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Effect of the environment on the dendritic morphology of the rat auditory cortex

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Abstract

The present study aimed to identify morphological correlates of environment-induced changes at excitatory synapses of the primary auditory cortex (A1). We used the Golgi-Cox stain technique to compare pyramidal cells dendritic properties of Sprague-Dawley rats exposed to different environmental manipulations.

Sholl analysis, dendritic length measures, and spine density counts were used to monitor the effects of sensory deafness and an auditory version of environmental enrichment (EE). We found that deafness decreased apical dendritic length leaving basal dendritic length unchanged, whereas EE selectively increased basal dendritic length without changing apical dendritic length. On the contrary, deafness decreased while EE increased spine density in both basal and apical dendrites of A1 layer 2/3 (LII/III) neurons.

To determine whether stress contributed to the observed morphological changes in A1, we studied neural morphology in a restraint-induced model that lacked behaviorally relevant acoustic cues. We found that stress selectively decreased apical dendritic length in the auditory but not in the visual primary cortex. Similar to the acoustic manipulation, stress-induced changes in dendritic length possessed a layer specific pattern displaying LII/III neurons from stressed animals with normal apical dendrites but shorter basal dendrites, while infragranular neurons (layers V and VI) displayed shorter apical dendrites but normal basal dendrites. The same treatment did not induce similar changes in the visual cortex, demonstrating that the auditory cortex is an exquisitely sensitive target of neocortical plasticity, and that prolonged exposure to different acoustic as well as emotional environmental manipulation may produce specific changes in dendritic shape and spine density.

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Keywords

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Introduction

The morphological and physiological characteristics of the central nervous system are the result of a combination of genetic and environmental factors (Bartoletti et al., 2004). Several studies report anatomical and physiological consequences of exposure to a sensory enriched environment (EE) (Diamond et al., 1964; Volkmar and Greenough, 1972). Many studies report the effects of EE on synaptic transmission in the hippocampus (Artola et al., 2006; Duffy et al., 2001; Foster and Dumas, 2001; Irvine and Abraham, 2005) as well as in the prefrontal cortex (Kolb and Gibb, 1991; Seib and Wellman, 2003) and in the parietal cortex (Leggio et al., 2005b). Comparatively less information is available on the effects of different types of environment on the auditory cortex.

In spite of representing the most abundant neocortical type of neuron and in spite of their varied functions, pyramidal neurons of the neocortex display a remarkable degree of similarity in their dendritic arborization pattern, among different brain areas and even between different cortical layers: their cell bodies are surrounded by a dense arborization of basal dendrites with multiple proximal branches, accompanied by a single apical dendrite projecting towards the pial surface and eventually branching as it enters layer I (Larkman and Mason, 1990). This stereotyped shape allows to perform systematic morphological analysis possible regardless of the variability in the details of individual neuronal morphology. Although the functional implications of the peculiar morphology of pyramidal neurons are not known, it is likely that apical and basal dendrites correspond to different circuit inputs producing specific contributions to pyramidal cell excitability and long term synaptic plasticity (Cauller et al., 1998; Dudman et al., 2007).

Using a previously developed model of auditory environmental enrichment (EE), we demonstrated large increases in surface auditory evoked potentials (AEPs), in the number of action potentials recorded in the auditory cortex (Engineer et al., 2004), and in the degree of auditory gating (paired pulse depression) recorded with both epidural and intracortical electrodes (Percaccio et al., 2005). We also found electrophysiological evidence that glutamatergic synapses in layer II/III of the auditory cortex are selectively enhanced by a protocol of auditory enrichment (Nichols et al., 2007), demonstrating that the auditory cortex is a sensitive target for neural plasticity. A question remains open on whether different environmental conditions may affect the morphological properties of excitatory neurons in the auditory cortex, and whether these modifications are paralleled or shared by other sensory cortices. In this study we addressed this question by testing the effect of four environments: a) an enriched auditory environment with behaviorally relevant sounds, b) a standard environment with sounds without behavioral relevance, c) the absence of sounds in a noise-induced deafness rat model, and d) a non-auditory environmental manipulation consisting of a restrain-induced stress rat model.

The Golgi-Cox staining technique was used to reveal morphological features and compare rats raised with a specific behavioral protocol with their littermate controls. We found that the auditory cortex is a particularly sensitive target for non-auditory environmental manipulations, and that environmental manipulations at opposite ends of the sensory intensity exert neurotrophic pressures paralleling the extent of their sensory input, producing distinctive environment-dependent morphological patterns.

Methods and Materials

All methods and procedures were in accordance with guidelines set by NIH for Ethical Treatment of Animals and received the approval of the University Committee on Animal Research at the University of Texas at Dallas and at the Universidad Catolica del Norte, Chile. Efforts were made to minimize the number of animals used and their possible discomfort.

Enriched environment and deafness groups

Each litter of 14-26-day-old Sprague–Dawley rats used in the study was split into two groups: one group was subjected to a specific environmental condition while the other littermates were housed under standard housing conditions as respective control groups. One set of animals was exposed to an enriched auditory environment while the other group was subjected to a deafening protocol.

Standard Environment

Rats were housed in groups of two or three for eight weeks, in a 26 × 18 × 18 cm-large cage, and exposed to sounds that included vocalizations from 30-40 other similarly housed rats. Rats in standard as well as all other environments received food and water *ad libitum*, were on a reverse 12-h light/dark cycle provided with constant temperature and humidity, and were exposed to sounds of cleaning, feeding, and room traffic.

Enriched Environment

The enriched environment was similar to that used by Engineer et al., and Percaccio et al. (Engineer et al., 2004a; Percaccio et al., 2005b). After weaning, Sprague-Dawley rats were housed for five weeks in a room separate from the main rat colony within a large cage (45 × 76 × 90 cm) which had four levels linked by ramps. Rats entering a level elicited a unique sound by passing through hanging chains and wind chimes hung over the entrance of each level. A rat stepping on two of the three ramps triggered magnetic switches at the bottom of the ramps which activated different tones (2.1 or 4 kHz). A motion detector set off an electronic chime when rats neared the water source. Rats on the exercise wheel evoked a tone (3 kHz Piezo-electric speaker) and activated a small green light emitting diode with each rotation. Each movement-activated sound had unique spectral and temporal features that provided behaviorally meaningful information about the location and activity of other rats in the cage.

Rats were exposed to 74 randomly selected sounds every 2-60 s from a CD player, seven of which triggered a pellet dispenser (Med Associates, St. Albans, VT, USA) to release a sugar pellet to encourage attention to the sounds. The sounds included simple tones, amplitude-modulated and frequency-modulated tones, noise burst, and other complex sounds (rat vocalizations, classical music, rustling leaves, etc.). The rewarded tracks included interleaved tones of different carrier frequencies (25-ms long, 4-,5-,9-,12-,14-, and 19-kHz tones with interstimulus intervals ranging from 50 ms to 2 s) and frequency modulated sweeps (1 octave up or down in a 140- or 300-ms sweep with interstimulus intervals ranging from 80 to 800 ms) for 24h/day. All sounds were <75 dB SPL, provided 24 h a day and spanned the entire hearing range of the rat (1–45 kHz). Rats reach sexual maturity at 5-6 weeks, so after four weeks of an enriched environment, a vasectomized male rat was added to the cage to encourage natural social interactions appropriate for the age.

Deafening Protocol

The deafening protocol was similar to the procedure used by Lorito et al. (Lorito et al., 2006). Twenty-six day-old rats were anesthetized with Sodium Pentobarbital (32.5mg/kg IP)

and body temperature was maintained at 37°C. In a double-walled sound attenuating chamber, anesthetized rats were subjected to 4 hours of continuous white noise (108 dB), band-pass filtered (1-16 KHz), and played through loudspeakers placed 5 cm from the animal's ears. Following exposure, animals were allowed to recover from anesthesia and placed into their respective housing condition for 5 weeks.

To test the effectiveness of the deafening protocol we measured the startle response before and after noise exposure. Auditory brain stem responses were also measured, 5 weeks after the noise exposure, immediately before rats were sacrificed.

Auditory Evoked Potential Responses

Rats were anesthetized with sodium pentobarbital (65 mg/kg IP) and body temperature was maintained at 37°C. Epidural auditory evoked potential recordings were made after inserting a tungsten electrode (300 kΩ) through a small hole made in the cranium overlying the auditory cortex, determined by anatomical landmarks. White noise bursts (25 ms duration, 75 dB spl) were presented in an electrically shielded sound attenuating booth. Sound production and signal acquisition were performed via Tucker-Davis System 3 hardware and Open-EX software suite. All recordings were performed by the same experimenter blind to the animal hearing condition.

Acoustic Startle Response

Animals were tested in a specially designed acoustic dampening booth for validating the effectiveness of the deafening protocol. Rats were placed in a 20 cm^3 wire mesh cage that rested on a startle platform (Lafayette Instrument Co.) that constantly recorded level of activity via a piezoelectric transducer. Platform and cage were located in a double walled, 67 cm^3 particleboard booth. Acoustic foam 5 cm thick lined the walls of the inner booth, except for on the floor and on the door. Startle stimuli were delivered through an RP2.1 Tucker-Davis station via an Optimus Bullet Horn Tweeter mounted roughly 20 cm above and to the side of the center of the cage. The speaker was calibrated in each booth using an ACO Pacific microphone (PS9200-7016) from various locations within the mesh cage to approximate the movement of the rat's head. Performance was video monitored for each training session through a video camera mounted above the cage in each booth.

Startle stimuli consisted of 65 dB, 75 dB, 85 dB, 95 dB, or 102 dB white noise bursts 50 ms in duration presented once every 15-45 seconds (30 seconds on average). Response waveforms were collected from the startle platform within 500 ms of the noise presentation, sampled at 10 kHz through an RP2.1 (Tucker-Davis Technologies) and processed using MATLAB.

Restraint stress protocol

Littermate rats (Sprague Dawley; 180-200 g, ~ 50 days old at the start of the experiment) were housed in groups of two or three under a 12/12 light/dark cycle (lights on at 7:00 A.M.) with ad libitum access to food and water in a temperature-controlled room. Rats were randomly assigned to a control group ($n = 7$), and a stress group ($n = 7$). Control animals were housed in separate cages and rooms and not subjected to any type of experimental stress. Restraint-stressed rats were placed in a plastic rat restrainer (6 cm diameter × 12 cm long; 6 cm diameter × 20 cm long as the rats grew) in their home cages for 2 h daily, beginning at 10 A.M.-noon for 15 consecutive days. Restraint occurred during the dark phase of the light cycle.

The following additional parameters were measured to monitor the overall effects of the stress paradigms: percentage gain in body weight (net change in weight after experiment ×

100/weight at the beginning of experiment), anxiety level as determined by performance in the elevated plus maze, and relative adrenal weight (wet weight of adrenal glands in mg × 100/body weight in grams).

Spontaneous motor activity

Twenty-four hours after completion of the stress protocol each rat was individually analyzed in the following order: spontaneous motor activity and elevated plus-maze. First, each rat was placed during 30-min into a Plexiglass cage (30 × 30 × 30 cm) located inside a soundproof chamber. The floor of the cage was an activity platform connected to an electromechanical counter (Lafayette Instrument Co, Lafayette, IN, USA). The spontaneous motor activity was measured in number of counts generated by the electromechanical counter (counts/30 min).

Elevated Plus-Maze

Each rat was individually placed in an elevated plus-maze, consisting of two open arms (50 × 10 cm each), two closed arms (50 × 10 × 20 cm each) and a central platform (10 × 10 cm), arranged in a way so that the two arms of each type were opposite to each other. The maze was elevated 1 m above the floor. At the beginning of each trial, animals were placed at the center of the maze, facing a closed arm. During a 5-min test period, we recorded: a) the number of open arm entries, b) the number of closed arm entries, c) the time spent in open arms, and d) the time spent in closed arms. Entry into an arm was defined as the animal placing all four limbs onto the arm. The maze was wiped clean with 5% ethanol solution after each trial. All trials were conducted between 10 A.M. and 2 P.M.

Morphological data analysis

Animals were euthanized under deep anesthesia with sodium pentobarbital. Brains were removed quickly and processed using FD Rapid GolgiStain™ kit (FD Neuro Technologies, Inc., Ellicott city, MD, USA). The Golgi technique only stains a small percentage of neurons and is a potential source of variability in the number of neurons stained, dendritic length, and number of dendritic spines. For this reason this study was conducted by comparing results from brains from treated vs. untreated offspring from same birth litter in the same experiment, where the two samples were processed using same solution, incubation times and experimenter, in order to minimize intra-sample variability. Coronal sections were cut at 150 μm on a sliding cryostat (Microm, Walldorf, Germany). Sections were collected serially, dehydrated in absolute alcohol, cleared in xylene, and coverslipped. Slides were coded before quantitative analysis, and the code was revealed only after the analysis was completed. The rules for the selection of the neurons for morphologic analysis are specified below.

Morphological data analysis for restraint stress protocol

Although most neurons studied in the stress and littermate control rats were from layer II-III primary auditory cortex, part of the study included analysis from the same layer in the visual cortex as well as from layer V-VI in both auditory and visual cortex, for comparison. The morphometric analysis of the pyramidal neurons was restricted to those located between bregma −3.8 mm and interaural 5.2 mm in the primary auditory cortex (area Te1), and between bregma −6.3 mm and interaural 2.7 mm in the primary visual cortex (area Oc1). Pyramidal neurons were defined by the presence of a basilar dendritic tree, a distinct, single apical dendrite, and dendritic spines. Neurons with somata in the middle third of sections were chosen to minimize the number of truncated branches. We chose only well-differentiated pyramidal neurons, which showed more tertiary or basal dendrites. Pyramidal neurons from layers V-VI of our study were characterized by the thinner apical trunk, which

tapers to a fine diameter and terminates, without forming a terminal arbor, before reaching layer 1 (Harris et al., 1996; Larkman, 1991). On the other hand, pyramidal neurons from layers II-III were characterized by a terminal arbor in layer 1 (Harris et al., 1996). The experimenter selected at random 10 layer II-III pyramidal neurons and 10 layer V-VI pyramidal neurons from the primary auditory cortex (5 from each hemisphere), and as many pyramidal neurons from the visual cortex. Neurons fulfilled the following selection criteria: (1) presence of un-truncated dendrites, (2) consistent and dark impregnation along the entire dendritic field, and (3) relative isolation from neighboring impregnated neurons to avoid overlap. This number of neurons selected for each animal has been previously used (Harris et al., 1996). In order to reduce experimental bias, the experimenter had no knowledge of the source of the sample. Camera lucida tracings (500X, BH-2, Olympus Co., Tokyo, Japan) were drawn from selected neurons and then scanned (eight-bit grayscale TIFF images with 1200 dpi resolution; EPSON ES-1000C) along with a calibrated scale for subsequent computerized image analysis. Custom-designed macros embedded in NIH Image 1.6 software were used for morphometric analysis of digitized images. In each selected neuron the dendritic length and the number of branch (bifurcation) points were determined. The measurements from selected neurons were averaged to get a single value of dendritic length and the number of branch points from each rat (stress, $n = 7$; control, $n = 7$), and group means were obtained from each subject.

Morphological data analysis for environmental enrichment and deafness

Sholl analysis (Sholl, 1953) was performed over 5 neurons from each hemisphere (10 neurons per animal), from the auditory cortex of 6 animals in each condition for deafness and EE. Each group was compared with an equal number of littermate controls. For Sholl Analysis a series of concentric rings at 20 μm intervals (calibrated to the final magnification of 400X) was transferred to a transparency sheet. Using the center of the soma as the reference point, the total number of intersections between rings and each dendritic branch was counted separately for apical and basal dendrites. Dendritic length was measured and averaged for each animal and then for each condition. A map wheel was calibrated to the final magnification of 400X and the dendritic length of apical and basal dendrites was measured separately.

Spine density was also measured and averaged for each animal and then for each condition. Spine density was measured by tracing the length of a dendritic terminal (~ 20 μm) at 2000X final magnification using a Camera Lucida system, computing the length of the dendritic segment and counting the number of spines along the segment. Spine density was quantified for apical and basal dendritic terminals of each sample separately.

Statistical analysis

The percentage gain in body weight, relative adrenal weight, spontaneous motor activity, and anxiety were analyzed by a Student's paired t-test. The morphological studies were analyzed using the Mann-Whitney *U*- test, or the F-test wherever appropriate, depending on whether or not data were normally distributed. Results were expressed as the mean ± s.e.m. values. A mínimum criterion of probability level $P < 0.05$ was accepted as indicative of significant difference.

Results

Deafness and environmental enrichment

We compared dendritic morphology of layer II/III neurons in deafened animals, and in animals which underwent an auditory version of an enriched environment, with their respective littermate controls. Animals were deafened with a noise-exposure protocol similar

to what has already been described (Lorito et al., 2006). The effectiveness of our environmental enrichment protocol was demonstrated in several earlier studies (Engineer et al., 2004b; Nichols et al., 2007; Percaccio et al., 2005a).

Startle and Auditory Brainstem Responses (ABR)—In order to assess the effectiveness of our deafness protocol we compared the auditory brainstem response (ABR) to a 75 dB, 20 ms-long white-noise burst in control animals vs. animals that underwent the deafening protocol (see Methods). ABR in treated animals showed no evoked response, compared to the typical shape of the ABR from a control rat (example in fig. 1A, mean in fig. 1B, n = 7 each). As a behavioral test for verifying animal deafness we measured the startle response of treated vs. control animals (see Methods). Sounds of 80 dB or louder evoked a significant larger response in control vs. treated animals, confirming the effectiveness of the deafening protocol (fig.1C, n = 7 each group, asterisks (*) indicates statistical differences at $P < 0.05$, unpaired *t*-Student test).

Dendritic Morphology

Dendritic Length: Dendritic lengths of basal and apical dendrites were compared in deafened and EE rats vs. their respective controls. While basal dendrites of deafened animals did not display any difference compared to control ($P = 0.295$, Mann-Whitney *U* test, n = 6 and n = 5, respectively) apical dendrites of deafened animals displayed a significantly shorter length ($P < 0.007$, Mann-Whitney *U* test, n = 6 and n = 5, respectively). Conversely, basal dendrites of EE rats were significantly longer than control ($P < 0.0001$, Mann-Whitney *U* test, n = 6) while no differences were detected between apical dendrites in EE vs. control ($P = 0.926$, Whitney *U* test, n = 6). Examples of layer II/III neurons and their camera lucida drawing are shown in fig. 2A for deafened animals and in fig. 2B for animals in EE. Control indicates littermate untreated rats for each condition (to each left). Graphs in fig. 2C and fig. 2D compare mean ± s.e.m. of deafened and EE rats with their respective controls in basal and apical dendrites. Table 1 reports the direction of the changes in dendritic length following deafening or EE.

Sholl analysis: Sholl analysis was performed on apical and basal dendrites separately for all neurons in each group. Radius of the shell in μm and number of intersections per shell were plotted on the x- and on the y-axis, respectively. While basal dendrites of deafened rats were no different from their respective controls (fig.3A, left), EE rats basal dendrites showed a significantly increased number of intersections over the full range of radii when compared with their littermate controls (fig. 3A, right). Sholl analysis was also performed on apical dendrites of treatment rats and compared with control rats from the same litter. The irregular shape of the Sholl graph compared to basal dendrites is due to their larger shape variability with respect to the center of the apical dendrite. The number of intersections in three radii/bins of deafened rats was significantly lower compared with the corresponding bins for the control animals (fig. 3B, left). Deafened rats (absence of stimulation) exhibit a trend with fewer intersections for distances larger than 250 μm (fig. 3B, left). No differences were detected at any radii between EE and their controls (fig. 3B, right).

Spine density: In order to test whether changes in spine density accompanied and paralleled changes in dendritic arborization, spine densities were measured and averaged from 6 rats in EE and deafened animals and compared with their littermate controls. All comparisons indicated statistically significant differences. Both basal and apical dendrites from deafened animals had much lower spine density than control (examples in fig. 4A, left; mean ± s.e.m. in the graphs in fig. 4B, left: basal dendrites: $P < 0.001$; apical dendrites: $P < 0.001$, Whitney *U* test, n = 6). On the contrary, both basal and apical dendrites of EE rats displayed a higher spine density compared to their littermate controls (example in fig. 4A, right; mean ± s.e.m.

in the graphs in fig. 4B, right: basal dendrites: $P < 0.001$; apical dendrites: $P < 0.001$, Whitney *U* test, n = 6). This analysis suggests that a change in spine density does not necessarily come about with a change in dendritic length, while changes in dendritic length and morphology might occur without changes in spine density.

Effects of chronic restraint stress

While it is natural to assume that morphological changes in the auditory cortex might be brought about by modality-specific conditions, it is less obvious to predict whether non-auditory conditions might specifically affect the morphology of the auditory cortex. We addressed this question by using restraint stress. We evaluated the effectiveness of the paradigm with behavioral and physiological parameters, and compared the morphology in pyramidal cells from restrained rats with their littermate controls. In order to check whether this non-modality specific treatment, also affected non-auditory areas, in parallel, we studied the morphology of neurons of the primary visual area in the same sample.

Physiological stress markers—We analyzed the effects of chronic stress in body and adrenal weights. Statistical analysis revealed a significant reduction in percentage body weight gain after 15 days of stress (stress: 5.0 ± 1.6%, $n = 7$; control: 11.3 ± 1.3%, $n = 7$; $p < 0.05$). In addition, stress caused a significant adrenal hypertrophy (relative adrenal weight, stress: 15.4 ± 2.2%, $n = 7$; control: 10.1 ± 2.0%, $n = 7$; $p < 0.05$). These results are consistent with earlier studies.

Spontaneous motor responses and anxiety—Figure 5*A* shows the effects of chronic restraint stress on spontaneous motor activity. Student's t-test revealed that stress did not affect the motor activity of the rats (control: 1064 ± 98, $n = 7$; stress: 1024 ± 115, $n = 7$). A significant reduction in both percentage of open-arm entries (stress: 23 ± 6.9%, $n = 7$; control: 41 ± 2.6%, $n = 7$; $p < 0.05$) and percentage of time spent in open arms (stress: 9 ± 3.0%, $n = 7$; control: 23 ± 2.3%, $n = 7$; $p < 0.01$) was found in the elevated plus maze that is indicative of an enhanced anxiety response in the stressed animals (Fig. 5*B*).

Dendritic morphology of the pyramidal neurons from the auditory and visual cortex—The morphology of layer II/III neurons of the primary visual cortex did not display any differences between control and stressed rats as shown in the representative photomicrographs of Golgi-impregnated pyramidal cells (above) and corresponding camera lucida drawing (below) in fig. 6A. On the contrary, basal dendrites of auditory cortex neurons of layer II/III showed a significant decrease in dendritic length as well as in branch number, although apical dendrites appeared unchanged, as shown in the example in fig.6B. The bar graphs in fig. C and D report the mean ± s.e.m. of dendritic length and branch number for visual and auditory cortex respectively, asterisks (*) represent statistical significance.

We also investigated the layer specificity of these morphological changes by repeating similar measurements in neurons from infragranular layers V/IV. Similar to the previous finding in layer II/III, we found no significant morphological differences between control and stressed rats in neurons of the visual cortex (example in fig. 7A), while we detected a selective withdrawal of apical dendrites in auditory cortex LII/III neurons (example in fig. 7B). Mean ± s.e.m. of dendritic length and branch number are reported in the bar graphs in fig. 7C and D, for visual and auditory cortex, respectively (open bars represent control, filled bars, stressed rats, asterisks (*) represent statistical significance as above). Table 2 reports the direction of the changes in dendritic length following restrain. These data suggest that auditory cortex but not visual cortex neurons are sensitive to restrain-induced stress, and that the changes of dendritic patterns are layer-specific.

Discussion

We compared Golgi-stained primary auditory cortex dendrites in neurons from young adult rats subject to different environmental conditions with their littermate controls. The environmental manipulations tested, which included deafening, an auditory version of EE, and repeated sessions of physical restraint, produced measurable changes in the dendrites of auditory cortical neurons, indicating that the primary auditory cortex is capable of relatively rapid and massive reorganization following changes in sensory input. The observed changes in dendritic structure following physical restraint with no obvious alteration of the acoustic environment suggest that sensory stimulation is not the only environmental determinant of AC neuronal dendritic morphology.

Excitatory synapses in the auditory cortex are particularly sensitive to sensory deprivation (Lu et al., 2008). Our deafening protocol produced a withdrawal of apical dendrites and a decrease in basal and apical spine density, indicating that the absence of auditory stimulation induced neuronal atrophy in the auditory cortex. The decrease in spine density of pyramidal cells after deafness or sensory deprivation is in agreement with previous studies (Lu et al., 2008; McMullen and Glaser, 1988; McMullen et al., 1988a; McMullen et al., 1988b). Different from our results, these studies did not reveal changes in dendritic length following deafness. This might be a consequence of the use of different species, different age of the lesion, or the monoaural vs. binaural site of the lesion. Our results and the specific changes in the morphology of non-pyramidal cells following deafness (McMullen et al., 1988b) suggest multiple avenues of morphologic alterations concurring to shift cortical balance between excitation and inhibition to the advantage of the latter, in deafness. Concurrent cause for the loss of dendritic spines could be an abnormal corticotropin metabolism (Chen et al., 2008) associated with a decrease in the baseline of social interactions (Silva-Gomez et al., 2003).

On the contrary, we observed that EE significantly increases apical dendritic length and spine density in pyramidal neurons in the auditory cortex. Also in this case our data concur with previous studies in other brain areas suggesting that an enriched sensory environment has a dendrotrophic and spinogenic effect. The corresponding changes in dendritic shape and length, and in spine density may be caused by increased concentration and gradient in neural growth factors.or other neurotrophic molecules synthesized in an activity-dependent manner. BDNF enhances neurogenesis induced by exposure to an enriched environment and may play a critical role in linking a complex environmental stimulation with adult neurogenesis (Rossi et al., 2006).

Similar results were shown in the hippocampus, where EE produces an enhancement of dendritic branching and spine density (Bindu et al., 2007), as well as in pyramidal neurons of the parietal cortex (Leggio et al., 2005a), and in somatosensory cortex (Johansson and Belichenko, 2002) as well as in several other brain areas (Hickmott and Ethell, 2006; Sala et al., 2008; Schreiner and Winer, 2007).

This effect resembles the action of learning, which induces an increase in spine density in the hippocampus and in the motor cortex (Kleim et al., 2004; Leuner et al., 2003). The corresponding changes in dendritic shape and length, and in spine density may be caused by increased concentration and gradient in neural growth factors or other neurotrophic molecules synthesized in an activity-dependent manner (Gordon et al., 2006; Johansson and Belichenko, 2002; Tanaka et al., 2008). Spike-timing dependent plasticity (STDP) determines the sign of synaptic changes (Froemke et al., 2005), and is likely acting by modifying synaptic structure (Froemke et al., 2007; Harvey and Svoboda, 2007). Since dendrites represent the major site of synaptic connectivity, the pattern of dendritic branching

and the dendritic arbor distribution are determinant factors of neuronal signal integration (Grudt and Perl, 2002; Koch and Segev, 2000; Lu et al., 2001).

While our observations prompt to a positive correlation between sensory stimulation and spine density, they do not explain the details nor the specificity of the basal vs. apical dendritic changes, alternatively present or absent following different conditions. For instance, it is not clear what mechanism underlies the deafness-induced decreases in apical but not basal dendritic length, in spite of the fact that basal dendrites presumably receive the bulk of thalamocortical input. A reason for this could be that the arborization of the apical dendrites in supragranular layers could be more prone to a decrease in postsynaptic excitation compared to the more proximal dendrites which receive a substantial amount of synthesized proteins around the perykarial region. Similarly, our data fail to explain why different environmental conditions induced spine density increases or decreases without changes in dendritic length or shape.

In our stress paradigm, animal restraint induced dendritic reorganization in pyramidal neurons in supragranular as well as infragranular layers in the AC, as reported in previous studies in other brain areas (Cook and Wellman, 2004; Murmu et al., 2006; Radley and Morrison, 2005; Radley et al., 2005). A recent study showed a reduced apical dendritic spine motility in the visual cortex compared to auditory and somatosensory cortices (Majewska and Sur, 2006). The fact that rewiring visual input to auditory cortex fails to decrease auditory cortex spine motility (Majewska and Sur, 2006) suggests that a high degree of neural plasticity is a distinctive property of the AC. The absence of dendritic reorganization in the visual cortex confirms previous studies on other brain areas, suggesting that the auditory cortex is more prone to stress-induced plasticity than the visual cortex. This is not surprising in view of the fact that the auditory and the visual cortical systems differ considerably in their connectivity with the amygdalar complex (McDonald, 1998). In fact the primary auditory cortex, receiving input from the medial geniculate nucleus of the thalamus, projects efferents to higher order auditory areas which in turn process complex stimuli including biologically relevant sounds (Brugge JF, 1985; Kishan et al., 2008; Mascagni et al., 1993; Shi and Cassell, 1997). Secondary and tertiary rat auditory cortices send monosynaptic efferents to the lateral amygdala (McDonald, 1998). In addition to this, the lateral amygdala receives direct afferents from the primary auditory cortex (LeDoux et al., 1991; Romansky LM, 1993). On the contrary, visual information received in the primary visual cortex is sent to higher-order visual processing (McDonald, 1998) areas, from where visual information is furthered to the temporal cortex before being processed by the lateral amygdala (McDonald, 1998). Henceforth, more direct interactions of the amygdala with auditory cortices, compared to that between amygdala and visual cortices, could explain why stress is more likely to produce structural changes in the auditory cortex than in the visual cortex, paralleling our findings at mesencephalic levels (Dagnino-Subiabre et al., 2005).

One possibility to explain the selectivity of the stress-induced atrophy in A1 and not in V1 is that A1 atrophy is indirectly produced by stress-related plasticity in the basolateral amygdala (Vyas et al., 2002; Vyas et al., 2004). Evidence indicates that an intact basolateral amygdala is essential for the development of associative neuronal plasticity in the medial geniculate nucleus during aversive learning (Maren et al., 2001), and might also influence plasticity at cortical levels like the auditory cortex. Chronic stress-induced hypertrophy of basolateral amygdala pyramidal-like neurons may produce an increase in the local excitatory activity of the lateral amygdala. The increase in excitatory activity associated with changes in intracellular calcium concentration may induce neural plasticity (Johnston, 2004) and neurotoxicity (Sapolsky, 2000) and morphologic changes in the medial geniculate efferents to the amygdale, which in turn could spread to the upper levels of the auditory pathway,

including the primary auditory cortex. Conversely, neurons of the primary visual cortex, which are target of efferents of the lateral geniculate nucleus of the thalamus, do not project to the lateral amygdale (McDonald, 1998), whose stress-induced pyramidal-like cell hypertrophy might less likely affect visual cortical neurons.

Another possible explanation of our results is that the stress-related dendritic atrophy in the primary auditory cortex was induced by an increase of glucocorticoid receptors in these cortical areas, as proposed previously for the stress-induced hippocampal atrophy (Sapolsky, 2000). Glucocorticoids, secreted during stress, may contribute to neuronal remodeling by affecting neuronal energetics, glutamate accumulation, and calcium-dependent degeneration (Sapolsky, 2000).

It would be important to assess whether environmental-induced changes in the cortex are primary or are rather induced by parallel changes at the earlier relays in the anatomical auditory pathway (i.e. thalamus, colliculus, or other). Although this question is of great theoretical interest it would be difficult to answer to it with anatomical studies, due to the large extent of the back-projections from the auditory cortex (Winer, 2005).

We speculate that the dendritic changes documented here in pyramidal neurons of the primary auditory cortex provide a broad, dynamic, scaffolding for adaptation to the auditory environment, but could also make the animal susceptible to negative symptoms associated with stress and other sensory-related disorders. Comparable alterations could be induced by psychosocial stress in humans and might have a role in the development of depressive disorders.

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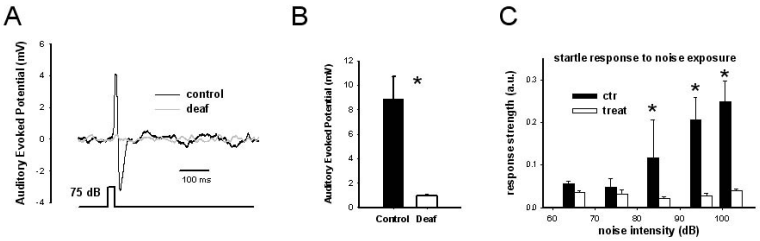

Figure 1. Auditory evoked potentials

A: average of 7 consecutive traces in control and 7 traces in the deafened group. B AEP Peak 1 mean + s.e.m. for control (black) and for the deafened group (empty) bar. The two groups differ statistically ($p < 0.001$, $n = 7$ each). C: startle response in control (black bars) or deafened animals (empty bars). Responses for the control group are stronger than responses for the treated animals for test sound levels ≥ 80 dB.

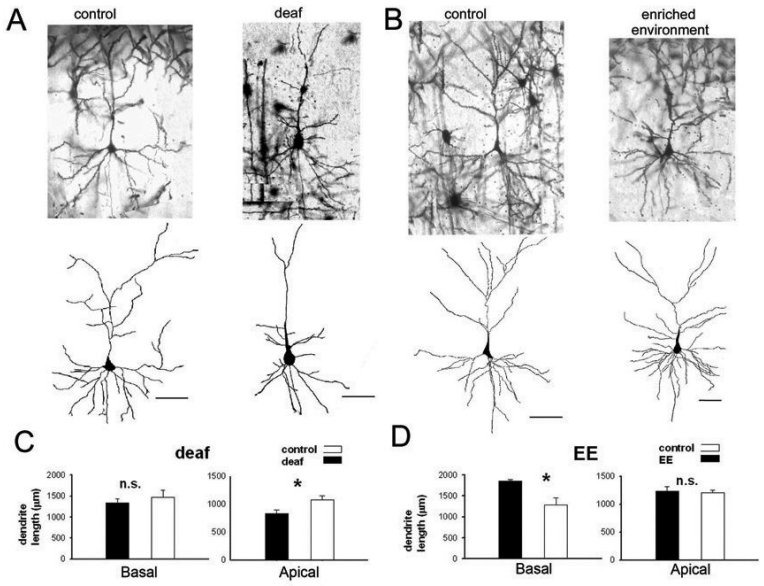

Figure 2. Effects of deafness (left) or EE (right) on neuronal morphology of layers II-III auditory cortex

A: Photomicrographs and camera lucida tracings of representative Golgi-impregnated pyramidal neurons from layers II-III in control vs. littermate deafened rats. All scale bars, 20 µm. B and C: Morphometric analysis of neurons shown in A. Deafness significantly reduced the total apical dendritic length of pyramidal neurons compared with control rats (n = 6 animals each group) while leaving basal dendritic length unchanged. EE produced a different picture in which dendritic length of basal neurons was increased, while there were no change in apical dendrites (n = 6 animals each group).

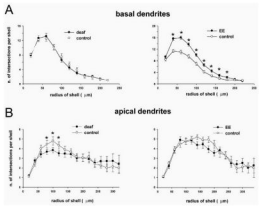

Figure 3. Sholl analysis of EE and deafened vs. their littermate controls

A: Sholl analysis of basal dendrites. Deafness does not change dendritic shape whereas EE increases the number of intersections per radius of shell. B: Sholl analysis of apical dendrites. Deafened rats induces a decrease in the number of intersections per shell while EE animals do not display any change. These results are in agreement with data in fig. 5, which shows changes in dendritic length.

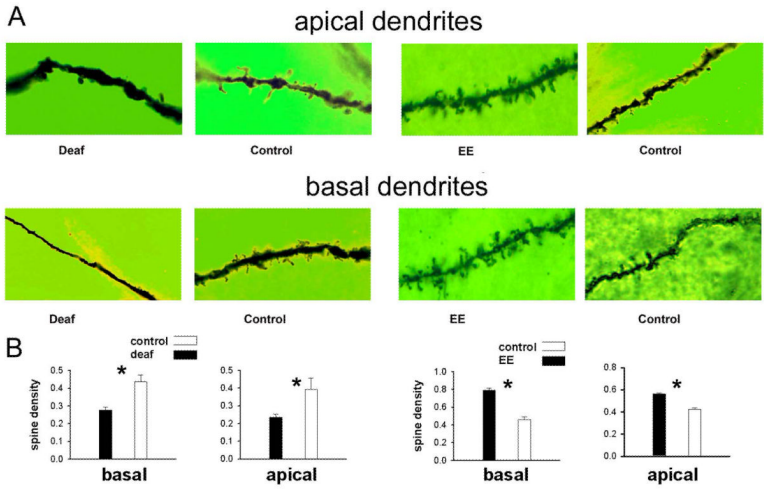

Figure 4. Dendritic spine density is an indicator of sensory activity

A: representative examples of photomicrographs of apical and basal dendrites (above and below, respectively), of deafened vs. littermate control (left), and EE vs. littermate control (right). B: Average values for spine densities in deafened vs. control (left) and in EE vs. control (right) in basal and apical dendrites. The data show that spine density is a function of the level of activity of the auditory cortex.

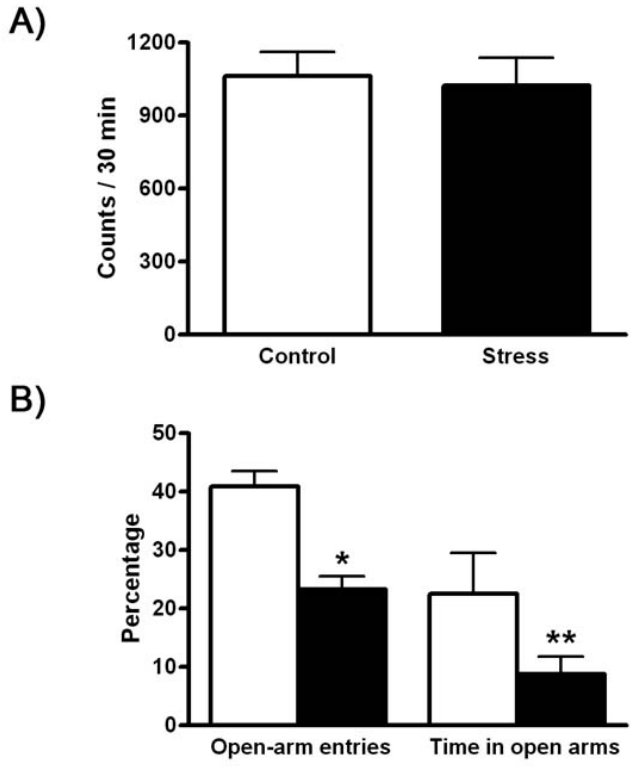

Figure 5. Behavioral tests in the experimental animals

A: Effect of chronic restraint stress on spontaneous motor responses and anxiety in rats. Chronic stress did not affect the motor activity of the experimental animals. Bars represent the total spontaneous motor activity in a 30-min observation period. B: Stress increases anxiety in the elevated plus maze. Fifteen days after restraint, stressed rats show decreases in the time inside and in the entries onto open arms of the elevated maze, indicating an increase in anxiety. Data represent the means ± SEM of 7 rats for control group and 7 for stress group. Comparison was made by a Student's t-test ($p < 0.001$ compared with control group).

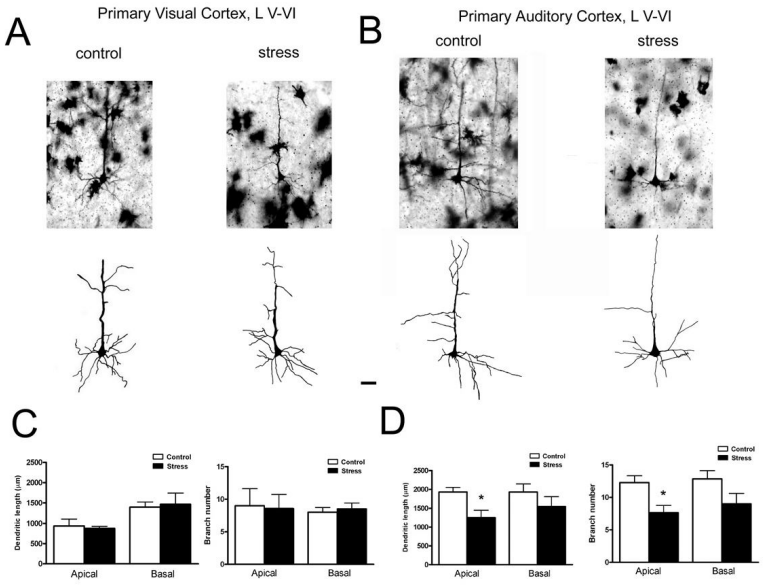

Figure 6. Chronic restraint affects differentially neuronal morphology in layer II-III of the auditory vs. visual cortex

A: Photomicrographs and camera lucida tracings of representative Golgi-impregnated pyramidal neurons from layers II-III of the primary auditory and visual cortex in control and stressed rats. Scale bar, 20 μm. B and C, Morphometric analysis of neurons shows in A. After 10 days of restraint stress (n = 7), the total basal dendritic length and the branch number of pyramidal neurons from layers II-III of the primary auditory cortex was significantly reduced compared with control rats (n = 7). There were no stress-induced changes observed (stress; n = 7; control; n = 7) in total dendritic length or branch number of pyramidal neurons from layers II-III of the primary visual cortex.

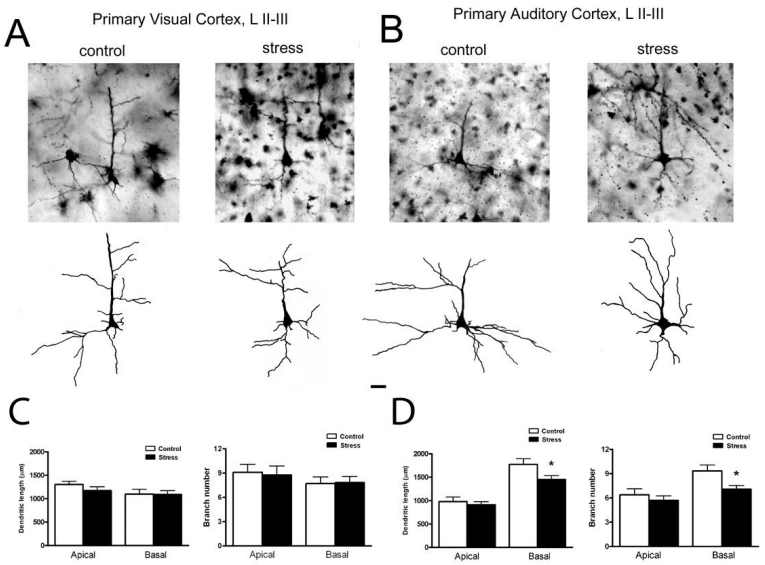

Figure 7. Different effects of chronic stress on the neuronal morphology of the layers V-VI of the auditory and visual cortex

A and B, Photomicrographs and *camera lucida* tracings of representative Golgi-impregnated pyramidal neurons from layers V-VI of the primary auditory (A) and visual cortex (B) in control and stressed rats. Scale bar, 20 μm. C and D: Morphometric analysis of neurons shows in A and B. After 10 days of chronic restraint stress ($n = 7$), the total apical dendritic length and the branch number of pyramidal neurons from layers V-VI of the primary auditory cortex was significantly reduced compared with control rats ($n = 7$). There were no stress-induced changes observed (stress: $n = 7$; control: $n = 7$) in total dendritic length or branch number of pyramidal neurons from layers V-VI of the primary visual cortex.

Table 1

Effect of different behavioral conditions on the dendritic length of neurons of layer II/III of the auditory cortex. Arrow down (↓) indicates a statistically significant decrease, arrow up (↑) indicates an increase, no ch. indicates no change.

Dendritic length L2/3 AC1	**EE**	**Deaf**
Basal	↑	no ch
Apical	no ch.	↓

Table 2

effect of restraint on the dendritic length of neurons of the auditory cortex. As above, arrow down (↓) indicates a statistically significant decrease, arrow up (↑) indicates an increase, no ch. indicates no change.

Dendritic length restrained rats AC1	**L 2/3**	**L 5/6**
Basal	↓	no ch.
Apical	no ch.	↓