3aPP12. Prolonged low-grade noise exposure induces aging-like functional and structural changes in cortical auditory pathways

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Age-related impairments in the primary auditory cortex (A1) include poor tuning selectivity, neural desynchronization and degraded responses to low-probability or "oddball" sounds. These changes have been for the most part attributed to reduced inhibition in the aged brain. Since many of these changes can be partially reversed with auditory training, it has been speculated that they might not be purely degenerative but might rather represent negative plastic adjustments to noisy or distorted auditory inputs. To test this hypothesis, we exposed young adult rats to low-grade broadband noise for 6 weeks and then compared the effect of this exposure on several aspects of A1 function and structure. We found that the impact of noise exposure on A1 tuning selectivity and responses to oddball tones was almost indistinguishable from the effect of natural aging. These changes were paralleled by alterations in A1 inhibitory interneuron populations in the exposed group. Moreover we found that noise exposure reduced the anatomical and functional connectivity of A1 to downstream cortical fields. These results support the hypothesis that age-related changes in cortical auditory pathways might have a strong activity-dependent component, making them potentially preventable and reversible.
ABSTRACT

Age-related impairments in the primary auditory cortex (A1) include poor tuning selectivity, neural desynchronization and degraded responses to low-probability or “oddball” sounds. These changes have been for the most part attributed to reduced inhibition in the aged brain. Since many of these changes can be partially reversed with auditory training, it has been speculated that they might not be purely degenerative but might rather represent negative plastic adjustments to noisy or distorted auditory inputs. To test this hypothesis, we exposed young adult rats to low-grade broadband noise for 6 weeks and then compared the effect of this exposure on several aspects of A1 function and structure. We found that the impact of noise exposure on A1 tuning selectivity and responses to oddball tones was almost indistinguishable from the effect of natural aging. These changes were paralleled by alterations in A1 inhibitory interneuron populations in the exposed group. Moreover we found that noise exposure reduced the anatomical and functional connectivity of A1 to downstream cortical fields. These results support the hypothesis that age-related changes in cortical auditory pathways might have a strong activity-dependent component, making them potentially preventable and reversible.

INTRODUCTION

A decline in auditory perceptual abilities is an almost universal aspect of natural aging. The vast majority of aging rats develop A1 abnormality that relate directly to auditory perceptual deficits experienced by aging humans. These include poor tuning selectivity, neural desynchronization and an impaired response to low probability or “oddball” tones [1-4]. While degenerative changes in the peripheral auditory system often contribute to these impairments, abnormalities in temporal aspects of the coding of auditory signals seem to arise primarily in the central auditory system [5, 6]. These have been documented in auditory brainstem nuclei and the primary auditory cortex [4, 7, 8]. Furthermore, age-induced changes appear to be amplified centrally by reduced inhibition thought to result from a “negative” plastic compensation to peripheral degeneration [8, 9]. How peripheral degeneration might induce these changes remains however elusive. A reasonable possibility is that this occurs in response to a chronic, progressive reduction in the signal-to-noise ratio of auditory signals transmitted by aging middle and inner ear. Showing that this process is mostly the fruit of activity-dependent plasticity and therefore not purely degenerative, would bear the interesting implication that it might also be reversible due to its inherently plastic nature. We tested this hypothesis here by exposing young adult rats (5 months old) to continuous low-grade broadband noise for a period of 8 weeks. At the end of this noise exposure, we examined several aspects of A1 sound processing in this young-exposed group and compared them to A1 responses in healthy aged rats (24 months). We also documented in both groups the density of parvalbumin positive (PV+) cells, a population of inter-neurons known to be reduced by aging [9]. Finally, we examined the functional and anatomical integrity of the interconnection between A1 and the frontal cortex. Studies performed in humans suggest that such long cerebral pathways are negatively affected by the aging process [10]. Overall, our results indicate that the effect of low-grade broadband noise exposure on A1 is almost indistinguishable from the effect of natural aging.

EXPERIMENTAL PROCEDURES

All experimental procedures used in this study were approved by the Montreal Neurological Institute and McGill University Animal Care Committees.

Mapping of A1

Twenty-two Long-Evans rats aged 5-24 months were anesthetized with a ketamine/xylazine/acerpromazine (65/13/1.5 mg/kg, i.p.). Supplemental doses of dilute anesthetic were given as required to maintain the rat in an areflexic state while preserving a physiological breathing rate. The cisterna magnum was drained of cerebrospinal fluid to minimize cerebral edema. The skull was secured in a head holder leaving the ears unobstructed. The right temporalis muscle was reflected, auditory cortex was exposed and the dura was resected. The cortex was maintained under a thin layer of silicone oil to prevent desiccation. Recording sites were marked on a digital image of the cortical surface. Cortical responses were recorded with 32-64 channels tungsten microelectrodes arrays (TDT, Alachua). The electrodes were lowered orthogonally into the cortex to a depth of 470-600µm (layers 4/5), where vigorous stimulus-driven responses were obtained. The neural signal was amplified (10,000x), filtered (0.3–3 kHz), and monitored on-line. Recordings in VO were achieved using linear

32ch Neuronexus probes positioned with a stereotaxic apparatus (Bregma 4.5mm, Lateral 1.2mm, Deep 4.0mm). Acoustic stimuli were generated using TDT System III (Tucker-Davis Technology, Alachua, FL) and delivered to the left ear through a free field calibrated speaker. A software package (OpenEx; TDT, Alachua, FL) was used to generate acoustic stimuli, monitor cortical response properties on-line, and store data for off-line analysis. The evoked spikes of a single neuron or a small cluster of neurons were collected at each site. Frequency-intensity receptive fields (RF) were reconstructed by presenting pure tones of 63 frequencies (1–48 kHz; 0.1 octave increments; 25ms duration; 5ms ramps) at eight sound intensities (0–70dB SPL in 10dB increments) to the contralateral ear at a rate of one tone stimulus per second. The stimuli used for the determination of responses to oddball tones were ten minute-long trains of 50 ms duration pips presented at 5 pulses per second at a sound intensity of 70 dB SPL. Each train had a commonly occurring frequency (standard) with a probability of occurrence of 90% and a pseudo-randomly distributed oddball frequency pips presented 10% of the time with no repetition. The two frequencies in the train had constant separation of 1 octave and were chosen so they would be contained within the RF of the recorded neuron and elicit strong reliable spiking responses. Electrical stimulation in A1 to elicit responses in VO were achieved using 32 channels microwire array covering the surface of A1 and lowered to a depth of 400 μm. A 5 μs biphasic pulse of 200 μA was delivered simultaneously in all channels while simultaneously recording in VO.

**Noise Exposure**

Young adult rats aged 5 months were housed for 8 consecutive weeks (24h/day, 7 d/w) in a sound attenuated chamber equipped with a speaker reproducing continuous broadband random noise covering 0.1-80 kHz and presented at 65 dB SPL. The noise stimulus was generate with custom MatLab routines and played back using an Apple computer via a MOTU UltraLite-mk3 Hybrid Interface sampling at 192 kHz.

**Tracer Studies**

To reveal axons connecting A1 and VO, a mixture of biotinylated dextran amine conjugated to Fluororuby (Dextran,Tetramethylrhodamine, 10,000 MW Lysine fixable, Invitrogen) and cholera toxin subunit B (CTB: # C-34775, Invitrogen) was injected in A1 with a Hamilton syringe positioned at a cortical depth of 500μm. A single mixture injection of the two tracers was made at a rate of 13 nl/second with a total volume of 2μl [20% v/v Fluororuby,5% v/v CTB]. After the injection, a silicone based polymer (Kwik Cast Sealant: #KWIK-CAST, World Precision Instruments Inc. Sarasota, FL) was placed on the cortex and the skin sutured. The rats then recovered and on day 7 were perfused, and their brain was fixed and sectioned as described below.

**Histology**

At the end of recording sessions, the rats received a high dose of pentobarbital (85mg/kg i.p.) and were perfused intracardially with saline followed by 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) at pH 7.2. Their brains were removed and placed in the same fixative overnight for further fixation and then transferred to a 30% sucrose solution, snap-frozen, and stored at -80°C until sectioning. The brains were cut in 40 μm sections on a freezing microtome. Sections were washed 3 times with PBS for 5 minutes and then 4 times with PBS GT for 15 minutes prior to being incubated overnight at room temperature in anti-PV (for anti-PV:#P3088; dilution: 1:10,000; Sigma Chemicals). The following day, sections were washed 4x15 minutes in PBS GT and incubated in secondary anti-sera (for PV+, Cy2:#715-545-151; dilution: 1:800; Jackson ImmunoResearch). For tracer studies sections were incubated first with a polyclonal anti-fluororuby (anti-FR) (for anti-FR: #A6397; dilution: 1:20,000; Invitrogen). The sections were then washed 4x15 minutes in PBS-GT and incubated in secondary antisera (Cy3(rabbit):#711-165-152; dilution: 1:800) for 45 minutes. Sections from young and aged rats were always processed together in pairs during immunostaining procedures to limit discrepancies related to antibody penetration, incubation time, and post-sectioning age/condition of tissue. A Zeiss LSM 510 Meta confocal microscope was used to assess fluorescence in the immunostained section in both injected and non-injected rats. Z-stacks were acquired from LSM 510 and reconstructed in 3D using Image J (Image J, Image Processing and Analysis in Java, NIH). Quantification of PV+ cells, and synaptic boutons were performed in Image J and MetaMorph imaging software(Molecular Devices Systems, Toronto, ON) respectively. All quantification was assessed in 300 to 400 μm wide A1 sectors extending from layer 1 till the underlying white matter.
RESULTS

Impact of Noise Exposure on Tuning Selectivity in A1

The average tuning curve width (BW10) was significantly increased by 7 to 27% in the noise-exposed group. The maximal effect was found for low frequency tuned (4.6 ± 0.3 octaves) neurons (1.27± 0.11 vs 1.01 ± 0.05; p < 0.001, t-test). We also found that BW10 was uniformly increased in the aged group, as previously reported in another strain of rats [4]. In the aged group, the BW10 was 9 to 43% greater, with the greatest effect also for low frequency tuned neurons (1.43± 0.11 vs 1.01 ± 0.05; p < 0.001, t-test). Of note, sound intensity threshold in A1 were not significantly altered after noise exposure (p>0.2).

**FIGURE 1.** Chronic noise exposure reduces tuning selectivity in A1 similarly to aging. (A) Representative cortical receptive fields recorded in A1 in young, young NE and aged groups. (B) Average tuning curve width (BW10, bandwidth 10 dB above threshold) by CF in all experimental groups. Young: n=4, neurons=205; Young NE: n=4, neurons =198; Aged: n=5, neurons=259 Values shown are mean ± s.e.m. *: P<0.05, **: P <0.001: t-test.

Impact of Noise Exposure on Responses to Low Probability (Oddball) Stimuli

We compared the effect of noise exposure and aging on rare stimulus detection in A1 by presenting trains of identical, repeated tones (standard) and introducing occasional deviant (oddball) frequencies in the background of these ‘distractors’ while recording neural activity in A1. Exponential functions were fitted to the normalized response rates to oddballs and standard tones in all experimental groups to obtain a quantitative measure of maximal suppression (asymptote, normalized units) of background tones and their separability from rare tones. Representative normalized responses to these tones are...
presented in Fig 2. We found in the noise-exposed group and as expected in the aged group, a significant reduction in the average suppression of responses to standard tones (Young vs noise-exposed mean standard asymptote of normalized response rate; 0.20±0.01 vs 0.38±0.05, p<0.001; Young vs aged mean standard asymptote of normalized response rate; 0.20±0.01 vs 0.36±0.07, p<0.01). No significant difference in the overall magnitude of responses to oddballs was found in either group (p>0.2). Overall, the effect of noise exposure and aging translated into a diminished response gap between standards and oddballs (asymptote difference between oddballs and standards; Young 0.43±0.06; Noise-exposed 0.21±0.08, p<0.01 relative to young; Aged; 0.22±0.06, p<0.01 relative to young).

Both Noise Exposure and Aging Reduce the Number of PV+ Cells in A1

Parvalbumin positive (PV+) cortical neurons are part of a group of inhibitory inter-neurons which play an important role in stimulus selectivity and novel stimulus detection in A1. These have previously been shown to be affected by aging [3, 11]. We examined the number of PV+ cells in A1 in our 3 experimental groups. We observed a 16% reduction in PV+ cells in A1 following noise exposure (p<0.05), which was not significantly different from the decrease seen in the aged group.

FIGURE 3. Impact of noise exposure on inhibitory interneurons in A1. (Left) High power photomicrographs demonstrating the density of parvalbumin (PV) immunoreactive neurons (pointed by arrow heads) in the young, young NE and aged groups. (Right) Quantitative analysis of the number of average number of PV+ cells per high power field in all layers of A1 in all experimental groups. Number of hemispheres examined: Young = 8, Young-NE =8, Aged=10. *: P<0.05, **: P<0.01: t-test. Error bars are s.e.m. Scale bar: 50 µm.
Noise Exposure Degrades Functional and Anatomical Connectivity Between A1 and the Frontal Field VO

To examine the impact of noise exposure on the interconnection between A1 and the frontal field VO we quantified the magnitude of the LFP response in the superficial cortical layers of VO following direct electrical stimulation in A1 (Fig. 4A) and measured the density of synaptic boutons of A1 axons reaching VO (Fig. 4B, see methods). VO is the only frontal cortical field receiving direct A1 projections and is involved in the discrimination of sensory stimuli during behavior [12, 13]. The average peak latency of the LFP response in VO was increased in both the noise-exposed group and the aged group compared to the young (Young: 85±7 ms, Young NE: 105±14 ms (p < 0.01, t-test), Aged 110±16 ms (p < 0.01, t-test)). A significant decrease in the average amplitude was also observed in the noise exposed group as well as the aged group (Young: 9.1±1.3 µV, Young NE: 7.3±1.1 µV, p < 0.05, t-test; Aged: 5.8±1.1 µV, p < 0.01, t-test). Noise-exposure resulted in a reduction of the density of synaptic boutons on A1 axon reaching VO in all layers (Young:0.135±0.0095, Young NE:0.109±0.0131, Aged: 0.080±0.0107, p<0.05).

**FIGURE 4.** Degraded anatomical and functional connectivity between A1 and the frontal cortex after noise exposure. (A). Average local field potential (LFP) responses in VO following electrical stimulation in A1. Note the reduced amplitude and increased latency of the first response in VO with aging and after noise exposure. (B) Number of synaptic boutons following anterograde tracer injection was quantified in VO. Young: n=3; Young NE: n=3; Aged: n=3. Scale bar= 10 µm. 10 *= P<0.05, **: P<0.01: t-test. Error bars are s.e.m.
CONCLUSION

We have shown here that prolonged exposure to low-grade broadband noise can induce functional and anatomical changes in A1 that mimic closely those caused by natural aging. These include a reduction in tuning specificity, poor detection of low-probability tones, a decrease in A1 PV+ cells and degraded connectivity between A1 and the frontal field VO. Since sound intensity thresholds in A1 were not affected by the noise exposure, we conclude that it is “noisy” neural activity in auditory pathways induced by the exposure, not cochlear or peripheral degeneration that is at the origin of these changes. This study has limitations however as it does not reveal if noise-exposure results in perceptual deficits similar to those previously described in aged rats [5, 14]. These results also do not indicate whether these changes would reverse after a return to a quieter environment. These ideas will be the subject of future experiments in the laboratory.

These experiments represent a first step in a series of experiments aimed at understanding the role of auditory experience on the emergence, prevention and remediation of age-related auditory decline. In theory, our findings could also apply to other sensory systems and ultimately unveil the still elusive mechanism involved in brain aging.

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