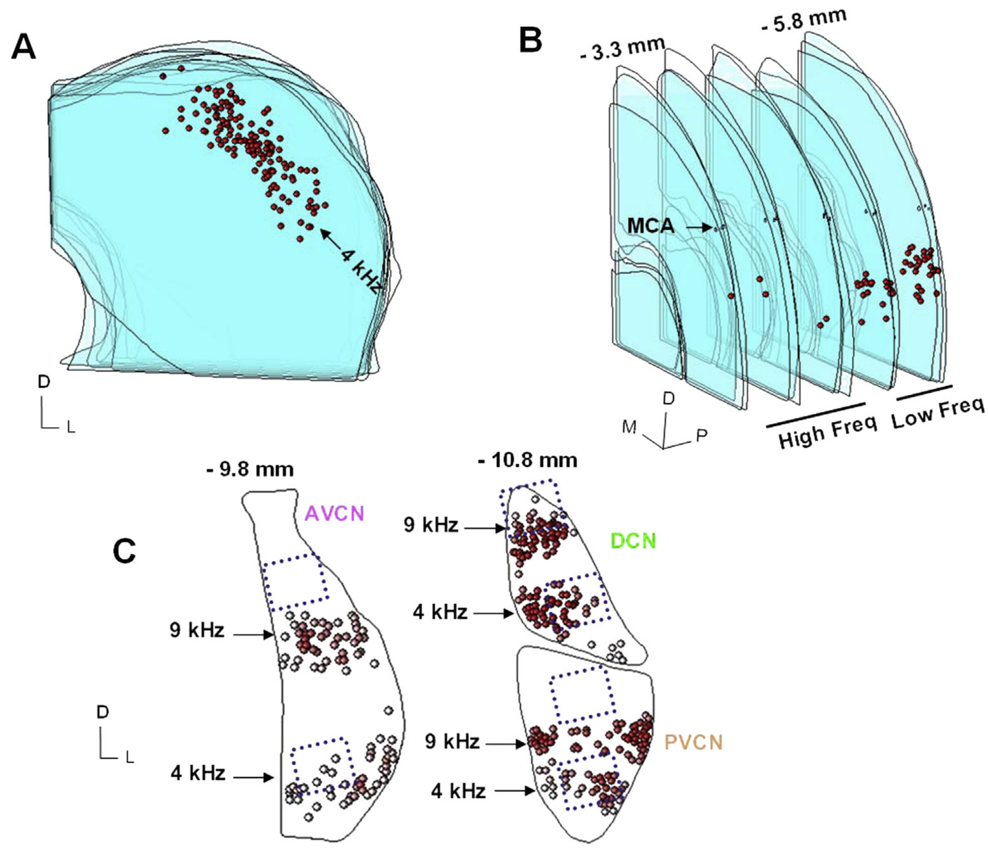

Fig. 1. Confirmation of the low- and high-frequency regions in A1, IC and CN using c-Fos immunohistochemistry in response to 30-min tone stimulation at 4 or 9 kHz. Each round symbol represents a darkly-stained nucleus, and the red intensity is proportional to the proximity of neighboring reaction product. Overlaid results from 3 animals showing the 4-kHz immuno-reactivity falling within the low-frequency regions in A1 (A), and the IC (B). C: Results in the CN from 6 rats (at approximate sections shown in Fig. 3C). In each subdivision, the sampled area of high-frequency neurons is marked by the upper rectangle, and low-frequency neurons the lower rectangle. AVCN – anteroventral cochlear nucleus, PVCN – posteroventral cochlear nucleus, DCN – dorsal cochlear nucleus, MCA – middle cerebral artery. Localization of slices given in mm from bregma. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

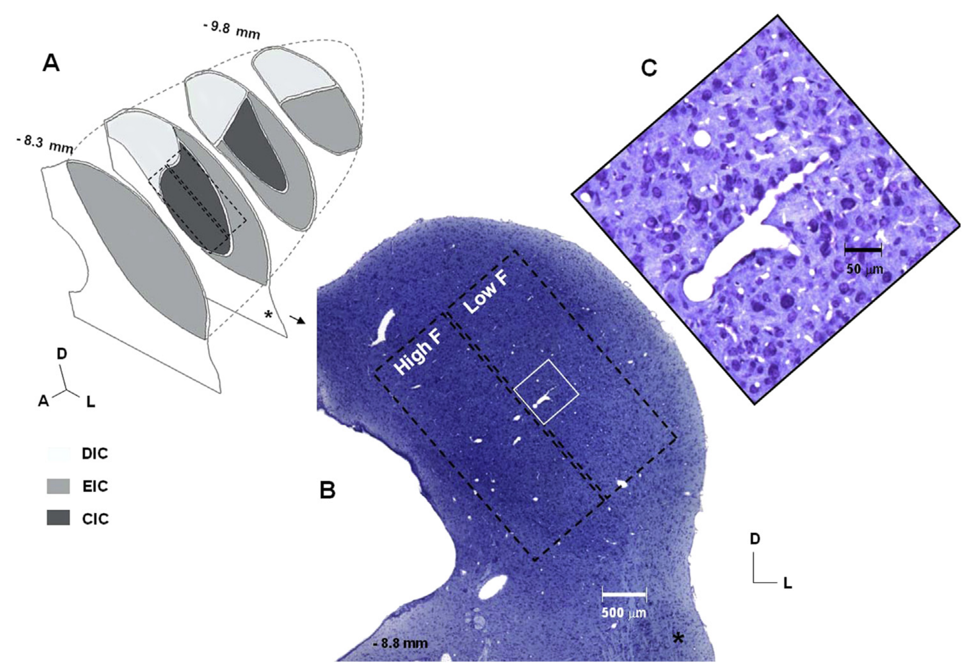

Fig. 2. Regions of neuronal sampling in the IC. A: outlines of an IC showing nuclear subdivisions in coronal sections marked relative to bregma (DIC: dorsal IC; EIC: external IC; CIC: central IC). B: photomicrograph of a toluidine blue-stained section near the middle of the IC showing the low- and high-frequency regions (dashed rectangles). C: magnified view of the CIC (area marked by the white rectangle in B) showing individual cells and vascular lumens. Localization of slices given in mm from bregma.

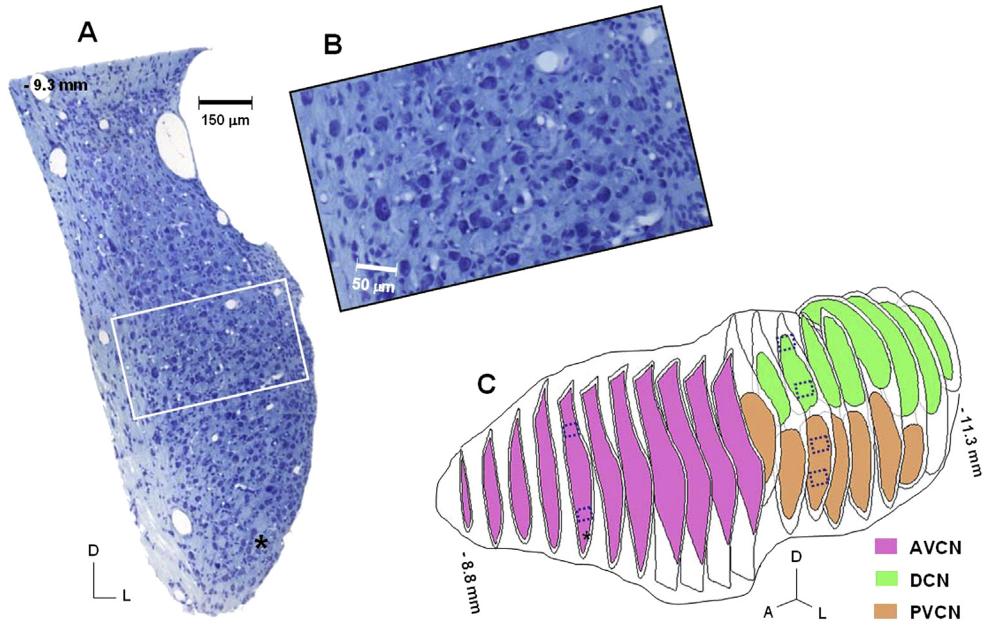

Fig. 3. Regions of neuronal sampling in the cochlear nucleus (CN). A: photomicrograph of a toluidine-blue stained section near the middle of the antero-ventral CN (AVCN; the pink section marked by an asterisk in C). B: enlarged view of the AVCN (area marked by the white rectangle in A). C: outlines of the CN showing the nuclear subdivisions in coronal sections (~130 µm intervals; PVCN: postero-ventral CN; DCN: dorsal CN). The low- and high-frequency regions of each subdivision are marked (dashed outlines) based on the results of c-Fos staining (see also Fig. 1C). Localization of slices is given in mm from bregma. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.5. Quantifying neuronal size and statistical analyses

After determining the areas of sampling, histological sections were digitally photographed (CoolSnap, Leica DMLB, 1000×) and images from areas of interest (e.g., Fig. 2B–C; Fig. 3A–B) were merged in the *z*-axis using an image-analysis system with a confocal-like function (Image Proplus, Media Cybernetics; Prior Proscan). We analyzed only cells showing the presence of at least one nucleolus (Supplementary Fig. 4). This criterion ensured that the cells selected were neurons and were cut roughly through the mid-portion of the cell body. For each neuron, the boundaries of the nucleus and perikaryon were manually digitized (Summa Graphics; Xu et al., 1990) and the area estimated (Image Proplus). Cell volumes were finally extrapolated with spherical approximation ($\text{vol} = 4\pi r^3/3$) based on the equivalent radius derived from the digitized area. For technical details please see our earlier report (Lu et al., 2009b).

For each brain in the first part of experiment ($n = 26$ rats), we analyzed morphometrically the following: (a) 1 set of midbrain sections (3 sections/set, 7 µm intervals), (b) 6 sets of cortical sections (3 sections/set, 0.5 mm intervals), and (c) 2 sets of CN sections (3 sections/set, 7 µm intervals). Together, we analyzed 1541 neurons in A1, 1194 in the IC, and 503 in the CN. In addition, 140 neurons in the visual midbrain (superior colliculus) were analyzed for comparison. In the second part of experiment ($n = 6$ rats), we determined the histological locations of c-Fos labels in the A1, IC and CN after 4 kHz stimulation, and those in CN after 9 kHz stimulation. To determine the region in the IC activated by 9 kHz please refer to our earlier c-Fos study (Lu et al., 2009a). For reasons unclear to us, the c-Fos labels in A1 after 9 kHz stimulation were too weak to be analyzed and results are therefore not presented here.

Volume values of nuclei and perikarya were finally displayed on logarithmic scales. The volume relationship between the nuclei and perikarya were estimated by linear regression. The effects of sound exposure on cell sizes were assessed using Student's *t*-test (unpaired), two-way ANOVA or F-tests wherever appropriate, with statistical significance set at $p < 0.01$. Furthermore, due to the relatively small number of animals we had studied (5 rats/group), additional statistics was done using three-way analyses of variance containing one repeated factor to isolate the sound-exposure effect from other effects related to individual animal variations or developmental changes. For statistical analyses we used the software Graphpad-Prism (v4), SPSS (v17) and SAS (v9.3).

3. Results

3.1. Changes in neuronal size in the control group during the sampled period

In the control brains (Supplementary Fig. 5), cell volumes in A1 showed an age-dependent drop in the experimental period from postnatal day 25–29 (perikaryon: postnatal day 25: 2483 ± 956 *vs* postnatal day 29: 2060 ± 850 µm^3, $p < 0.001$; nucleus: postnatal day 25: 727 ± 268 *vs* postnatal day 29: 602 ± 235 µm^3, $p < 0.001$; Student's *t*-test). In the IC, although the nuclear volume shrank from postnatal day 25–29 (postnatal day 25: 391 ± 172 *vs* postnatal day 29: 322 ± 168 µm^3, $p < 0.001$; two-way ANOVA, $F(1,1) = 9.05$), a similar age-dependent drop was not found with the perikaryon volume (postnatal day 25: 1399 ± 761 *vs* postnatal day 29: 1452 ± 845 µm^3, $p > 0.05$, Student's *t*-test). When compared across the frequency map in A1, low-frequency cells were typically smaller than the high-frequency cells (perikaryon postnatal day 25: low- 2094 ± 804 vs high- 2630 ± 967 µm^3, $p < 0.0001$; postnatal day 29: low- 1870 ± 805 vs high- 2142 ± 856 µm^3, $p < 0.01$; two-way ANOVA, $F(1,1) = 0.002$). No similar frequency-dependence in cell size was found in the IC suggesting that the development of neurons in the IC is likely more mature than in A1.

3.2. Effects of sound exposure on perikaryon and nuclei

Because of the multi-dimensional complexity of results (i.e., A1 *vs* IC, postnatal day 25 *vs* postnatal day 29, low *vs* high frequency, control *vs* exposed), first is presented a global view in Fig. 4 without segregating the results from the low and high frequency regions, and then are shown in greater details the frequency-dependent changes in Fig. 5.

Sound exposure (for either 3 or 7 days) may or may not produce a change in cell size (Fig. 4). The change, when occurred, was invariably an enlargement (perikaryon and nucleus showing same/different degrees of expansion). During the same observation time window (postnatal day 25–29) the sound-induced enlargement was typically found in parallel with a normal developmental change in cell size, which was a drop in size (e.g., see Fig. 4 lower panels: nucleus volumes in the control groups). Furthermore, such enlargements often led to a reduced slope of the regression line calculated between volumes of the perikaryon and nucleus (Fig. 6; $p < 0.0001$, F-test; Supplementary Fig. 6, Supplementary Table 2).

After sound-exposure for 3 days (postnatal day 25), a small increase in cell volume was found only in A1 (Fig. 5 left panels; perikaryon, an increment of ~8% on group basis, or 5.5 ± 6.6% per animal basis; for details of alternative statistics on individual animals here and in other parts of this paper, please see Supplementary Table 3). This sound-exposure effect was only found in high-frequency neurons (control 2630 ± 967 *vs* exposed 2844 ± 1049 μm^3, $p < 0.005$; Student's *t*-test).

After exposure for 7 days (postnatal day 29), the sound-induced enlargement became greater and extended to all frequency neurons in both the cortex and midbrain (Fig. 5). This volume change was present both in perikaryon and nucleus (Fig. 4) and was much larger than that found on postnatal day 25 (A1 perikaryon: control 2060 ± 850 *vs* exposed 2728 ± 1015 μm^3, an increment of ~32% on group basis, $p < 0.0001$, or 29.6 ± 3.5% per animal basis; nucleus: control 602 ± 235 *vs* exposed 805 ± 273 μm^3, an increment of ~34% on group basis, $p < 0.0001$, or 32.0 ± 4.1% per animal basis; IC perikaryon: control 1452 ± 845 *vs* exposed 2421 ± 1.345 μm^3, an increment of ~67% on group basis, $p < 0.0001$ or 76.1 ± 17.3% per animal basis; nucleus: control 322 ± 168 *vs* exposed 555 ± 217 μm^3, an increment of ~72% on group basis, $p < 0.0001$ or 64.1 ± 8.0% per animal basis; all Student's *t*-tests). Using our sampling criteria, more high-frequency neurons were sampled from the deep layers of A1 (in both the exposed and control groups), for reason that is unclear to us. Related to this, sampled high-frequency cells in A1 were larger than those in the low-frequency region (postnatal day 29 perikaryon: high- 3033 ± 1113 *vs* low- 2269 ± 673 μm^3, $p < 0.0001$; nucleus: high- 901 ± 281 *vs* low- 659 ± 185 μm^3, $p < 0.0001$; Student's *t*-test). However, between the exposed and control A1, sampled neurons did not differ in terms of their cortical depths (Supplementary Fig. 7), excluding the possibility that a sampling bias had produced the observed sound effects. Despite a difference in cell size, the sound-exposure effects from layer III to V appeared similar.

We also compared the mean cell sizes obtained from individual animals (rather than comparing pooled data from the individual groups), and found similar sound-exposure effects on the nucleus and perikaryon volumes measured on postnatal day 29 (7 days sound-exposure). Since the repeated measures for each animal were reduced to a single value, the sound-exposure effect became less significant (still down to $p < 0.05$, Wilcoxon rank sum test; Supplementary Table 4). Additional statistics done using three-way analyses of variance containing one repeated factor also showed that the observed effects of sound exposure were highly significant on postnatal day 29, and were not due to contributions by variations in individual animals. Details of the statistical analyses are shown in Supplementary Text 1.

Between A1 and IC, we found clear differences in the sound effects (Figs. 4 and 5). First, regarding the time course, IC neurons

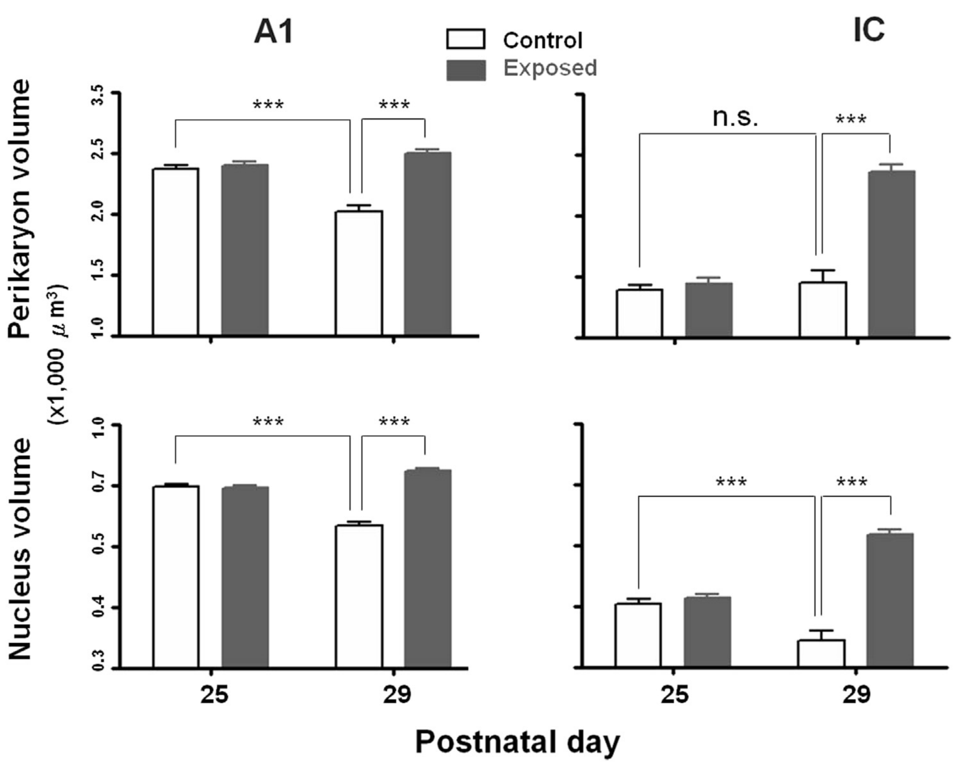

Fig. 4. Summary of the volume data (mean and SD) on perikaryon and nucleus in the control and sound-exposed groups. Here data from the high- and low-frequency regions were pooled together and analyzed for comparison in A1 and IC (mean ± SD; ***$p < 0.0001$, Student's *t*-test). Note clear enlargement of both volumes in IC on postnatal day 29. No similar change appeared on postnatal day 25. See also Fig. 5.

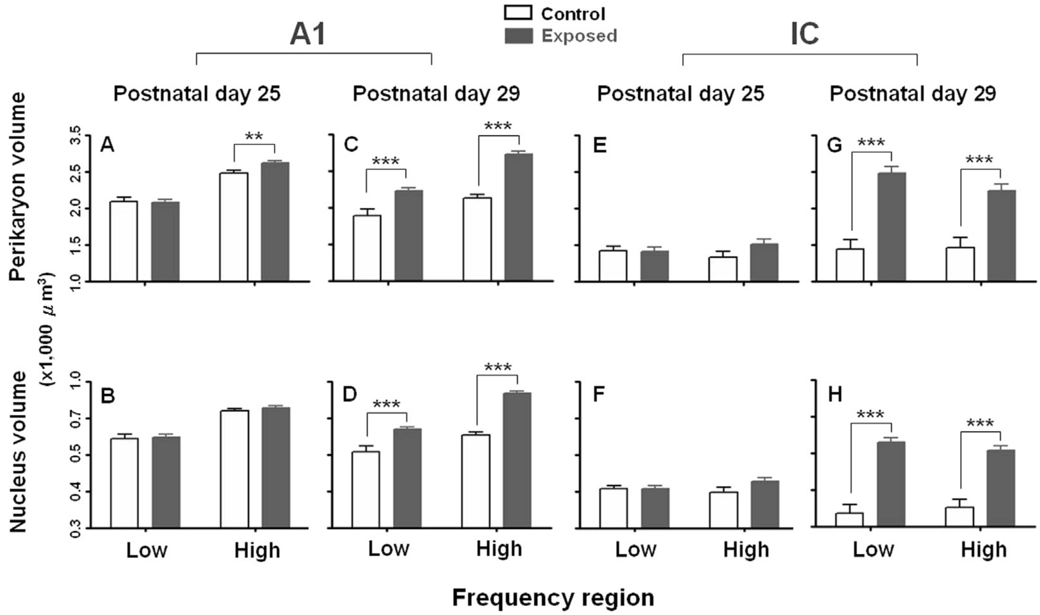

Fig. 5. Comparison of changes in perikaryon and nucleus with the high- and low-frequency regions analyzed separately (mean ± SD; $^{**}p < 0.001$, $^{***}p < 0.0001$, two-way ANOVA). Note the only sound-induced change found on postnatal day 25 was with perikaryon in the high-frequency region in A1 but not in the IC. Note in A1 and the IC the sound-effects on postnatal day 29 appeared different between the high- and low-frequency regions.

showed an enlargement on postnatal day 29 but not on postnatal day 25. Second, regarding frequency-dependence, A1 neurons showed a greater enlargement in the high-frequency region (42% increment, *vs* 21% in low-frequency, on group basis), while the opposite was found in the IC (56% increment in the high-frequency *vs* 81% in the low-frequency, on group basis). Third, IC neurons, which were smaller in size (perikaryon: IC 1563 ± 943 *vs* A1 2500 ± 992 μm^3, $p < 0.0001$, Student's *t*-test on group basis), showed sound-exposure effects that were proportionally greater than those in A1.

In contrast, no sound-exposure changes in cell size were found in any subdivisions of the CN, regardless of which frequency region examined (Fig. 7A). Similarly no changes were found in the visual midbrain (Fig. 7B).

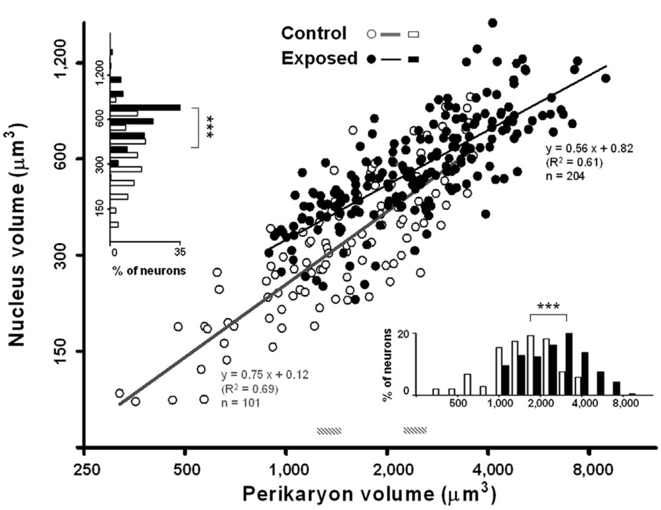

Fig. 6. The relationship between perikaryon and nucleus volumes in the IC of the control and exposed groups (showing results of postnatal day 29). Each symbol represents a neuron, and the volume relationship was fitted by linear regression. Neurons in Supplementary Fig. 4 were chosen with sizes near the mean values (bottom hatched bars) ($^{***}p < 0.0001$, Student's *t*-test).

While we were able to estimate the cortical layers from which the neurons were sampled, we were unable to determine the exact cell types based only on the soma shape and cell size. Furthermore, there is no simple way in the toluidine blue stained sections to limit the sampled cells to a single type. Similar uncertainty about cell types concerns the subcortical structures we studied (auditory and non-auditory).

4. Discussion

4.1. Paradoxical enlargement of high frequency neurons in A1

Our principal finding was that the cortical change in the neuronal size, induced by sound exposure in the prescribed postnatal period (week 4), does not reflect a simple activity-driven change that has occurred earlier in the brainstem. If the change were driven solely by neural over-activity evoked by the exposing tone, one would expect a greater change in the 4-kHz regions (or low-frequency cells). Paradoxically, on postnatal day 25, perikarya in the high-frequency region of A1 showed a slight sound-induced enlargement (together with a concomitant change in the slope of the regression line calculated between the volumes of perikaryon and nucleus), whereas no change was not found in the IC. This apparent effect on expanding high-frequency neurons in A1 became clearer towards postnatal day 29. In fact, an additional experiment done after completion of the study also confirmed similar changes occurred on postnatal day 27 (i.e., after 5 days of sound exposure, Supplementary Fig. 9). Results strongly suggested that the driving force on neuronal enlargement observed in the high frequency region of A1 is only indirectly linked to the sound-evoked over-activity of low frequency neurons.

That sound-induced plastic changes are marked and persistent in the cortex, but subtle and transient in the IC has been reported by Miyakawa et al. (2013). Their electrophysiological findings in the IC (multiple unit recordings), which were obtained after 7 kHz repetitive tone stimulation, revealed only a small expansion in the bandwidth of frequency tuning but no best frequency (BF) clustering around the exposing tone. In an earlier experiment with

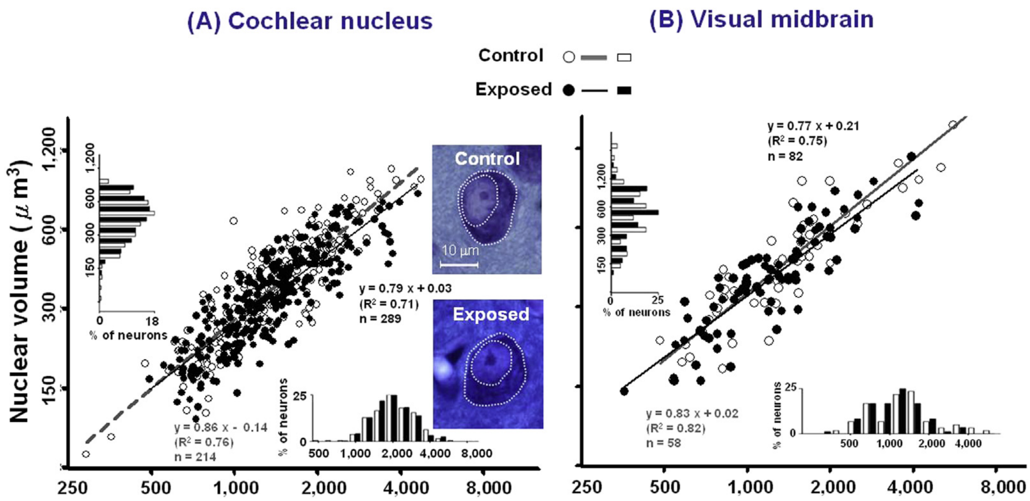

Fig. 7. The relationship between perikaryon and nucleus volumes for the CN (A), and visual midbrain (superior colliculus) (B). Note minimal changes after sound exposure on postnatal day 29. Captions are otherwise the same as in Fig. 6.

single unit recordings in the IC, Oliver et al. (2011) reported BF clustering following exposure to a 14 kHz repetitive tone, with additional complex changes in frequency response area. The discrepancy in results between Oliver et al. (2011) and Miyakawa et al. (2013) is likely related to differences in experimental procedures such as the choice of exposing tone (14 vs 7 kHz) and the recording method (single vs multiple units). Hence, the relationship between the cortical and subcortical changes could vary across different studies. In the literature on plasticity, it is not uncommon to find a discrepancy in results following early sound exposure, as the plastic changes are extremely sensitive to technical differences in methodology. For example, presenting an FM tone (3–5 kHz) or a steady tone (4 kHz) during early postnatal period produced different effects on BF clustering in the rat IC, with steady tone being a more powerful stimulus (Poon et al., 1990; Poon and Chen, 1992). In any event, that cortical change in the neural response to sound could occur with or without a parallel change in the subcortical structure that is consistent with our finding of different patterns of cell expansion between A1 and the IC.

4.2. Marked enlargement of neurons in both low- and high-frequency regions of the IC

In contrast to the paradoxical change at the high frequency region in A1, the marked enlargement of neurons in the low-frequency region of the IC is more consistent with sound-induced over-activity. It is conceivable that at this ‘auditory hub’ differential influences of the ascending and descending systems sum to produce larger enlargement of neurons, and this change is more comparable across frequency regions. Such a summed effect is supported by the extraordinary large cell-enlargement (67–72%, postnatal day 29). Such cell enlargements were not due to sound-induced changes in the overall size of the neural structure being studied, as the gross anatomical measurements of the IC or the brain were similar between the control and exposed groups (Supplementary Fig. 10).

In electrophysiological studies, tone exposure not only produced BF clustering in IC and expansion of laminae around the exposing frequency, but also produced marked changes in response property (threshold elevation) extending to a wide range of frequencies (Oliver et al., 2011). This is consistent with our observation of cell size expansion throughout the low and high frequency regions in the IC.

4.3. Difference between changes in perikaryon and nucleus volumes

The expansion of the perikaryon in A1 following sound exposure for 3 days was found earlier than that of the nucleus. This finding is consistent with different developmental changes between perikarya and nuclei in the control group, i.e., with the nuclear size continued to drop while the perikaryon size had likely stopped shrinking (e.g., IC, postnatal day 29). Results suggest that the mechanisms for regulating nucleus and perikaryon volumes are different and this difference should be taken into consideration in the comparison of their sound-induced changes.

4.4. Interaction between normal development and sound-effects during postnatal week 4

During our observation window (postnatal week 4), the volume of perikarya or nuclei is regulated dually by: (a) the ongoing development and (b) the sound-effect. In the control A1, cell size (both perikaryon and nucleus) showed an age-dependent drop during this week. This finding is consistent with an ongoing maturation process that involves a temporal drop in the mean cell size (Nixdorf-Bergweiler, 1998). Across the tonotopic map, low-frequency cells were found to be smaller. The finding of smaller cells in the low-frequency region is consistent with the maturation process that starts first in the low-frequency region before expanding later to high-frequencies (Lippe and Rubel, 1985; Rubel and Fritzsch, 2002). In the IC, although the nuclear volume shrank from postnatal day 25–29, a similar age-dependent drop did not apply to the perikaryon. Results are consistent with (a) an earlier development in the IC followed by a later development in A1, and (b) the development of perikaryon size precedes that of the nucleus (Lippe and Rubel, 1985; Rubel and Fritzsch, 2002).

4.5. Possible implication of the descending auditory system and tinnitus

The finding of cell expansion in the IC and A1 that occurred basically around the same time, if not preceded by the cortical change, is likely related to the strong inter-connections between the two neural centers. Besides ascending pathways, there are extensive descending connections from A1 to IC (Saldaña et al., 1996). Auditory cortex is well known to modulate spectral selectivity of lower auditory nuclei (Yan and Sug, 1996). Electrical stimulation at local sites of IC could shift frequency tuning of neurons nearby and this effect is abolished by cortical inactivation, suggesting that IC stimulation involves or goes through the cortex in order to produce the effects (Zhang and Suga, 2005). Using tone exposure, A1 neurons show a critical period for frequency-tuning in postnatal week 3 (Zhang et al., 2001). During the following week (week 4), neuronal sizes in A1 continued to drop along its development. This period of week 4 is special for sound-induced plastic changes, as loud sound exposure to young rats during this week can lead to audiogenic seizure in adulthood (Saunders et al., 1972). It is conceivable that during week 4, given the weakened plasticity in ascending auditory pathways, the less-known descending auditory system may still be plastic. Sounds, typically much louder than that used in this experiment, are known to cause cochlear damages leading to tinnitus, which is typically perceived as high-pitch sounds (Adjamian et al., 2009). Over-activity, especially in the high-frequency region of the cortico-collicular pathway is implicated in the pathogenesis of tinnitus (Eggermont, 2008; Norena and Eggermont, 2003). It is conceivable that prolonged sound exposure even to a moderate level during this time period could have led to tinnitus-like perception in our animals through actions of the descending auditory system. This possibility is supported by our preliminary observation of acoustic pinna reflexes, which showed an elevated threshold of ~15 dB lasting up to 4 months in the sound-exposed rats. This observation is consistent with a masking effect of tinnitus on soft sounds. Future experiments on the descending auditory system of sound-exposed animals could provide evidence for its involvement in tinnitus.

5. Conclusions

The effects of early sound-exposure on expanding neuronal sizes are different between the cortical and subcortical structures. The paradoxical changes at the high-frequency region of A1 in particular cannot be explained by sound-induced over-activity at the low-frequency region with peripheral origins. Other structures for example the descending auditory system could be involved. While a clear expansion of cell size was found in both the A1 and IC at day 29, a slight expansion is found in the high frequency region of A1 at day 22, suggesting the changes could be synchronized between A1 and IC, if not slightly led by the cortex. The present results do not however exclude the possibility that other functional changes occur at different time course along the auditory pathways. Limited by the technical approach (e.g., 7 μm sections), the present study failed to reveal sound-driven cell enlargements in smaller neurons, or plastic changes that might have occurred in other parts of the brain that had not been examined. These techniques and results can potentially be used as a springboard to studies of activity dependent plasticity.

Acknowledgments

We thank Dr Iain Bruce for reading the manuscript and Maria Chiu for comments. Supported by NSC, Taiwan, grants 99-2320-B-006-020, 100-2923-006-001, 101-2911-I-006-511, and GACR P303/11/J005, P304/12/1342.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.heares.2014.05.005.

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