Age-related changes in the guinea pig auditory cortex: relationship with brainstem changes and comparison with tone-induced hearing loss

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Keywords: aging, auditory brainstem response, hearing loss, local field potential, presbycusis, tuning curves

Abstract
Elderly people often show degraded hearing performance and have difficulties in understanding speech, particularly in noisy environments. Although loss in peripheral hearing sensitivity is an important factor in explaining these low performances, central alterations also have an impact but their exact contributions remained unclear. In this study, we focus on the functional effects of aging on auditory cortex responses. Neuronal discharges and local field potentials were recorded in the auditory cortex of aged guinea pigs (>3 years), and several parameters characterizing the processing of auditory information were quantified: the acoustic thresholds, response strength, latency and duration of the response, and breadth of tuning. Several of these parameters were also quantified from auditory brainstem responses collected from the same animals, and recordings obtained from a population of animals with trauma-induced hearing loss were also included in this study. The results showed that aging and acoustic trauma reduced the response strength at both brainstem and cortical levels, and increased the response latencies more at the cortical level than at the brainstem level. In addition to the brainstem hearing loss, aging induced a 'cortical hearing loss' as judged by additive changes in the threshold and frequency response seen in the cortex. It also increased the duration of neural responses and reduced the receptive field bandwidth, effects that were not found in traumatized animals. These effects substantiate the notion that presbycusis involves both peripheral hearing loss and biological aging in the central auditory system.

Introduction
Aging impairs auditory function. In humans, quality of life is then dramatically affected especially because of reduced speech intelligibility, particularly in noise (Bergman et al., 1976; Crandell et al., 1991; Willott, 1991; Frisina & Frisina, 1997; Abel et al., 2000; Divenyi et al., 2005; Kim et al., 2006; Hwang et al., 2007). At least three main causes have been proposed for age-related declines in spoken language comprehension (CHABA, 1988; Crandell et al., 1991). Peripheral factors are obviously the first. Studies assessing peripheral deficits by auditory brainstem responses (ABRs) revealed that, in correlation with the level of age-related hearing loss, the response strength is reduced, the amplitude-intensity function is shallower, the latencies and interpeak intervals are prolonged, and the latency-intensity function is steeper (Cooper & Gates, 1991), but up to 80–90% in populations with co-occurring sensorineural loss (Stach et al., 1990). Aging provokes changes in neurotransmitter systems from the brainstem to cortex. In particular, a down-regulation of glycinergic and GABAergic inhibitory neurotransmission was observed (review in Caspary et al., 2008), possibly compensating for a partial peripheral deafferentation of auditory central nuclei. Finally, some age-related changes in speech understanding might stem from cognitive deficits (deficit in learning, memory, problem solving or reasoning), which can exacerbate difficulties in auditory processing and speech recognition (CHABA, 1988; van Rooij et al., 1989; van Rooij & Plomp, 1990, 1992; Gordon-Salant & Fitzgibbons, 1997; Fitzgibbons et al., 2006; Houtgast & Festen, 2008).

These three potential causes can be inter-related as presbycusis is potentially a complex state that reflects pathological changes along the entire auditory system (review in Canlon et al., 2010). For instance, age-related changes in the central auditory system may be both sequelae of peripheral loss and the results of aging (for reviews see Willott, 1991; Kiessling et al., 2003). Despite recent studies (e.g.
Hughes et al., 2009), the functional consequences in the auditory cortex of such intertwined pathologies are still largely unknown.

The present study had three main purposes. First, we quantified age-related changes in the auditory cortex in terms of the auditory threshold, response strength, bandwidth of tuning and response latency from electrophysiological recordings collected in aged guinea pigs. Second, to clarify the contribution of age-related changes occurring in the auditory cortex vs. those occurring at more peripheral levels, we compared several of these parameters with those derived from ABRs collected from the same animals. Third, to evaluate whether the potential relationships between cortical changes and brainstem changes are specific to aging, we compared the results obtained in aged guinea pigs with those obtained in adult guinea pigs submitted to acoustic trauma (pure tone 5 kHz, 120 dB, 2 h).

Materials and methods

Subjects

Three groups of adult (male and female) pigmented guinea pigs free of middle ear infection and exhibiting a normal Preyer reflex (Bohmer, 1988) were used as subjects. The animals were housed in a humidity- (50–55%) and temperature-controlled (22–24°C) facility on a 12 h/12 h light/dark cycle (light on at 07:30 h) with free access to food and water.

The group of young animals (n = 4) was composed of young adults (age 3–4 months, 490–590 g). These animals were able to reproduce but did not reach their adult weight; they should be considered as adolescents. The group of traumatized animals (n = 6) was composed of animals (age 10–12 months, 985–1210 g) with permanent hearing loss due to a single 2 h exposure to a traumatic sound at 2–3 months of age [binaural pure tone of 5 kHz at 120 dB sound pressure level (SPL)] (see Gourevitch et al., 2009). The ABRs of these traumatized animals were followed from a few days before the trauma to 45 days after the trauma to make sure that most of the hearing loss detected in these animals does indeed come from the trauma and not from aging. The aging group (n = 6) was composed of aged animals (age 36–42 months, 840–1070 g). Domestic guinea pigs can live 5–6 years, but in laboratory conditions they rarely live more than 4 years. Thus, the aged animals studied here were in the last 25% of their life span. All animals came from our own breeding colony. All procedures conformed to the European (86/609/EEC) and French (JO 887-848) legislation on animal experimentation (which are similar to those described in the Guidelines for the Use of Animals in Neuroscience Research of the Society of Neuroscience).

Audiometry

The protocol for ABR recording was detailed previously (Gourevitch et al., 2009). Briefly, 2 days before the experiment, hearing thresholds were evaluated from ABRs under isoflurane anesthesia (2–2.5%). ABRs were obtained for clicks (100 μs, alternating rarefaction and condensation clicks) and for tone bursts (10 ms, rise–fall time 2 ms) of different frequencies (0.5, 1, 2, 4, 5, 8, 16, 24, 32 kHz). Responses to clicks were only used to make sure that the subcortical electrodes and the speaker were correctly positioned. For each stimulus, responses were obtained by averaging 500–750 repetitions of each stimulus (rate 15 stim/s). The stored waveforms were examined by the two authors and the threshold was defined as the lowest level (dB re: 20 μPa) at which a clear waveform could be observed. For each averaged waveform, latency was taken at the positive extrema of wave III (P3) because the P3 wave showed the best signal-to-noise ratio among other waves and thus could be easily identified at any stimulation level, even close to threshold. The P3 wave obtained from monaural stimulation may be attributed to generators in the trapezoid body, close to the superior olivary complex (Wada & Starr, 1983a,b; Simha et al., 1988). The activity strength for ABR was estimated by the peak-to-peak amplitude between P3 and the following negative extrema of wave III (N3). The ABR latencies used in statistical tests were taken at 70 dB SPL in control conditions and at 85 dB SPL at most in traumatized and aged animals when thresholds were above 70 dB. Latencies were only taken at frequencies between 1 and 24 kHz because (i) waveform shapes were distorted by microphonic potentials at 0.5 kHz (Batra et al., 1986) and (ii) thresholds were too high at 32 kHz for traumatized and aged animals.

Hearing loss was assessed by comparing the ABR thresholds obtained in the present study with a set of ABR thresholds collected from 12 normal-hearing guinea pigs. The four young adult animals used in the present study were part of this reference group.

Animal preparation and recording procedures

Guinea pigs were anesthetized by an initial injection of diazepam (4 mg/kg, i.p.) followed by urethane (1.2 g/kg, i.p.). Additional doses of urethane (0.5 g/kg, i.p.) were systematically delivered when reflex movements were observed after pinching the hindpaw (usually once or twice during a recording session). Under this anesthetic, the strength of cortical evoked responses and the breadth of tuning were not significantly different from those obtained in undrugged animals (Massaux et al., 2004; Huertz et al., 2009). The number of supplementary doses was similar in the different groups. The body temperature was maintained at around 37°C by a heating pad throughout the experiment. The stereotaxic frame supporting the animal was placed in a sound-attenuating chamber (IAC, model AC1).

A large opening was made in the temporal bone and the dura mater was removed under microscopic control. The location of the primary field (AI) was estimated based on the pattern of vasculature and previous studies (Edeline et al., 1993; Manunta & Edeline, 1999; Wallace et al., 2000). Neuronal clusters [multiunits (MUs)] were recorded with an array of 16 tungsten electrodes (33 μm in diameter, impedance < 1 MΩ) (Tucker Davies Technologies) arranged in an 8 × 2 configuration with 0.25 mm electrode separation in a row and 0.5 mm separation between rows of electrodes. The array was oriented such that all electrodes were simultaneously touching the cortical surface. The recording depth was between 500 and 1000 μm, which corresponds to deep layer III and layer IV in the guinea pig auditory cortex (Wallace & Palmer, 2008). The signals were amplified 10 000 times (Tucker Davies Technologies, Medusa) and were then processed by an RX5 multichannel data acquisition system (Tucker Davies Technologies). Spikes were extracted from a 0.6 to 10 kHz band-pass-filtered signal and local field potentials (LFPs) from a 5 to 305 Hz band-pass-filtered signal. Thus, we simultaneously extracted spikes and LFP data for each of the 16 electrodes. At the end of the recording session, a lethal dose of pentobarbital (200 mg/kg) was administered to the animal.

Mapping of the auditory cortex

For each animal, during each recording session, the 16 electrode array was placed successively at two or three cortical locations to sample neuronal activity in the entire tonotopic field AI. Given the size of the array (2000 × 500 μm total area), when placed in a given position, it can cover at least four to five octaves of the tonotopic representation in all groups of animals. The first cortical location was systematically
chosen to reach the representation of low frequencies (< 1–2 kHz) in the AI along the large blood vessels of the Sylvian pseudo-sulcus (Wallace et al., 2000). The following locations targeted higher frequencies at the border between the AI and the primary-like dorsocaudal area. Thus, the sampling systematically covered the whole tonotopic map of the AI. This procedure is illustrated in Supporting Information Fig. S1, which shows an electrode array in the first position for an aged animal.

**Acoustic stimulation**

Acoustic stimuli were generated in Matlab and transferred to an RP2.1-based sound delivery system (Tucker Davies Technologies) and then to a speaker (Fostex FE87E). The speaker was placed at 2 cm from the guinea pig’s right ear, a distance at which it produced a flat spectrum (± 3 dB) between 140 Hz and 36 kHz. Calibration of the speaker was made by sending noise and pure tones to the speaker via the RP2, with the output of the speaker being recorded by a microphone (Bruel & Kjaer 4133) coupled to a pre-amplifier (Bruel & Kjaer 2169) and a digital recorder (Marantz PMD671).

The characteristic frequency and tuning properties of neurons were determined using 81, 97 or 129 gamma-tone frequencies, covering five (1.1–36 kHz), six (0.14–9, 0.28–18 or 0.56–36 kHz) or eight (0.14–36 kHz) octaves, respectively, presented at intensities between −5 and 95 dB SPL at crest, in 10 or 20 dB steps (85 and 95 dB were only used for animals with hearing loss). At a given intensity, each frequency was repeated eight times in pseudo-random order at a rate of 2.35 Hz. Tone pips had gamma envelopes given by: \( \gamma(t) = (t/4)^{2} \exp(-t/4) \), where \( t \) is time in ms. The duration of these tones over half-peak amplitude was approximately 15 ms, whereas the total duration of the tone was 50 ms.

**Data analysis**

Spectro-temporal receptive fields (STRFs) obtained from MUs were determined by constructing post-stimulus time histograms for each frequency with time bins of 1 ms. All spikes falling in the averaging window (starting at the stimulus onset and lasting 100 ms) were counted. Thus, STRFs were matrices of 100 bins in the ordinate (frequency) by the number of spikes divided by the time bin (1 ms) and by the number of stimulus repetitions. STRFs obtained from LFPs were computed by a similar procedure, except that the LFP waveforms (0–100 ms after stimulus onset) were averaged for each tone frequency. All STRFs were smoothed with a uniform 5 x 5 bin window.

At a given intensity, the best frequency (BF) of each MU- or LFP-based STRF was defined as the frequency-time bin where the highest activity was recorded. For each MU- or LFP-based STRF, peaks of significant response were identified using the following procedure: a positive peak in the MU-based STRF was defined as a contour of positive firing rate (MUs) above the average level of the baseline activity (estimated from the first 10 ms of STRFs at all intensities) plus six times the SD of the baseline activity. For LFPs, the same procedure was used: the threshold for peaks was the averaged baseline plus three times the SD of the baseline activity. For a given cortical site, the ‘threshold’ was defined as the lowest intensity at which a significant peak remained visible. The BF obtained at threshold was defined as the characteristic frequency of this site. For a given cortical site and a given intensity, several measures were extracted from the peaks. The total bandwidth was defined as the sum of all peak widths in octaves; the minimum and maximum contour times of all peaks taken together defined the ‘first spike’ and ‘last spike’ latencies respectively for spikes, and ‘first deflection’ and ‘last deflection’ for LFPs. The ‘response duration’ was the time difference between the first and last spike (MUs) or deflection (LFPs) latencies, the ‘peak latency’ (which was the P3 latency for ABRs) was the latency at the bin showing the highest response, and the ‘response strength’ (which was the P3–N3 amplitude range for ABRs) was the number of spikes in the peaks for MUs and the volume of the peaks for LFPs. Examples of ABRs, MU- and LFP-based STRFs obtained at several intensities in a young animal are presented in Fig. 1 (see also Supporting Information Fig. S2 for an old animal). The different measures described above are illustrated in this example. In the ABRs presented in Fig. 1A, the waves have a longer latency and lower amplitude with decreasing tone intensity. Figure 1B and C shows classical V-shaped tuning curves obtained by constructing MU- and LFP-based STRFs at several intensities. Note that several significant peaks may appear with decreasing intensity (e.g. see at 55 dB SPL in Fig. 1B). As previously described (Norena & Eggermont, 2002), MU- and LFP-based STRFs showed very similar patterns, but LFP-based STRFs were typically broader than MU-based STRFs (Fig. 1B and C).

For statistical tests, non-parametric analyses were preferred to parametric analyses, given the absence of a Gaussian hypothesis for the distribution of studied parameters. Unless otherwise specified, throughout the entire article, comparisons between two groups were carried out with bilateral Mann–Whitney U tests (Mann & Whitney, 1947). The risk level in one direction of the test was 2.5%. When parameter values were compared between the three groups, three tests were performed. In that case, the risk level in one direction was fixed at 2.5% divided by 3, i.e. at a value of 0.83% according to the Bonferroni correction. In the analyses provided in the Supporting Information (Figs S4 and S5), the results were split according to the BF into three frequency bands: below (0–2.5 kHz), around (2.5–10 kHz) or above (10–36 kHz) the trauma frequency (5 kHz). In that case, when parameter values were compared between the three groups of animals and for each of the three frequency bands, nine tests were realized. Hence, the risk level in one direction was fixed at 2.5% divided by 9, i.e. at a value of 0.28% according to the Bonferroni correction.

**Results**

For a given placement of the microelectrode array, not all electrodes provided physiological signals. Therefore, the total number of recordings analyzed here for the MU/LFP signals was 171/192, 106/198 and 198/298 for young, traumatized and aged animals, respectively. The counts for each analysis are reported in the Supporting Information (Table S1). The proportion of cortical sites exhibiting no spike-based STRFs (STRFs without significant patterns) or very low firing rates (< 0.06 spikes/s) was 17.8% in young animals; it was significantly greater in aged animals [38%; proportion test (Kanjii, 1999), \( Z = 4.98 \), \( P = 6 \times 10^{-7} \)] and in traumatized animals [57.4%; \( P = 5 \times 10^{-6} \) with the other two groups]. The proportion of cortical sites with no LFP-based STRF was 7.7% in young animals and 6.9% in aged animals (\( Z = -0.36, P = 0.72 \)) and significantly higher in traumatized animals (20.5%; \( P < 2 \times 10^{-7} \) with the other two groups).

In the following paragraphs, we will present threshold differences between the three groups, first at the brainstem level and then at the cortical level. Next, we will show that, in addition to threshold differences, the parameters quantifying the STRFs revealed striking differences between young and hearing-impaired animals but also two major differences between aged and traumatized animals.
Auditory brainstem response thresholds and cortical thresholds

Consistent with previous studies (Norena et al., 2003; Gourevitch et al., 2009), animals traumatized with a 5 kHz pure tone showed the largest hearing loss around and above the trauma frequency. The increased threshold was up to 60 dB SPL between 4 and 8 kHz (Fig. 2) but it was < 30 dB at 0.5 and 32 kHz. In contrast, aged animals showed a relatively flat hearing loss ranging from 15 dB at 32 kHz up to 40 dB at 1–2 kHz. The four young animals used here were in the range (from 2–24 kHz) or slightly better than the set of 12 healthy control animals (< 2 kHz and at 32 kHz).

Figure 3 provides the ABR thresholds and cortical thresholds for each animal. For the young animals (top row in Fig. 3A and B), the ABR thresholds of each animal (unbroken lines) were very close to those of the reference group (dotted lines); the cortical thresholds derived from the MUs (dots in Fig. 3A) or LFPs (dots in Fig. 3B) were only slightly higher than the ABR thresholds (except in the low frequencies for animal no. 20). For the traumatized animals (middle row in Fig. 3A and B), the ABR thresholds were much higher than the reference thresholds (unbroken lines vs. dotted lines) and the cortical thresholds from the LFPs were slightly above the ABR thresholds. For the aged animals (bottom rows in Fig. 3A and B), the ABR thresholds were higher than the reference thresholds but remained roughly parallel to them (unbroken lines vs. dotted lines). In this group, except for animal no. 5, the cortical thresholds were much higher than the ABR thresholds (dots vs. unbroken line).

The scattergram presented in Fig. 4 summarizes, for all animals, the extent of the ‘cortical hearing loss’ as a function of the brainstem hearing loss detected from ABRs. The brainstem hearing loss detected from the ABRs was defined by the difference between the ABR of a given animal and the reference ABRs (from the 12 reference animals).

Fig. 1. Individual examples of ABR, MU and LFP recordings from a young animal (no. 17) and quantification of the different parameters. (A) ABRs were obtained for several intensities (in ordinate). The ‘threshold’ was defined as the lowest intensity at which a reliable potential remained visible and is indicated by a square around the corresponding value. For each intensity, the P3 (+) and N3 (black dot) peaks were identified; the peak latency (P3 latency) and response strength (P3–N3 amplitude) were measured. STRFs obtained for MU (B) and LFP (C); recordings at several intensities (in ordinate). Each STRF is plotted as a function of frequency (in abscissa, see above and below the plots) and time (in ordinate, see on the right of B). For both MUs and LFPs, significant positive peaks (MUs, LFPs) and negative peaks (LFPs) were identified and their contours were represented by an unbroken line (MUs, positive peaks; LFPs, negative peaks) and dotted line (LFPs, positive peaks). Rebounds, i.e. activities of any polarity occurring after the main peak and separated from it, were excluded (‘x’ in B and C). Thresholds for MUs and LFPs are defined in the same way as for ABRs. At a given intensity, the BF is the frequency of the largest peak and its latency was extracted (peak latency). The characteristic frequency (CF) is the BF at threshold. The total bandwidth was defined as the sum of frequency width of contours, in octaves. For each STRF, the minimum and maximum contour times of all peaks define the first spike and last spike latencies, respectively. For LFPs, these latencies were estimated in the same way but they are called first deflection and last deflection, respectively. The response duration was the time difference between the first and last spike (MUs) or deflection latencies (LFPs).

Fig. 2. Average hearing loss estimated from ABRs as a function of frequency in the three groups of animals. Vertical bars indicate the SE. For a given animal, at a given frequency, the hearing loss was computed by the difference between its own threshold minus the threshold averaged over a population of 12 healthy young animals. The shaded area is the trust interval (2.5–97.5%) for these 12 healthy young animals.
The cortical hearing loss was defined as the difference between the best cortical LFP threshold and the ABR threshold for each frequency tested in the ABR audiogram. For a given cortical site, the cortical threshold was the lowest intensity at which we could observe a peak in the LFP-based STRF including the ABR frequency or closer than a quarter an octave from it. Because LFPs represent the summation of electrical events coming from large neuronal populations, comparing the thresholds from ABRs and cortical LFPs can be viewed as an estimate of hearing deficits having a central origin (assuming that this comparison is not biased by differences between the waveform stimuli used to test the ABRs and LFPs). In Fig. 4, the young animals (having no hearing loss) showed a mean ‘cortical hearing loss’ of 11 dB, which simply indicates that the cortical thresholds were 11 dB above the ABR thresholds. The traumatized animals had the largest brainstem hearing loss (45 dB) among the three groups (Mann–Whitney U test, \(P < 10^{-7}\)) but a mean cortical hearing loss of 14 dB, which was not different from that in young animals (Mann–Whitney U test, \(Z = -1.17, P = 0.24\)). In contrast, the aged animals showed a mean brainstem hearing loss of 29 dB (higher than the young animals, \(Z = 7.46, P < 10^{-15}\)) and a cortical hearing loss of 29 dB, significantly higher than the other two groups (\(P < 10^{-15}\)).

Several parameters were systematically quantified at each recording site from both MU-based and LFP-based STRFs (see Materials and methods and Fig. 1). These parameters included response strength, response latency (that of the first spike/first deflection and that of the peak at the BF), response duration and tuning width. For each group, Fig. 5 provides representative examples of MU-based and LFP-based STRFs obtained in the three groups. These examples illustrate the three following points. First, in traumatized animals, the MU-based and LFP-based STRFs showed a lack of cortical response at the trauma frequency (except at 85 dB for animal no. 12). Second, the bandwidth of tuning curves was much smaller in aged animals compared with young and traumatized animals. This result is clearly illustrated by the length of the gray lines representing the tuning width in Fig. 3A and B (these lines are much shorter for the aged animals). Third, the duration of the response was longer in the aged animals compared with that observed in the young and traumatized animals (this effect can also be observed in the Supporting Information Fig. S2).
Fig. 4. ‘Cortical hearing loss’ as a function of ‘brainstem hearing loss’. Each point represents an animal and a frequency at which the ABR threshold was determined. For a given animal, at a given frequency, the brainstem hearing loss (in abscissa) is the difference between the ABR threshold of that animal and the threshold averaged over the population of control animals. For each frequency at which the ABR was quantified, the cortical threshold was defined as the lowest intensity at which a peak was detected in the LFP-based STRF. The cortical hearing loss (in ordinate) was computed as the difference between the cortical LFP thresholds and the ABR thresholds from the same animal. Each point was shifted by random values between −3 and +3 dB to separate the points. For each group, the larger symbol represents the mean and the ellipse around the mean has a major and minor radius equal to the SD along the abscissa and ordinate axis.

Figure 6 presents the group data for the parameters obtained from MU-based and LFP-based STRFs together with the parameters derived from the ABR quantification. As these measures were obtained at fixed suprathreshold intensities (70–95 dB, Fig. 6A–D and H), it was important to determine if similar results hold around threshold. Therefore, values for the response strength, peak latency and total bandwidth were also derived close to threshold (Fig. 6E–G). In Fig. 6, arrows indicate statistically significant differences between groups.

Similarities between traumatized and aged animals

A first striking common effect observed in aged and traumatized animals was the decrease in response strength observed in both ABRs and cortical responses at suprathreshold level (Fig. 6A). The difference in response strength was largely reduced close to threshold, only remaining significant for LFPs (Fig. 6E). Second, the peak latency (Fig. 6B) and the latency of the first spike (MUs)/first deflection (LFPs) (Fig. 6D) were longer in aged and traumatized animals compared with young animals, an effect that was not present in ABRs (Fig. 6B). Indeed, ABRs showed a difference of at most 0.5 ms between young vs. aged/traumatized animals, whereas this difference was of more than 5 ms at the cortical level. However, analysing the latency shift/intensity function for ABRs/MUs/LFPs (see Supporting Information Fig. S3) indicates that this shift of 0.5 ms at the brainstem level and of 5 ms at the cortical level would be equivalent to a 40 dB decrease in SPL for young animals. Thus, this differential effect on latencies in the cortex vs. the brainstem might simply result from the hearing loss detected in the aged and traumatized animals.

Note that, when the results from Fig. 6 were divided in three frequency bands according to the BF, similar differences were also observed between young animals vs. traumatized and aged animals (see Supporting Information Fig. S4A, B and E). The peak latencies did not reveal drastic differences between groups at threshold. The main difference was that the LFP and ABR peak latencies were longer in the young group (Fig. 6F), probably because of a lower threshold in young animals.

Differences between traumatized and aged animals

At suprathreshold intensities, two major differences were noted between aged and traumatized groups. First, the total bandwidth was significantly smaller in the aged animals compared with the young and traumatized animals (Fig. 6C). Second, the response duration was significantly longer in the aged animals compared with the young and traumatized animals (Fig. 6H). These effects on the response duration and total bandwidth held true when the results were split into three frequency bands (i.e. below, around and above the trauma frequency) (see Supporting Information Fig. S4D and F).

Surprisingly, close to threshold the total bandwidth in the trauma group was larger than in the other groups (Fig. 6G) but there was no difference between young and aged animals. This suggests that the type of threshold shifts has a major impact on the effects on bandwidth. In aged animals, the flat hearing loss across frequencies generated a normal bandwidth around threshold, as if all of the tuning curves were simply moved up. In contrast, in traumatized animals exhibiting similar (or even larger) threshold shifts, the concentration of hearing loss around the trauma frequency led to unaffected thresholds for distant frequencies (e.g. as in Fig. 5B) so that, around threshold, the total bandwidth was actually larger than in the other two groups.

Consequences of decreasing sound intensity

We then aimed to determine how the parameters describing the tuning properties were modified by a decrease in sound intensity. This question was addressed first when this decrease in intensity occurred far above threshold (in the range of 70–95 dB SPL), and second when it occurred close to the threshold of the MU/LFP signals. Figure 7 shows the variations of the previously described parameters (peak latency, response strength and total bandwidth) when the sound intensity was decreased by 10 dB, at both suprathreshold level (Fig. 7A–C) and around threshold (Fig. 7D–F).

Similarities between traumatized and aged animals

At both suprathreshold level and threshold, the decrease in response strength was larger in traumatized and aged animals than in young animals (Fig. 7A and D). This effect was observed in both MU and LFP recordings at the cortical level, but not in the ABR. Similarly, at both suprathreshold and threshold levels, the bandwidth reduction of the cortical recordings was larger in the traumatized and aged animals compared with young animals (Fig. 7C and F). Finally, the increase in peak latency was also larger in the traumatized and aged animals compared with young animals at suprathreshold; an effect observed in both the ABRs and cortical recordings (Fig. 7B).

Differences between traumatized and aged animals

Only a few differences were noted between the traumatized and aged animals; the latency shift at the brainstem level was larger in traumatized animals than in aged animals. Values of the latency shift/intensity function (available in Supporting Information Fig. S3A) confirm this result at any SPL. All other differences between traumatized and aged animals were detected from the cortical LFPs. At suprathreshold level, the effects on response strength, peak latency and total bandwidth were more pronounced in aged animals than in traumatized animals. At threshold, the effects on response strength and total bandwidth were also more pronounced in aged animals than in traumatized animals. Analyzing the effects from Fig. 7 according to frequency bands (around, above or below the frequency of the acoustic
trauma) did not reveal additional effects (Supporting Information Fig. S5).

These results indicate that analyzing the consequences of an intensity decrease mostly revealed differences between young animals vs. aged/traumatized animals in terms of response strength and bandwidth (and latency at suprathreshold only). At the cortical level, differences between aged and traumatized animals were rather small in magnitude and were only observed from LFP recordings, in terms of response strength and bandwidth.

Discussion

We compared MU/LFP-based cortical STRFs obtained in young adult guinea pigs with (i) those obtained in aged (> 36-month-old) animals exhibiting a broad 30 dB hearing loss and (ii) those obtained in animals (9 months old) previously submitted to an acoustic trauma and exhibiting a severe (40–60 dB) hearing loss at and above the trauma frequency. In young animals, the thresholds observed in the auditory cortex were slightly above (11 dB) those obtained with ABRs (Fig. 4). The difference between ABRs and cortical thresholds was similar in traumatized animals (14 dB) but was much larger for the aged animals (29 dB) (Fig. 4). In both aged and traumatized animals, the evoked responses were reduced in ABRs and in cortical recordings. Specific effects of aging compared with those due to acoustic trauma involved an increased duration of evoked response and a reduced bandwidth of cortical STRFs (Fig. 6C–H). When decreasing the sound intensity, aged and traumatized animals showed larger changes than young animals in bandwidth and response strength (Fig. 7).

Methodological considerations

In analyzing the present data, we made between-groups comparisons on ABRs and cortical evoked responses. When delivering the sound
frequencies, we used short linear ramps (2 ms) for ABRs and gamma-shaped longer ramps at the cortical level. Such a difference between ramp lengths could be questioned but essentially stems from physiological considerations; ABRs are onset responses that only need a few periods of the tone to be triggered (Suzuki & Horiuchi, 1981). A longer rise-time deteriorates the synchronization of responses from individual nerve fibers and subsequent auditory nuclei. Thus, short ramps are necessary. However, if the ramp is too short (the

Fig. 6. Averaged group data for the parameters derived from ABRs and cortical STRFs (based on MUs and LFPs). Data are from the highest sound pressure level tested for each cortical site (i.e., in the range 75–95 dB SPL), except in F, where it is the threshold SPL, and in E and G, where it is 10/20 dB above the threshold of the site. (A and E) Response strength (P3–N3 amplitude for the ABRs; number of spikes in the peaks of each site for MUs; volume of the peaks of each implanted site for LFPs). (B and F) Latency of the response peaks. (C and G) Total bandwidth of peaks for each cortical site. (D) First spike (MU data) and first deflection (LFP) latency of peaks. (H) Duration of the responses, i.e., last spike (MU data) and last deflection (LFP) minus first spike/deflection latencies of peaks. One cortical site provided only one value except in E and G, where STRFs were possibly extracted at both threshold +10 dB and at threshold +20 dB when the data were available. Counts for each plot are reported in Table S1 (see Supporting Information). Mean values are represented ± SE values. Arrows indicate when the values from one group significantly differ from another group (Mann–Whitney bilateral test, see Materials and methods).

Fig. 7. Averaged group data for the percentage of change in ABR and STRF parameters obtained when the intensity was decreased to 10 dB at suprathreshold levels (> 70 dB; top panels) or at 10/20 dB above threshold (bottom panels). (A and D) Changes in response strength (P3–N3 amplitude range for ABRs; number of spikes in the peaks from STRFs for MUs; volume of the peaks from STRFs for LFPs). (B and E) Changes in latency for the peaks for the ABRs, MU- and LFP-based STRFs. (C and F) Changes in total bandwidth of STRFs for MU- and LFP-based STRFs. One site can be included several times in the average when recordings were performed at several SPLs within the intensity range (on the left). Counts for each plot are reported in Table S1 (see Supporting Information).
There was a large 40 dB threshold shift between pre-trauma and acoustic trauma. First, we have followed over time the ABR threshold hearing loss detected in the trauma group mainly came from the cortical (Huetz et al., 1998; Capsius & Leppelsack, 1996). For example, in two recent studies, we did not detect significant differences between the response strength and breadth of tuning in waking and urethane-anesthetized animals, at both the thalamic (Massaux et al., 2004) and cortical (Huetz et al., 2009) level.

We compared the data obtained from young animals (2–3 months old) with those obtained from 1-year-old animals submitted to an acoustic trauma at the age of 2–3 months. Thus, it could be argued that the effects observed on the ABR audiogram of traumatized animals result from two factors: the acoustic trauma and the aging process occurring between 3 and 12 months. Two results suggest that the hearing loss detected in the trauma group mainly came from the acoustic trauma. First, we have followed over time the ABR threshold shift produced by the acoustic trauma up to 45 days after the trauma. There was a large 40 dB threshold shift between pre-trauma and 45 days post-trauma (Wilcoxon paired test, $Z = 2.201, P = 0.0277$) (see Supporting Information Fig S6A and B). On average, the brainstem hearing loss detected at 12 months of age was only 13 dB higher than that detected at 45 days after the acoustic trauma, a small but significant difference ($Z = 2.023, P = 0.043$). Second, the ABR threshold obtained for a group of seven normal animals (8–12 months old) was much lower than the ABR thresholds observed in the trauma group (15 vs. 59 dB, Mann–Whitney test, $Z = 2.857, P = 0.0043$) (see Supporting Information Fig S6C). These results confirm that little (Nozawa et al., 1996) or no (Ingham et al., 1998) change in hearing threshold can be detected in 12–15-month-old guinea pigs compared with young animals.

Peripheral and brainstem alterations

Cochlear alterations have long been assessed through ABRs, which partially reflect brainstem activities, even if it is often assumed that ABRs give results similar to those obtained at the cochlea’s output (e.g. Boettcher et al., 1996). Indeed, the threshold of the compound action potential increases in parallel with the threshold of the ABR in aged animals (Hellstrom & Schmiedt, 1990; Mills et al., 1990). Our results obtained in aged guinea pigs are consistent with previous studies. First, the hearing loss found here with clicks (30 dB) is similar to that previously reported using 3–5-year-old guinea pigs (Ingham et al., 1998). Second, in aged animals, we observed a somewhat flat hearing loss of 25–40 dB up to 24 kHz, as previously described in other species (Butler & Gastel, 1979; Henry et al., 1980; Kujawa & Liberman, 2006), including humans (Lee et al., 2005). Several effects of aging have been associated with hearing loss. For instance, the amplitudes and slopes of the compound action potential input/output functions were decreased in aged gerbils; ABRs exhibited slightly longer latencies and a decreased amplitude especially when threshold elevations were substantial (Harkins, 1981; Hunter & Willott, 1987; Harada et al., 1999). In agreement with these studies, the ABRs recorded here indicated a significant reduction of the P3–N3 amplitude. The intensity decrease led to significant latency differences between young and aged animals at suprathreshold levels (75–95 dB) but not near threshold (Fig. 7B and E). This indicates that the latency-intensity functions were steeper in aged than in young animals at suprathreshold levels, consistent with the results obtained in aged gerbils (Boettcher et al., 1993b) and aged guinea pigs (Ingham et al., 1998). However, we did not observe a shallower amplitude-intensity function as reported in elderly humans (Boettcher et al., 1993a). It could be argued that our aged guinea pigs were not old enough to display all of the changes observed during aging, but it is between 2 and 3 years old that important differences in the compound action potential threshold and ABR magnitude were found in guinea pigs (Nozawa et al., 1996; Proctor et al., 1998).

Alterations in the central auditory system

During aging, many structural alterations have been described at the first auditory stages (review in Frisina & Walton 2006). For example, a loss of octopus cells, a slight decrease in their size, an increase in the packing density of glial cells and dendritic changes ranging from minor to total loss of primary branches were reported in the cochlear nucleus of old mice (Willott & Bros, 1990). Undoubtedly, the most striking structural change that has been described in aged animals relates to inhibitory neurotransmission (Ling et al., 2005; Caspary et al., 2008). Changes in glycnergic and GABAergic receptors were found from the cochlear nucleus (Krenning et al., 1998; Caspary et al., 2001; Milbrandt & Caspary, 1995; Wang et al. 2009; Willott et al., 1997) to the inferior colliculus (IC) (Gutiérrez et al., 1994; Milbrandt et al., 1996; Milbrandt et al., 1997; Caspary et al., 1999) and to the auditory cortex (Ling et al., 2005).

Apart from studies focusing on temporal processing (e.g. Barsz et al., 2002; Ison & Allen 2003; Lee et al. 2002; Mendelson & Ricketts, 2001; Schneider et al. 1998; Walton et al., 1998), relatively few studies have assessed the impact of aging on functional properties of auditory neurons. At the brainstem level, studies in aged C57 mice have emphasized an age-related elevation of thresholds mostly in the ventral cochlear nucleus (Willott et al., 1991), whereas the thresholds were similar or moderately affected in the dorsal cochlear nucleus, IC and AI (Willott, 1986, 1991; Willott et al., 1988, 1991, 1993). Despite differences in the stimuli waveforms used when recording ABRs and LFPs, there was a relatively good match between the ABR thresholds and the cortical thresholds for young animals. In contrast with young animals, the difference between ABRs and cortical thresholds was larger for aged animals than for young animals (Figs 3 and 4). Assuming that differences in the stimulus envelope do not invalidate our results, this suggests that the hearing loss detected at the cortical level is larger than that detected at the brainstem level, which clearly favors the possibility that central alterations add to more peripheral alterations. A recent study (Khouri et al., 2011), investigating the consequences of aging in the IC of old gerbils, reported that the spontaneous activity and the Q100m were not different in 3-month-old vs. 3-year-old animals despite the fact that the cortical neurons had...
higher thresholds (11 dB higher). Also, the IC neurons of aged animals were found to be less selective than those of young animals for temporal parameters when the duration and the gap between brief noises were evaluated. At the cortical level, alterations of the tonotopic map corresponding to threshold elevation were observed in C57 aged mice, so that virtually the entire auditory cortex became devoted to the middle frequencies (especially 10–13 kHz), for which sensitivity remained high (Willott et al., 1993). The same phenomenon has been noted in the IC (Palombi & Caspary, 1996). In fact, similar results were obtained from our aged guinea pigs. As very few MUs and LFPs responded to low and high frequencies (even when the hearing loss was moderate, as in animal nos. 18 and 19, see Fig. 3A and B), the tonotopic map was mostly composed of middle frequencies.

At suprathreshold levels, we found that both cortical and brainstem responses were decreased in aged and traumatized animals (Fig. 6A). For MU recordings, this decrease might imply that fewer neurons were recorded (instead of reduced evoked responses), but this is unlikely given that the results for LFPs and ABRs were similar. Age-related changes in response strength have not often been reported and are controversial. In aged rats, higher discharge rates to characteristic frequency tones have been noticed in the cochlear nucleus (Caspary et al., 2005), whereas there was a 12% reduction in discharge rate in the IC (Palombi & Caspary, 1996). In the primary auditory cortex (A1) of aged rats, a non-significant reduction of spontaneous activity was initially mentioned for single units showing classical V-shaped tuning curves (Turner et al., 2005), whereas increases were recently reported in the rat and primate auditory cortex (Hughes et al., 2009), and no change was found by other groups (Mendelson & Ricketts, 2001). In humans, the amplitude of middle-latency evoked potentials from the IC and thalamus, but not late-latency evoked potentials from the auditory cortex, increased with aging (Woods & Clayworth, 1986; Pekkonen et al., 1995; Amenedo & Diaz, 1998). As correctly noted by Burkard et al. (2007), the controversial effects of aging on early, middle and late evoked potentials found in the literature ‘simply reflect the delicate down-regulation of neural inhibition’. In other words, despite the fact that inhibition is down-regulated (review in Caspary et al., 2008), if excitation is also strongly attenuated, the net result can be a decrease in response strength as observed in our study. In fact, this can explain the apparent discrepancy between our results and those recently reported by Juarez-Salinas et al. (2010). Studying auditory cortex neurons in aged primates exhibiting no hearing deficit, i.e. having potentially no decrease in excitatory drive from subcortical stages, they observed an increase in evoked activity, which might reflect a pure decrease in inhibitory inputs without a concomitant decrease in excitatory inputs. This explanation can also fit with our results obtained near threshold: when the responses are analyzed relative to the hearing loss, only small differences (LFPs) or no difference (MUs, ABRs) were found in terms of response strength between young, traumatized and aged animals.

Interestingly, the sensitivity to an intensity decrease was greater at the cortical level than at the ABR level for both aged and traumatized animals, suggesting that the slope of the amplitude-intensity function was steeper at the cortical than at the brainstem level. Of course, it is important to exercise caution about this result because differences in the stimuli waveform between sounds used to test ABRs and cortical responses could bias direct comparisons between ABR changes and cortical changes. However, this result might indicate an additional age/trauma-related loss of dynamics (intensity range that can be processed) at the cortical level compared with the peripheral/brainstem level.

Very few studies have investigated the age-related effects on latencies at the brainstem or cortical levels and heterogenous results were described. Compared with young animals, the response latency of IC neurons in aged mice was found to be shorter (Simon et al., 2004) or unchanged (Palombi & Caspary, 1996), but the latencies of evoked potentials were increased in the IC and auditory cortex of aged guinea pigs (Dum, 1983) and humans (Woods & Clayworth, 1986; Lenzì et al., 1989). Here, the most significant difference between ABR- and MU/LFP-based results was the delayed peak latency at the cortical level; ABRs showed a difference of at most 0.5 ms between young and aged/trauamitized animals, whereas this difference was more than 5 ms at the cortical level (Fig. 6B). Potentially, this suggests that the timing of neuronal responses is considerably impaired between the trapezoid body (source of the P3 wave) and the cortical level. Similarly, central effects of aging have been reported in gerbils (Boettcher et al., 1996); no latency shifts have been detected in the compound action potential of the eighth nerve, whereas in the same animals, latency shifts have been reported in analyzing waves II and IV of the ABR. However, analyzing the latency shift/intensity function (Supporting Information Fig. S3) indicates that, in young animals, a similar 40 dB level change should lead to those shifts at both the brainstem and cortical levels. This is close to the range of 25–40 dB of brainstem hearing loss observed in aged animals (Fig. 2) and therefore we cannot discard the possibility that these changes simply result from a threshold change.

**Comparing effects of aging and noise-induced hearing loss**

In our study, differences were detected when comparing the cortical parameters in aged and traumatized animals. Two of them were particularly striking and were observed from both MU and LFP recordings. First, there was a large increase in response duration in aged animals (Fig. 6E). Similar effects were recently reported while testing neurons in aged rats with broadband noise; in all cortical layers, the response duration was increased and the post-excitatory suppression was shortened (Hughes et al., 2009). These response alterations can be viewed as the signature of an age-related loss of inhibition in the auditory cortex and, more generally, in the central auditory system.

A second striking effect concerns the bandwidth of STRFs obtained in the aged animals, which was smaller than that in the young and traumatized animals at suprathreshold levels but not at 10/20 dB above threshold (Fig. 6C–G). A reduction of 20–25% of the single-unit response area has also been emphasized in cochlear nucleus neurons of aged CBA mice (Willott et al., 1991). In contrast, in the IC of aged rats, an 18% increase in the breadth of the isointensity functions has been found at 30 dB above threshold (Palombi & Caspary, 1996). In the auditory cortex, this effect was not reported by Turner et al. (2005) who studied layer V neurons in aged rats and described that there were less classical V/U-shaped tuning curves and more ‘complex’ tuning curves in aged than in young rats. In fact, these ‘complex’ tuning curves corresponded to weak and unreliable auditory responses. As the ‘complexity’ of the tuning curves is difficult to quantify, we chose here to quantify classical parameters such as the bandwidth, peak latency and temporal response duration as markers of auditory processing. We quantified neuronal activities in layer III/IV and focused on reliable STRFs. In aged animals, we found a larger number of cortical sites with no responses or no spiking activity compared with young animals (see first paragraph in Results), which might be related to the observations by Turner et al. (2005). Nevertheless, this was not specific to the aged animals as the same phenomenon occurred in the trauma group. Obviously, it is also possible that, in our MU recordings collected in aged animals, some single units showed robust STRFs, whereas others had poor, or no,
STRFs. This might also explain (i) the lower evoked firing rate and (ii) the smaller bandwidth in the aged animals. Nevertheless, in traumatized animals, the strength of the cortical responses was as low as in aged animals, whereas the bandwidth was not as narrow. Note also that this decrease in response strength had already been observed from ABR recordings. This decrease in both response strength and bandwidth cannot easily be explained by a loss in cortical inhibition. However, interpreting all of the results only with changes in inhibition is a naive oversimplification. Shortly after an acoustic trauma, the synaptic mechanisms underlying receptive field changes include a selective gain of inhibition but also unmasking by selective loss of inhibition elsewhere in the neurons’ receptive field (Scholl & Wehr, 2008). Also, evoked responses can be delayed, prolonged and reduced in amplitude (see Fig. 6 in Scholl & Wehr, 2008), similar to the effects that we observed here in both aged and traumatized animals. Actually, a large panoply of structural and physiological changes occurs in the forebrain during aging. They include changes in neuronal intrinsic properties (e.g. changes in afterhyperpolarization), synaptic transmission, dendritic spine morphology, the structure of myelin sheaths (reducing conduction velocity and timing in neuronal circuits) and other potential subcellular events (see for review Burke & Barnes, 2006; Disterhoft & Oh, 2006; Dickstein et al., 2007; Kojima & Shirao, 2007; Mora et al., 2007; Luebke et al., 2010). Thus, it can be unwise to interpret all of the physiological results observed in aged animals simply in terms of gain/loss of inhibition. It is possible that the reduced bandwidth of cortical STRFs in aged animals results from reduced synaptic thalamic inputs, the thalamic level itself receiving reduced brainstem inputs (as suspected from the reduced ABR magnitude). Also, it is possible that auditory cortex neurons might undergo drastic morphological alterations such as massive decreases in dendritic spines as reported in other cortical areas (25–50%; see Luebke et al., 2010). Finally, the strength and density of horizontal connections, crucial to explain the breadth of cortical tuning (Kaur et al., 2004), could be modified with aging.

Conclusion

Our results indicate that aging alters auditory processing at both brainstem and cortical levels. At the cortical level, response strength decreases to the same extent as at the peripheral/brainstem level. However, aging also increases the response latencies and the response duration and reduces the receptive field bandwidth, effects that seem difficult to explain simply by cochlear degradations. These cortical effects probably have an important role in the lower speech recognition scores observed in elderly people.

Supporting Information

Additional supporting information can be found in the online version of this article:
Fig. S1. Left panel: the typical two-first positions chosen for placing the electrode array in the guinea pig auditory cortex. The first position was along the large blood vessels filling the dorso-ventral Sylvian pseudo-sulcus, which was easily found in all animals. The lowest best frequencies were found close to this sulcus (Edeline et al. 1993; Manunta and Edeline 1999; Wallace et al. 2000). The second position was perpendicular to the first one and reached higher frequencies, some of them possibly belonging to the tonotopic DC area known to have very similar properties to the AI area regarding the STRFs (Rutkowski et al. 2002). If a third position was tried, it was parallel to the second one, more ventral or dorsal. Thus, the sampling covered the whole tonotopic map of AI. Right panel: first position of the electrode array in an aged animal (#4) along the Sylvian pseudo-sulcus. Black dots indicate the penetration localization for the 16 electrodes. Note that despite this anterior position very close to the sulcus, no low CF or cortical response to low frequency tones < 1.5kHz was found in this animal at 75dB SPL (Fig. 2).

Fig. S2. Individual example of ABR, MU and LFP recording from an old animal (#5) and some of the quantified parameters (in bold italic).

Fig. S3. Latency shift for a 10dB decrease as a function of the intensity level for ABR (A), Multi-unit activity (B) and LFP (C).

Fig. S4. Averaged group results on effects of hearing loss or aging on several features of peaks in receptive fields of cortical neurons.

Fig. S5. Averaged group results on effects of hearing loss or aging in the case of a 10dB intensity level decrease for a SPL above 70dB (left panels) or 10/20dB above threshold (right panels).

Fig. S6. Evolution of ABR threshold in the traumatized animals.

Table S1. Size of sets of MUs and LFPs (in parenthesis) used in each figure, for each group and each frequency band.

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Acknowledgements

This work was supported by grants from the National Research Agency (ANR Neuro2006-021) and Fédération pour la Recherche sur le Cerveau (FRC) to J.-M. E. We thank J.J. Egggermont and G. Shaw for their generous help with the acquisition software, and M.-H. Basset for helpful discussions on the neurobiology of aging. Many thanks to Nathalie Samson and Pascale Leblanc-Veyrac for taking care of the guinea pig colony.

Abbreviations

ABR, auditory brainstem response; AI, primary field; BF, best frequency; IC, inferior colliculus; LFP, local field potential; MU, multiunit; SPL, sound pressure level; STRF, spectro-temporal receptive field.

References


