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1 Temporal alterations to central auditory processing without
2 synaptopathy after lifetime exposure to environmental noise

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5

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23 Abbreviations

24 ABRs: auditory brainstem recordings

25 BF: best frequency

26 CAPs: compound action potentials

27 DPOAEs : distortion product otoacoustic emissions

28 ESR: evoked-to-spontaneous firing rate ratio

29 FR: firing rate

30 IHC: inner hair cell

31 PSTHs: post-stimulus time histograms

32 RDS: random double sweep

33 SNR: signal-to-noise ratio

34 STRF: spectrotemporal receptive field

35 TMTF: temporal modulation transfer function

36 TTS: temporary threshold shift

37 VS: vector strength

38

39 **Keywords:** auditory cortex, auditory brainstem response, compound action potential, synaptic
40 ribbons, behavioral task, passive noise exposure.

41

42 Abstract

43 People are increasingly exposed to environmental noise, through the cumulation of
44 occupational and recreational activities, which is considered harmless to the auditory system if
45 the sound intensity remains <80 dB. However, recent evidence of noise-induced peripheral
46 synaptic damage and central reorganizations in the auditory cortex, despite normal audiometry
47 results, have cast doubt on the innocuousness of lifetime exposure to environmental noise. We
48 addressed this issue, by exposing adult rats to realistic and non-traumatic environmental noise,
49 within the daily permissible noise exposure limit for humans (80 dB SPL, 8 hours per day) for
50 between 3 and 18 months. We found that temporary hearing loss could be detected after six
51 months of daily exposure, without leading to permanent hearing loss or to missing synaptic
52 ribbons in cochlear hair cells. The degraded temporal representation of sounds in the auditory
53 cortex after 18 months of exposure was very different from the effects observed after only
54 three months of exposure, suggesting that modifications to the neural code continue
55 throughout a lifetime of exposure to noise.

56 Introduction

57 In recent decades, people have been exposed to increasing environmental noise, defined as
58 the sum of noise from transport, professional and recreational activities. The cumulative effect
59 of daily exposure to environmental noise, at loud but non-traumatic sound pressure levels, such
60 as 80 dB SPL (sound pressure level), has long been considered harmless to the auditory system,
61 even at the scale of a lifetime of exposure (ISO 1990).

62 This view is based principally on the absence of auditory threshold shifts after prolonged
63 exposure to such levels of noise in humans (SCENIHR 2008; Prendergast et al. 2017) and animals

64 (Canlon and Fransson 1995; Willott and Bross 2004; Noreña et al. 2006). However, normal
65 auditory thresholds do not fully reflect the health status of the peripheral auditory system:
66 auditory thresholds can recover from a temporary threshold shift (TTS), experienced in the
67 hours immediately following transient noise trauma. In rodents, a TTS is typically induced by
68 exposing animals for a few hours to 100 dB SPL, and is accompanied by a loss of synaptic ribbons
69 in the sensory inner hair cells, a decrease in the synchronized activity of the auditory nerve and
70 subsequent degenerations of auditory nerve fibers (Kujawa and Liberman 2009) with no change
71 in hearing thresholds, resulting in a “synaptopathy”. If hearing damage were proportional to
72 the acoustic energy received by the ear (Eldred et al. 1957), cumulative lifetime exposure to 80
73 dB SPL, which largely exceeds the energy delivered by synaptopathy-related protocols (100 dB
74 SPL for 2 hours), should induce a TTS leading to long-term synaptopathy and, possibly,
75 aggravated auditory aging.

76 Moreover, overstimulation with noise elicits auditory cortex plasticity. In adult animals, passive
77 exposure, for one to three months, to noise levels <85 dB SPL with spectral or temporal
78 narrowband acoustic content has been shown to modify the organization of the cortical circuits
79 and to reduce the response to this specific content (Noreña et al. 2006; Zhou and Merzenich
80 2012). This reorganization may be governed by homeostatic plasticity (Gourévitch et al. 2014)
81 and is partially reversible over a few weeks (Pienkowski and Eggermont 2012). Long-term noise
82 exposure may also favor subsequent plastic changes, as if a new critical window had been
83 opened in adulthood (Zhou et al. 2011; Thomas, Friedman, et al. 2019; Thomas et al. 2020), but
84 could also lead to auditory disorders, such as hyperacusis (Thomas, Guercio, et al. 2019).
85 However, it remains unclear how perception of auditory stimuli is altered by all these plastic
86 changes.

87 All the animal studies described above used relatively short periods of exposure, at the scale of
88 rodent life, and unrealistic types of environmental noise, such as broadband noise, noise bursts
89 or random pure tones. The potential of a lifetime of exposure to realistic environmental noise
90 to induce a TTS, to alter the neural representation of sounds, to impair behavioral performance,
91 or to exacerbate the auditory aging process is an issue of the utmost concern in auditory
92 neuroscience, but is currently unknown.

93 We evaluated the impact on the auditory system of adult rats of long-term (3 months) to
94 lifetime (18 months) exposure to realistic non-traumatic noise (80 dB SPL, 8 h/day). Unlike many
95 previous studies, we assessed the consequences of such exposure for the cochlea, auditory
96 nerve, brainstem, cortical response to various acoustic features and behavior in each animal.
97 Contrary to the prevailing view, exposure to noise at this moderate intensity (80 dB SPL) led to
98 a TTS occurring after six months, which was not accompanied by a classical pattern of
99 synaptopathy. We also found that three months of exposure led to degradation of the evoked-
100 to-spontaneous firing rate ratio in the auditory cortex, whereas a lifetime of noise exposure
101 led, instead, to degradation of the temporal representation of sounds. These findings suggest
102 that repeated daily noise exposure, at a moderate SPL, may alter progressively the auditory
103 system function over a period of years.

104

105 Methods

106 With the exception of noise exposure, the methods were as described in a previous
107 study (Occelli et al. 2019) for which the control dataset was studied for specific aging effects.

108 We therefore describe here only the most important points.

109

110 Subjects

111 Recordings were obtained from the primary auditory cortex of adult female Sprague
112 Dawley rats. The animals were obtained from Janvier Laboratories at the age of two months,
113 adapted for one month to the core animal facility, and housed for 3, 6, 12, or 18 months (the
114 groups are named according to these time periods) in either a classical facility (unexposed
115 animals) or a dedicated facility (exposed animals) with controlled humidity (50-55%) and
116 temperature (22-24 °C) conditions, under a 12 h light/12 h dark cycle (lights on at 7:30 a.m.)
117 with free access to food and water. At the end of experiments, animals were 6, 9, 15, or 21
118 months old. The initial number of animals was 10 for the 3-, 6-, and 12-month groups, and 20
119 for the 18-month group. We chose female rats, as they typically show less aggressive behaviors
120 than males in groups of a few animals (Schweinfurth 2020) and are as good as, if not better
121 than, male rats in shock avoidance tasks (Dalla and Shors 2009). Given the well-documented
122 susceptibility of female Sprague Dawley rats to mammary tumors (Davis et al. 1956; Freedman
123 et al. 1990; Fay et al. 1997; Jowa and Howd 2011), all aged animals were examined three
124 times/week by staff from the animal facility, and any animal with tumors was excluded from
125 the study. The final sample sizes for the various groups of animals are summarized in
126 Supplementary Table 1. The protocol was approved by the local ethics committee (Paris-Sud
127 University, CEEA No. 59, project 2014-25). Each animal was subjected to the sequence of
128 protocols displayed in Fig. 1b and detailed below.

129 Environmental noise exposure

130 The dedicated facility had thick walls and was specially built for the project. Four cages,
131 each housing three to four animals, were placed 1.5 m away (Supp. Fig. 1d) from a full-range
132 powerful speaker (Adam A8X). Environmental noise exposure for 20 minutes was generated at

133 a sampling rate of 96 kHz from a Dynamic Moving Ripple (Kowalski et al. 1996; Escabi and
134 Schreiner 2002) ranging between 100 and 40000 Hz with an instantaneous ripple density of 3
135 peaks/oct and an instantaneous modulation rate of 50 Hz. The stimulus was then amplitude-
136 modulated by a temporal envelope obtained by low-pass filtering (Butterworth, frequency
137 cutoff 5 Hz) a uniform white noise. The stimulus was rendered acoustically flat, by recording
138 the speaker output within a cage (preamplifier 2169, transducer 4133, Bruel&Kjaer, Marantz
139 PMD671 digital recorder), then inverting it and fitting a sixth-order IIR filter under Matlab
140 (Matworks), which was then applied to the acoustic stimulus. The SPL was then adjusted to
141 obtain 80 ± 1 dB SPL in the four cages (Supp. Fig. 1d), with a Bruel&Kjaer 2250 soundmeter. The
142 animals were exposed to the noise from 6 pm to 2 am each day, while they were in a waking
143 state (at least most of the time), under the control of the task manager of Windows (Microsoft).

144

145 Auditory brainstem recordings

146 At several times during exposure for the 18-month group, and three weeks after the
147 end of exposure, or at an equivalent age for control groups, auditory brainstem responses
148 (ABRs) were obtained under isoflurane anesthesia (2.5%), by differential recordings between
149 two subdermal electrodes (SC25, NeuroService) placed at the vertex and behind the mastoid
150 bone. RTLab software (Echodia) was used to average 500 responses during the presentation of
151 nine pure-tone frequencies (between 0.5 and 32 kHz) delivered by a speaker (Knowles
152 Electronics) placed in the right ear of the animal. For each frequency, the threshold was
153 determined by gradually decreasing the sound intensity (from 80 dB down to -10 dB SPL). The
154 ABR threshold was defined as the minimum sound intensity eliciting a well-defined and
155 reproducible wave II from the cochlear nucleus (Chen and Chen 1991).

156

157

158 Behavioral task

159 Rats were trained to discriminate between an amplitude-modulated white noise (4 Hz,
160 100% depth modulation; conditioned stimulus, CS+) and an unmodulated white noise (CS-) in
161 a two-compartment shuttle box. Both stimuli lasted 5 s, and they were presented a mean of 30
162 s apart (range: 20 s -75 s). The rat was required to change compartment on CS+ presentation.
163 A lack of response to the CS+ stimulus triggered a 0.3 mA footshock lasting for 10 s, which was
164 stopped immediately if the rat switched compartment. On presentation of the CS- signal, no
165 change in compartment was required. The CS+ and CS- stimuli were each presented 40 times
166 per session.

167 Performance was estimated by calculating the A' index (Verde et al. 2006), which is a
168 non-parametric analog of d' quantifying the discrimination between two stimuli, as follows:

169

$$170 \quad A' = 12 + (H - F)(1 + H - F)4H(1 - F) \text{ if } H \geq F$$

171 and

$$172 \quad A' = \frac{1}{2} + \frac{(F-H)(1+F-H)}{4F(1-H)} \text{ if } H < F$$

173 where H is the hit rate (the proportion of switches on CS+ presentation) and F is the false alarm
174 rate (the proportion of switches on CS- presentation). In our experiment, a successful session
175 was defined as a session in which $H \geq 0.5$ and $A' \geq 0.75$.

176 During the first 10 sessions, each rat was required to complete three sessions in a row
177 successfully, or training was stopped. Once the animal had reached this level of performance,
178 the second phase of the task began, in which we determined the smallest modulation depth
179 for which the rat discriminated between CS+ and CS-. Each session was split into two parts: an
180 initial "recall phase", during which the animal had to discriminate between 0% and 100%

181 modulated white noise for 20 random presentations of CS+ and CS-, followed by a test phase,
182 during which the animal had to discriminate between 0% and a particular modulation depth:
183 80%, 60%, 40%, or 20%. Only one value of the modulation depth was used in this second part
184 of the session, consisting of the highest modulation depth for which the animal did not yet
185 complete a successful session. The animal had a maximum of three sessions to perform
186 successfully at a given modulation depth before a lower modulation depth was selected. If the
187 animal satisfied this criterion, a lower modulation depth was tested at the next session. If the
188 animal did not satisfy the criterion after three sessions, or it satisfied this criterion only at the
189 lowest modulation depth (20%), training was stopped.

190

191 Extracellular recordings in the primary auditory cortex

192 *Acoustic stimuli*

193 Acoustic stimuli were generated in Matlab, transferred to an RP2.1-based sound
194 delivery system (TDT) and sent to a Fostex speaker (FE87E). The speaker was placed 2 cm away
195 from the right ear of the rat. At this distance, the speaker produced a flat spectrum (± 3 dB)
196 between 140 Hz and 36 kHz after calibration. The speaker was calibrated in a similar fashion to
197 the A8X speaker, using noise to estimate the transfer function of the speaker. The inverted
198 transfer function was applied to all sounds sent to the speaker. Spectrotemporal receptive
199 fields were determined with 97 gamma-tone frequencies (the product of a gamma distribution
200 and sinusoidal tone (Lyon et al. 2010)), covering eight octaves (0.14-36 kHz), presented in a
201 random order at a rate of 4.15 Hz and at 75 dB SPL. The frequency response area was
202 determined with the same set of tones presented from 75 to 5 dB SPL (5 dB steps, random
203 order) at a rate of 2 Hz. Each tone was presented eight times at each intensity.

204 We quantified the responses to a set of heterospecific guinea pig vocalizations,
205 corresponding to three representative examples of a whistle call used in a previous study
206 (Gaucher et al. 2013), concatenated into a one-second stimulus presented 25 times. The
207 vocalization was presented with and without two levels of white noise (60 and 70 dB SPL). We
208 then used a gap detection protocol, involving a 300 ms guinea pig whistle (the first call from
209 the set of three used above), split into two halves separated by a gap of 2, 4, 8, 16, 32 or 64 ms
210 of silence. A 1 ms ramp was used at the transition between vocalization and the silent gap, on
211 both sides of the gap. We used 25 repetitions of the stimulus for each of the six gap values.

212 Responses to amplitude-modulated white noise were tested with 15 presentations of
213 100% modulated white noise, at 2 Hz to 50 Hz. The first and second cycles of modulated white
214 noise were also used to study the impact on (i) rising time and (ii) on forward suppression,
215 respectively. Responses to modulation depth were assessed with 20 presentations of one
216 second of white noise at 4 Hz, with a modulation depth ranging from 0% to 100%. We also used
217 30 repetitions of a 50 ms chord (inter-stimulus interval 150 ms) including a tone at 4 kHz and
218 its harmonics of equal amplitude up to 40 kHz inserted in progressively decreasing white noise
219 (the decrease in noise SPL over time was linear). The signal-to-noise ratio ranged from +16 dB
220 to -16 dB over 6 seconds of stimulus. This stimulus was repeated 30 times. We also used 5
221 minutes of the random double sweep (RDS) stimulus previously designed by our team
222 (Gourévitch et al. 2015).

223

224 *Surgical procedure*

225 The animal received an initial dose of ketamine and xylazine (100 mg/kg i.p. and 15
226 mg/kg i.p., respectively) supplemented with lower doses of ketamine (20 mg/kg) and xylazine
227 (4 mg/kg) until reflex movements were no longer observed when the hind paw was pinched.

228 Liberal amounts of a local anesthetic (2% xylocaine) were injected subcutaneously into the skin
229 above the skull and the temporal muscles. The animal was placed in a stereotaxic frame, a
230 craniotomy was performed above the left temporal cortex, and the temporal bone was placed
231 in sterile saline. The opening was 9 mm wide and began at the point of intersection between
232 the parietal and temporal bones, at a height of 5 mm (Manunta and Edeline 1997, 1998, 2004).
233 The dura above the auditory cortex was carefully removed under binocular control without
234 damaging the blood vessels. At the end of surgery, a pedestal was built with dental acrylic
235 cement, to fix the animal's head without the earbars during the recording session. The
236 stereotaxic frame supporting the animal was placed in a sound-attenuating chamber (IAC,
237 model AC1).

238

239 *Recording procedure*

240 Data were collected from multiunit recordings in the core auditory cortex (AC, primary
241 auditory area AI, and anterior auditory field AAF). Extracellular recordings were obtained from
242 arrays of 16 tungsten electrodes (\varnothing : 33 μm , $<1\text{ M}\Omega$) composed of two rows of eight electrodes
243 separated by 1000 μm (350 μm between electrodes of the same row). A silver wire, used as the
244 ground electrode, was inserted between the temporal bone and the dura mater on the
245 contralateral side. The estimated location of AC was 4-7 mm posterior to bregma and 3 mm
246 ventral to the superior suture of the temporal bone (corresponding to the area of interest, AI,
247 defined by Paxinos and Watson (Paxinos and Watson 2005)). The raw signal was amplified by a
248 factor of 10,000 (TDT Medusa) and processed by a multichannel data acquisition system (TDT
249 RX5). The signal collected from each electrode was filtered (610-10,000 Hz) to extract multi-
250 unit activity. The trigger level was carefully set for each electrode so as to select the largest
251 action potentials from the signal. Online and offline examinations of the waveforms suggested

252 that the multi-unit activity collected here consisted of action potentials generated by three to
253 six neurons close to the electrode. At the beginning of each recording session, we set the
254 position of the electrode array such that the two rows of eight electrodes could sample neurons
255 responding to low to high frequencies in the rostro-caudal direction (see example in Supp. Fig.
256 3ci).

257

258 *Recording session*

259 The recording depth was 300-700 μm , corresponding to layer III/IV and the upper part
260 of layer V (Games and Winer 1988; Roger and Arnault 1989). We therefore mainly recorded
261 the largest excitatory pyramidal neurons of layers III and V (Games and Winer 1988; Humphrey
262 and Schmidt 1990; Smith and Populin 2001). Acoustic stimuli were presented at 75 dB SPL in
263 the following order: gamma-tones to determine the pure tone spectrotemporal receptive field
264 (STRF^{pt}, 5 min), followed by the frequency response areas (12 min), followed by the different
265 sets of vocalizations without noise (3 min) and with increasing noise levels (60, 70 dB SPL, 3 min
266 each). The gap detection protocol was then performed (3 min), followed by 3 min of
267 spontaneous activity, and then the depth-modulated noise (4 min), amplitude-modulated noise
268 (7 min), chords in noise (3 min), and the random double sweep (RDS, Gourévitch et al., 2015, 5
269 min) assessments. The presentation of this entire series of stimuli lasted 49 minutes. This set
270 of stimuli was used with the electrode array positioned at two to five locations per animal, in
271 the core auditory cortex.

272

273 *Quantification of responses to pure tones*

274 The STRFs^{pt} derived from multi-unit activity were obtained by constructing post-
275 stimulus time histograms (PSTHs) for each frequency, with 1 ms time bins. All STRFs^{pt} were

276 smoothed with a uniform 5x5 bin window. The best frequency (BF) was then defined as the
277 frequency at which the highest firing rate was recorded. A significant peak in the STRF^{pt} was
278 defined as a firing rate contour above the mean level of baseline activity (estimated from the
279 first 10 milliseconds of STRFs^{pt}) plus six times the standard deviation of the baseline activity.
280 For a given site, “bandwidth” was defined as the sum of all peak widths in octaves.

281

282 *Other stimuli*

283 We first constructed post-stimulus time histograms of the responses with a 2 ms time
284 bin and a 5 ms uniform smoothing window. Individual examples for depth modulation
285 transfer function, temporal modulation transfer function and gap detection are available in
286 Supp. Fig. 4ai,aii,aiii. A significant peak in the PSTH was defined as a firing rate exceeding the
287 mean + 4 standard deviations of the PSTH bin values corresponding to presumed
288 spontaneous activity over a time interval of 100- to 300 ms (depending on the recording time
289 for a given stimulus) starting 100 ms after the stimulus ended. Specific analyses were
290 performed as follows.

291

292 *Gap detection analysis*

293 We considered the neural response to be modulated by the presence of the gap if an
294 onset peak appeared in the PSTH, typically at the beginning of the second half of the
295 vocalization, immediately after the gap. The peak was considered significant if its maximum
296 amplitude was above the mean + 4 standard deviations of the PSTH values over a period of 50
297 ms immediately before the gap.

298

299 *Analyses of temporal modulation transfer functions (TMTFs) and depth-MTFs.*

300 TMTFs: for each modulation frequency or depth modulation, we calculated the vector
301 strength (Goldberg and Brown 1969) (VS), defined as a measurement of the degree of phase-
302 locking (or synchronization) of the spikes with the stimulus envelope. The VS ranged from 0 to
303 1. Depth-MTFs: for each modulation depth, we calculated the VS and took its value at the
304 modulation frequency, 4 Hz.

305

306 *Chords in noise analysis*

307 We considered the neural response to be modulated by the presence of the chord if an
308 onset peak appeared in the PSTH, typically within 0 to 100 ms after the beginning of acoustic
309 stimulation. The peak was considered significant if its maximum amplitude was above the mean
310 + 4 standard deviations of the spontaneous activity obtained in the period extending from 100
311 to 400 ms after the stimulus.

312

313 *End of the recording session*

314 After three to six hours of recording, the skull covering the temporal bone was carefully
315 placed back over the auditory cortex and secured in place with a very thin layer of dental
316 cement. The skin was cleaned and sutured to close the wound and an analgesic
317 (buprenorphine, 0.05 mg/kg, s.c.) and an antibiotic (Convenia, 0.8 mg/kg, s.c.) were injected
318 into the animal. The animal's health was monitored every six hours for 24 h, and the animal
319 was kept in a separate cage for a few days before being returned to the colony room. After two
320 to three weeks of recovery, the animals were sent to the INM (Montpellier) via a specialist
321 transporter (Sanitrans, France), for peripheral auditory system assessment.

322

323 **Peripheral auditory system assessments (DPOAEs, CAPs, ABRs)**

324 *Distortion product otoacoustic emissions (DPOAEs)*

325 DPOAEs were used to assess the functional integrity of outer hair cells. DPOAEs were
326 collected under anesthesia (a mixture of Zoletil 50 (tiletamine, 40 mg/kg) and Rompun
327 (xylazine, 3 mg/kg)). They were recorded in the external auditory canal with an ER-10C S/N
328 2525 probe (Etymotic Research Inc. Elk Grove Village, IL, USA) consisting of two emitters and
329 one microphone. The two primary tones were generated, and the distortion was processed by
330 the Cubdis HID 40133DP system (Mimosa Acoustics, Champaign, IL, USA). The two tones were
331 presented simultaneously, with f_2 sweeping from 0.5 kHz to 16 kHz in quarter-octave steps, and
332 the maintenance of a constant f_2/f_1 ratio of 1.2. The primary intensities of f_2 and f_1 were set to
333 60 and 55 dB SPL, respectively. For each frequency, the cubic distortion product $2f_1-f_2$ and the
334 neighboring noise magnitudes were measured and expressed as a function of f_2 .

335

336 *Compound action potential (CAP) of the auditory nerve*

337 Recordings were performed under anesthesia (Zoletil 50 (tiletamine, 40 mg/kg) and
338 Rompun (xylazine, 3 mg/kg)) in a Faraday-shielded anechoic soundproof cage. Rectal
339 temperature was measured with a thermistor probe, and maintained at $38\text{ }^\circ\text{C} \pm 1\text{ }^\circ\text{C}$ with a
340 heated blanket placed underneath the animal. Signals were generated, acquired and processed
341 with an NI PXI-4461 signal generator (National Instruments) controlled with LabVIEW software.
342 Bursts of pure tones (1 ms rise/fall time, 10 ms duration, 11 bursts/s, 200 presentations per
343 level, alternating polarity) were delivered by a JBL 075 loudspeaker (James B. Lansing Sound)
344 positioned 10 cm away from the ear tested, in calibrated free-field conditions.
345 Electrophysiological signals ($\times 20,000$) were amplified with a Grass P511 differential amplifier
346 with a 300 Hz to 3.5 kHz bandpass.

347 The CAP of the auditory nerve was recorded from an electrode located in the round
348 window niche (active) and two subcutaneous needle electrodes placed on the pinna of the ear
349 tested and in the neck muscles (ground). Intensity-amplitude functions were obtained, at each
350 frequency tested (1, 2, 4, 8, 16, 24, 32 kHz), by varying the intensity of the tone burst from 0 to
351 80 dB SPL, in 5 dB increments. CAP amplitude was measured between N1 and P1, with CAP
352 threshold defined as the dB SPL required to elicit a measurable response of greater magnitude
353 than the noise level.

354

355 **Immunohistochemistry**

356 *Quantification of GAD67 labeling*

357 At the end of the CAP recording session, the rats were deeply anesthetized with a
358 mixture of ketamine and xylazine (200 mg/kg body weight and 15 mg/kg, respectively, i.p.) and
359 transcardially perfused with 150 ml of saline and 1,000 ml of a fixative solution consisting of 4%
360 paraformaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.4). The brains were collected and
361 fixed in 4% PFA; they were then incubated in incremental concentrations of sucrose (10, 20 and
362 30%). Each brain was sliced (40 μ m sections) on a cryostat (HM550, Microm, Thermo Fisher
363 Scientific), from stereotaxic coordinates -4 mm to -6 mm relative to bregma (Paxinos and
364 Watson 2009, 6th edition). One in every four slices was stained with Nissl stain and three co-
365 authors (JME, FO, ND) examined the stained coronal sections to select the anterior-posterior
366 level corresponding to the center of the AI. One adjacent section (immediately before or after
367 the Nissl-stained slice) was used for GAD67 labeling. The brain slices were rinsed in 1 x PBS and
368 endogenous peroxidases were inactivated by incubation in 1 x PBS supplemented with 10%
369 methanol and 10% H₂O₂. The coronal sections were then washed and permeabilized in 2.5%
370 Triton X-100 in 1 x PBS (PBST). Nonspecific antigen sites were blocked by incubation with 5%

371 normal goat serum and 1% BSA in PBST. The sections were then incubated overnight at 4 °C
372 with the primary anti-GAD67 antibody (Euromedex, GeneTex) diluted 1:500 in the same
373 blocking solution. The sections were washed in PBST and incubated with a secondary antibody
374 (biotinylated anti-rabbit IgG antibody, EuroBio) for two hours at room temperature. Staining
375 was detected with an ABC kit (EuroBio), in accordance with the manufacturer's instructions.
376 Sections were then mounted on glass slides (Fisher) in 0.3% PB gelatin. On the third day, slides
377 were dehydrated and mounted in Eukitt (Fisher). Photomicrographs were taken with an upright
378 optical microscope (Olympus BX60) equipped with mapping software (MercatorPro;
379 ExploraNova, France). Immunolabeling was assessed in two predefined areas (800x300 μm)
380 manually delimited in the center of the AI, in the supragranular and infragranular layers. The
381 immunolabeled cells were counted by an experimenter blind to the age of the animal.

382

383 *Number of ribbon synapses per inner hair cell along the tonotopic axis*

384 The immunohistochemical method for assessing the number of synapses per inner hair
385 cell (IHC) has been described in detail elsewhere (Bourien et al. 2014; Batrel et al. 2017). Briefly,
386 the presynaptic IHC ribbons were identified with a mouse anti-CtBP2 antibody (1:500; BD
387 Biosciences, San Diego, CA). Glutamate receptors were labeled with a mouse antibody raised
388 against the C-terminus of the GluA2 subunit, IgG2a (1:200, Millipore, Billerica, MA). A 3D,
389 custom algorithm was used to detect the juxtaposition of pre- and post-synaptic structures in
390 stacked confocal images. Once the ribbons had been counted, the corresponding coding
391 frequency of each ribbon was inferred from the rat cochlear place frequency map (Müller
392 1991). A second-order polynomial was then fitted to synapse count as a function of position
393 relative to the cochlea apex (Meyer et al. 2009).

394 **Statistical analysis**

395 We mostly used ANOVA (one-way, two-way, three-way) to test for effects in our data.
396 Stimulus parameters were systematically considered to be categorical in ANOVA. Most ANOVA
397 tests were three-way, with exposure, exposure duration, and stimulus parameters as factors.
398 The total number of observations used to compute the second degree of freedom of such
399 ANOVA tests was therefore the number of animals, or cortical sites, for all groups (in
400 Supplementary Table 1), multiplied by the number of stimulus parameters for each protocol,
401 provided in Supplementary Table 2. In practice, it was lower than the theoretical maximum, as
402 certain combinations were unavailable. Following significant ANOVA test results, post-hoc
403 Student's *t*-tests were performed, without correction for multiple comparisons, for peripheral
404 auditory system testing (*n* is small and stimulus parameters, such as frequency, typically take
405 only a few values). Tukey-Kramer correction was used for cortical test results if the stimulus
406 parameter could take more than four values.

407 The statistical distribution of many parameters, including firing rates, is typically
408 skewed. We therefore applied a Log_{10} transformation to render these distributions Gaussian.
409 The robustness of ANOVA to small deviations from normality (Lix et al. 1996; Blanca et al. 2017)
410 and the large sample sizes of our groups (see Supplementary Table 1) ensured that ANOVA was
411 a valid option. Furthermore, there is currently no satisfactory non-parametric solution for two-
412 way and three-way tests.

413 **Data and code availability**

414 The datasets and code supporting the current study are available from the corresponding
415 author on request.

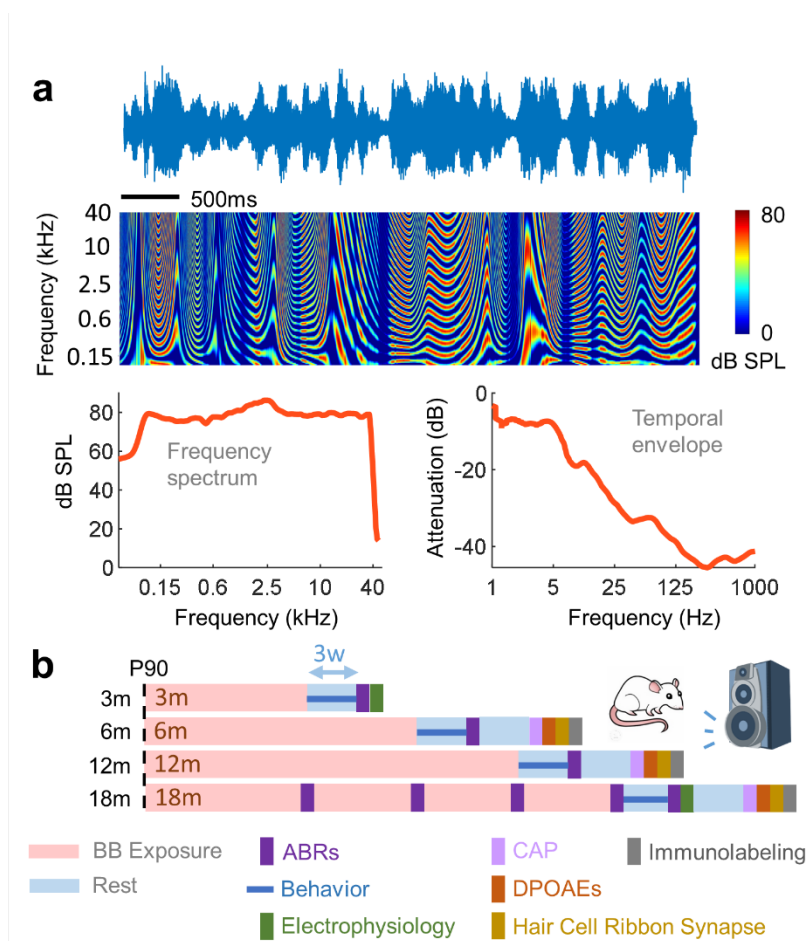
416

417 Results

418 Lifetime exposure and synaptopathy

419 Noise in urban, professional and leisure environments typically consists of continuous
420 broadband sounds with a low-pass temporal envelope (Supp. Fig. 1a-c). We designed a realistic
421 random noise mimicking these spectrotemporal properties, to assess the effects of lifetime
422 exposure to environmental noise on peripheral and central auditory processing (Fig. 1a). We
423 then exposed young (three-month-old) adult Sprague-Dawley rats to such noise at 80 dB SPL,
424 for 8 h per day over periods of 3, 6, 12 and 18 months (see suppl Table 1). By initiating exposure
425 in adult rats, we avoided the massive effects of developmental plasticity, which occur during
426 early exposure to noise (Barkat et al. 2011; de Villers-Sidani and Merzenich 2011; Bhumika et
427 al. 2020). The longest period of exposure (18 months) covers 70 to 80% of the typical lifespan
428 of Sprague-Dawley rats (680-760 days, see Davis et al. 1956; Durbin et al. 1966). Initial noise
429 exposure occurred post-sexual maturity, which is around P60 for female Sprague-Dawley rats
430 (Evans 1986). Although slightly exaggerated, our use of the word "lifetime" is consistent with
431 previous human studies implicitly dealing with noise exposure that occurred mostly during
432 adulthood (Prendergast et al. 2017; Valderrama et al. 2018). We analyzed the functional
433 properties of the peripheral and central auditory system of these rats three weeks after the
434 end of the exposure period, to investigate the long term, potentially permanent, effects (Fig.
435 1b). Specific aging effects were described in detail in a previous study on control animals from
436 the same cohort (Occelli et al. 2019). Briefly, we found that aging effects were very limited at
437 the periphery and moderate at the cortical level, also disfavoring any putative physiological
438 effects related to ambient noise in the control animal facility (<30dB SPL in the rat hearing

439 range). Unless otherwise indicated, all ANOVAs were three-factor tests (exposure x exposure
440 duration x stimulus parameter).



441

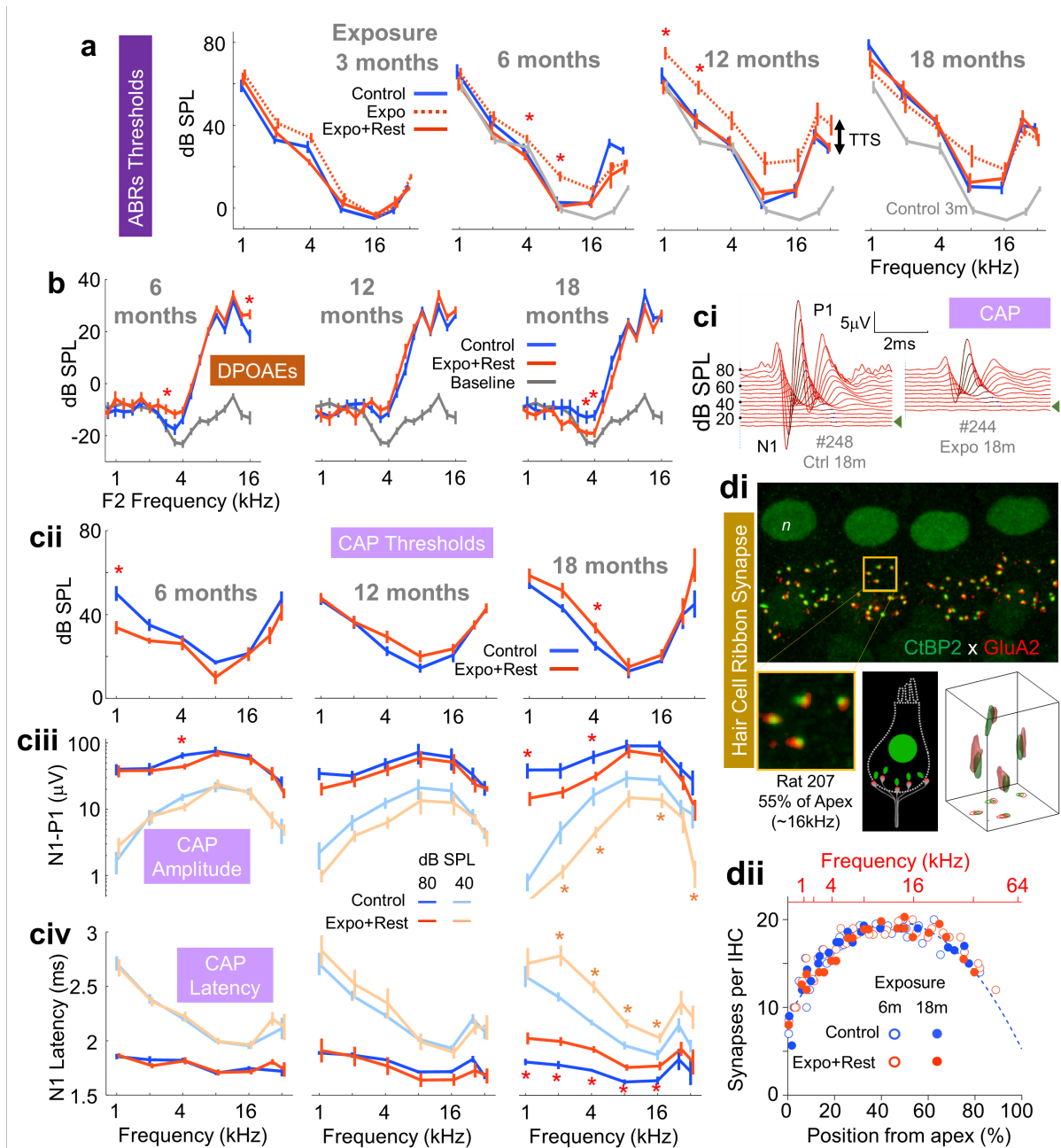
442 **Fig. 1: Lifetime exposure to a realistic environmental noise.** **a** We designed a broadband sound based on amplitude-
443 modulated dynamic moving ripples (Kowalski et al. 1996; Escabi and Schreiner 2002). A random low-pass temporal
444 envelope was applied to match environmental sounds. The overall spectrum was flat, to ensure that no particular
445 frequency band was favored. **b** Groups of P90 animals reared in a dedicated facility were exposed to this realistic
446 noise for 3, 6, 12 or 18 months. The sound intensity in the cage of the animal was 80 dB SPL (Supplementary Fig.
447 1d). At the end of the exposure period, each animal was subjected to the following protocols, in the following order:
448 a three-week behavior task; determination of auditory brainstem responses (ABRs); extracellular recordings in the
449 primary auditory cortex; two weeks of rest; functional assessments of the peripheral auditory system (CAP,
450 DPOAEs); immunolabeling of the cochlea and cerebral areas (see methods). For the animals exposed to noise for
451 18 months, ABRs were performed not only at the end of the exposure period, but also with no rest after 3, 6 and
452 12 months of exposure. The number of animals involved in each process is detailed in Supplementary Table 1.

453 It has been shown that temporary threshold shifts (TTS) following exposure to loud sounds (e.g.
454 100 dB SPL) can be accompanied by a “synaptopathy”: auditory thresholds return to normal
455 after a few weeks, but the response of the auditory nerve decreases, as do the number of
456 auditory fibers connected to inner hair cells and the distortion product otoacoustic emissions

457 (DPOAEs), all these effects being exacerbated by aging (Kujawa and Liberman 2009; Fernandez
458 et al. 2015; Hickox et al. 2017). Here, we explored whether hearing loss and/or synaptopathy
459 occurred after a lifetime of exposure to 80 dB SPL. We found that the auditory thresholds of
460 exposed rats, as determined by auditory brainstem recordings (ABRs), were similar to those of
461 age-matched control rats for all exposure durations, from 3 to 18 months (Fig. 2a, $F_{1,651}=1.99$,
462 $p=0.16$), suggesting that lifetime exposure (beginning early in adulthood) did not cause
463 permanent hearing loss. The largest ABR wave amplitudes were also unaffected (see Supp. Fig.
464 2b). Interestingly, the thresholds measured immediately after exposure, before the three-week
465 rest period, were significantly higher after six and 12 months of exposure (and after 3 and 12
466 months of exposure for ABR amplitudes, see Supp. Fig. 2b), suggesting that these animals
467 experienced a temporary threshold shift. After 18 months of exposure, this TTS was no longer
468 significant, probably because the rats were more than 21 months old and their auditory
469 thresholds had degraded due to aging, as previously shown (Ocelli et al. 2019).

470 We then investigated the peripheral auditory system of the animals after 6, 12 and 18 months
471 of exposure. We assessed the functional state of the outer hair cells by measuring DPOAEs.
472 There was a significant interaction between the effects of noise exposure and exposure
473 duration ($F_{2,1699}=1167$, $p<1e-10$, Fig. 2b), but discernible differences between the DPOAEs of
474 exposed and control animals were rare and restricted to the 3-4 kHz range, in which the
475 amplitude of DPOAEs is very low anyway. This suggests that noise exposure had little effect on
476 outer hair cell function after exposure to noise for three months or a lifetime. For the N_1 wave
477 of compound action potentials (CAPs), corresponding to wave I of ABRs and the activity of the
478 auditory nerve, noise exposure increased thresholds (Fig. 2cii, $F_{2,243}=880$, $p<1e-10$) but, again,
479 the magnitude of this effect was very modest (<10 dB for any frequency) and only significant at
480 4 kHz, after lifetime exposure. We found that latencies were longer (80 dB SPL, $F_{2,243}=13.2$,

481 $p < 1e-10$; 40 dB SPL, $F_{2,243} = 8.4$, $p = 3e-4$, Fig. 2civ) and CAP amplitude was lower (80 dB SPL,
482 exposure factor, $F_{2,243} = 12.2$, $p = 6e-4$; 40 dB SPL, $F_{2,243} = 4.9$, $p = 9e-3$) at many frequencies, after
483 lifetime exposure (Fig 2ciii, and Fig2civ). Indeed, for all thresholds, latencies and amplitudes,
484 significant differences in post-hoc tests were found between control animals and animals
485 exposed to individual frequencies only after 18 months of exposure. Latencies were the most
486 widely affected parameter of CAPs (Fig2civ), and the effects of noise exposure were visible both
487 at suprathreshold (80 dB SPL) levels and at a lower level (40 dB SPL). In the presence of a
488 synaptopathy, these effects would be accompanied by a decrease in the number of synapses
489 per inner hair cell (IHC). We estimated the change in the number of synapses per IHC from a
490 3D reconstruction of pre- and post-synaptic structures in stacked confocal images (Fig. 2di).
491 Exposure to noise for 6 or 18 months had no effect on the number of synapses per IHC
492 ($F_{1,111} = 0.04$, $p = 0.84$, Fig. 2dii). Overall, these results suggest that the peripheral auditory system
493 of rats is remarkably robust to moderate noise exposure for at least 12 months.



494
 495 **Fig. 2: Effects of broadband noise exposure on the peripheral auditory system.** *a* Average auditory thresholds
 496 obtained by auditory brainstem recordings (ABRs) for exposure durations of 3 (left) to 18 (right) months. There is a
 497 significant temporary threshold shift after 6 and 12 months of exposure (exposure factor, 3 months, $F_{1,215}=3.63$,
 498 $p=0.06$, 6 months, $F_{1,234}=23.79$, $p<1e-10$, 12 months, $F_{1,213}=39.32$, $p<1e-10$, 18 months, $F_{1,142}=0.86$, $p=0.35$, t -tests
 499 $*p<0.05$, for this and subsequent plots). *b* DPOAEs for exposure durations of 6, 12 and 18 months. The gray line
 500 indicates the noise level. *ci* Examples of CAP recordings obtained from a control animal (left) and an animal exposed
 501 (right) to noise at intensities ranging from 10-80 dB. *cii* CAP thresholds for exposure durations of 6, 12 and 18
 502 months. *ciii* CAP amplitude at 80 dB SPL (dark colors) or 40 dB SPL (light colors) for exposure durations of 6, 12 and
 503 18 months. *civ* CAP latency after noise exposure, presentation as in *ciii*. *di* Simultaneous labeling of presynaptic
 504 inner hair cell (IHC) ribbons with a mouse anti-CtBP2 antibody (green) and of postsynaptic glutamate receptors
 505 (GluA2 subunit, red). The lower left panel shows a magnification of labeling at the synaptic level. The lower central
 506 panel illustrates the theoretical expected juxtaposition of labeling for pre- and post-synaptic structures for a given
 507 hair cell. The lower right panel shows the use of a 3D, custom-developed algorithm to detect the juxtaposition of
 508 pre- and post-synaptic structures in stacked confocal images, and to identify the synapses for a given IHC. *dii*
 509 Number of synapses per IHC as a function of the position of the ribbon synapse along the cochlea (abscissa). The
 510 frequencies, corresponding to cochlear locations, are indicated on the top axis (red). Each dot represents the mean
 511 for six consecutive IHCs (Bourien et al. 2014). The dashed curve is a second-order polynomial fit to all data ($f(x) = -$

512 $0.005x^2+0.459x+8.5$, $r^2 > 0.9$, where x is the position relative to the apex, expressed in percent). **a,b,c** Error bars
513 indicated the SEM.

514 Cortical evoked response is affected by 3 months of moderate noise exposure

515 We used a battery of acoustic stimulations to investigate the effects of three to 18
516 months of exposure on the responses of auditory cortex neurons. Moderate noise exposure
517 has already been shown to affect these neurons (Noreña et al. 2006; Zheng 2012; Zhou and
518 Merzenich 2012; Thomas, Friedman, et al. 2019). Here, we derived the evoked-to-spontaneous
519 firing rate ratio (ESR) as a “normalized” measurement of firing rate (Manunta and Edeline 1997;
520 Novák et al. 2016; Pauzin and Krieger 2018). Furthermore, as firing rates typically have a skewed
521 statistical distribution, we converted this distribution to a gaussian form by applying a 20Log_{10}
522 transformation. The ESR is therefore expressed in dB. We provide a rationale for this choice in
523 Supp. Fig. 3^{bis}b.

524 The tuning properties of cortical sites are classically characterized by determining the
525 pure tone spectrotemporal receptive field (STRF^{pt}), defined as the time-frequency response of
526 neurons to pure tones at 75 dB SPL (Fig. 3ai). The best frequency (BF) is that eliciting the highest
527 firing rate. The distribution of BFs in our study was similar in all groups (Supp. Fig. 3b). Contrary
528 to several previous studies (Noreña et al. 2006; Zheng 2012), we found no significant effect of
529 our exposure regimen on tonotopy, i.e. on the topographic organization of BFs in the primary
530 auditory cortex and the anterior auditory field (Supp. Fig. 3c). However, the ESR at and around
531 the BF was reduced by three months of noise exposure ($F_{1,100213}=137$, $p<1e-10$, Fig. 3aii). This
532 decrease was not due to a change in the evoked firing rate (Supp. Fig. 3a). Instead, it was due
533 to the increase in (baseline) spontaneous firing rate observed after three months of noise
534 exposure ($F_{1,1513}=31.6$, $p<1e-10$, t -tests in Fig. 3aiii, see also Supp. Fig. 3a). The lower ESR led to
535 a decrease in the bandwidth of neurons, i.e. the range of frequencies to which cortical sites

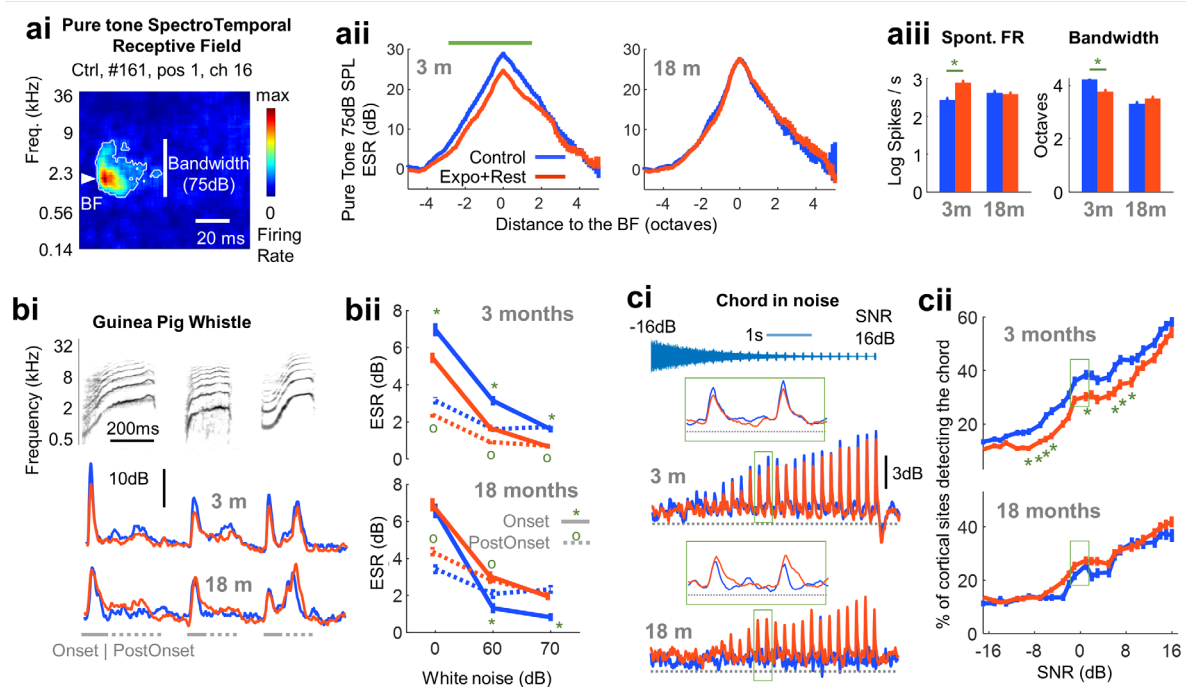
536 responded ($F_{1,1625}=39$, $p<1e-10$, t -tests in Fig. 3aiii) and a degradation of cortical auditory
537 thresholds (Supp. Fig. 3ei). By contrast, 18 months of noise exposure had no effect on ESR,
538 bandwidth, cortical thresholds or the spontaneous activity of cortical sites (Fig. 3aii,aiii and
539 Supp. Fig. 3eii).

540 We obtained similar results for ESR when pure-tone stimulation, as in STRFs^{pt}, was
541 replaced by broadband white noise with different rising times (from 10 to 250 ms, Supp. Fig.
542 3fi and fii). These results were also confirmed with natural sounds, including a guinea pig
543 whistle (Fig. 3bi). The mean response time revealed that the onset peak response occurring at
544 each salient part of the whistle was lower in rats exposed to noise for three months than in
545 unexposed rats of the same age ($F_{1,3200}=66$, $p<1e-10$, t -tests in Fig. 3bii). This decrease also
546 applied to the post-onset parts of the neural response ($F_{1,3204}=18.9$, $p<1e-10$, Fig. 3bii). The
547 results on the onset and post-onset responses were similar following the addition of 60 or 70
548 dB of noise to the vocalization. The addition of noise generally affected the onset more than
549 post-onset part, as also illustrated by the mean response time in the presence of noise (Supp.
550 Fig. 3^{bis}a). Here again, 18 months of exposure did not decrease, and indeed even increased the
551 onset and post-onset ESR for all noise levels (except post-onset, 70 dB, Fig. 3bii). The similar
552 post-onset ESR values between the three and 18 month groups for control animals is
553 noteworthy, and suggests that the onset was heavily degraded by the 60/70 dB noise in older
554 control animals, unlike the post-onset part.

555 We were intrigued by this finding that a lifetime of exposure to noise actually improves
556 the ESR in the presence of noise. We pursued our investigations further, by testing the response
557 to a chord (4 kHz tone and its harmonics, all with equal amplitude) presented in progressively
558 decreasing levels of noise (Fig. 3ci). The signal-to-noise ratio (SNR) of this stimulus ranged from

559 -16 to +16 dB. A neural response to the chord emerged from the noise at a SNR of about -4/-5
560 dB in all groups (Fig. 3cii). As shown by the mean time and the grouped data, chord detection
561 was reduced by exposure to noise for many SNR values for animals exposed to noise for three
562 months ($F_{1,48449}=175$, $p<1e-10$, t -tests in Fig. 3cii), but not in the group of animals exposed to
563 noise for 18 months. Interestingly, there was no interaction between exposure to noise for
564 three months and SNR level ($F_{29,48449}=1.4$, $p=0.07$). In other words, exposure to noise for three
565 months decreased the ESR to the chord regardless of the SNR of the stimulus, thereby altering
566 the ability of cortical sites to detect a salient sound in noise. Surprisingly, lifetime exposure to
567 noise did not impair nor improve the ESR significantly in the presence of noise.

568 Overall, our results for all the artificial and natural stimuli described above suggest that
569 a relatively “short” exposure to noise of three months, frequently considered in published
570 studies to be quite a long period of exposure given the lifespan of rodents, alters the response
571 of the core auditory cortex by increasing spontaneous activity, consequently reducing the
572 “signal-to-noise ratio” of the evoked onset response to sounds. However, these effects tend to
573 disappear rather than increase with the duration of exposure, and are no longer detected after
574 18 months of exposure.



575 **Fig. 3: Exposure to moderate noise for three months decreases the evoked response of the auditory cortex.** *ai* The
576 spectrotemporal receptive field (STRF^{pt}) is the discharge rate of a cortical site as a function of frequency (y-axis)
577 and time (x-axis) after acoustic stimulation with pure tones presented at 75 dB SPL. The best frequency of the
578 cortical site (BF), i.e. the frequency eliciting the highest discharge rate, and the bandwidth (frequency band to which
579 the cortical site responds) can be extracted from the STRF^{pt}. **aii** Averaged frequency profile of the STRF^{pt} (t-tests:
580 $p < 0.05$ for each frequency corresponding to the green line). **aiii** Spontaneous firing rate (left) and bandwidth of
581 STRFs at 75 dB SPL (right). T-tests: * $p < 0.05$, for this and all subsequent plots. **bi** Average response of all cortical
582 sites from each group to three guinea pig whistles (represented by the spectrograms at the top). We distinguished
583 the onset peak time intervals (gray solid line) from the post-onset time interval (gray dotted line). The onset
584 response was reduced after three months, but not after 18 months of noise exposure. **bii** This result was statistically
585 confirmed (see main text) by the quantification of onset and post-onset firing rates in response to the whistle in a
586 background of silence or in the presence of background noise. **ci** Average response of cortical sites to a chord
587 presented in progressively decreasing levels of noise. The peak value for the maximum response is reduced after 3
588 months of exposure for many noise levels, but not after 18 months. The green rectangles focus on the response for
589 two levels of noise presented in the insets. **cii** Percentage of cortical sites showing a significant peak response to
590 the chord in noise as a function of the signal-to-noise ratio (SNR) of the stimulus.

592 Lifetime exposure degrades some temporal aspects of the cortical response

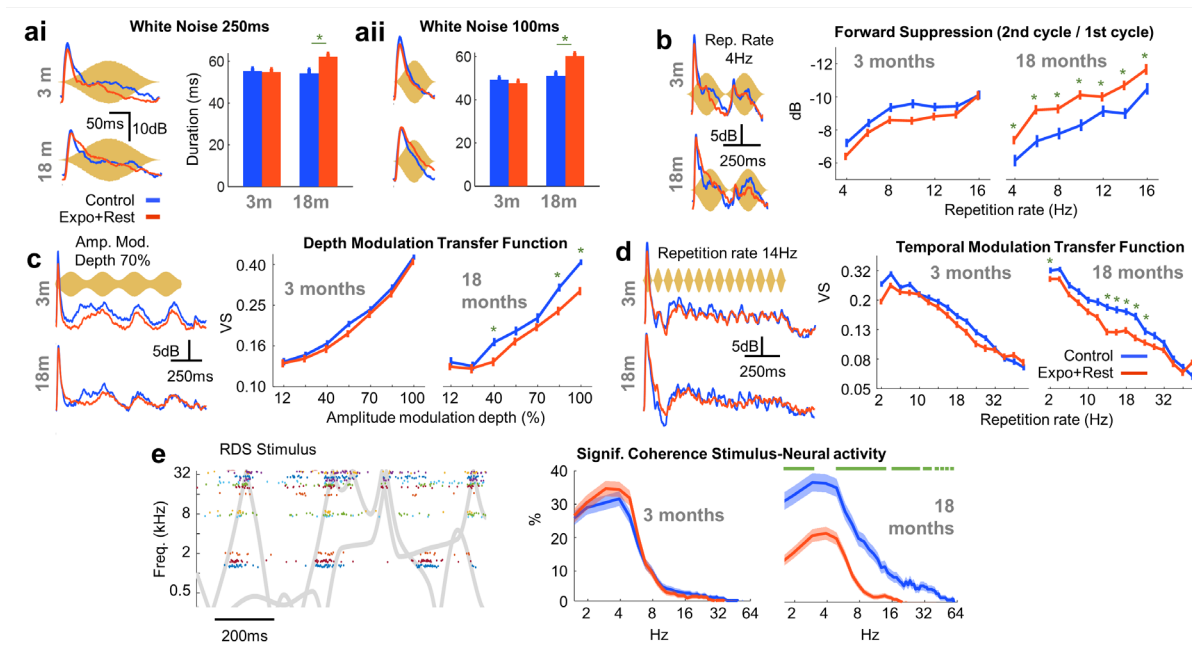
593 It was clear from our results (Fig. 3) that lifetime exposure affected the duration of the
594 cortical response (see prolonged post-peak activities in the green rectangle in Fig. 3ci). We then
595 investigated the temporal aspects of the cortical response (Fig. 4). We first showed that 18
596 months, but not three months, of noise exposure increased the duration of the peak response
597 (see methods) to white noise bursts, which were either 250 ms ($F_{1,1625}=6.4$, $p=0.01$, t-tests in
598 Fig. 4ai) or 100 ms long ($F_{1,1625}=14.4$, $p=2e-4$, t-tests in Fig. 4aaii). We suspected that such an
599 increase in the duration of the response might result from an exposure effect on neural

600 adaptation, and, thus, on the processing of sequences of acoustic events. A comparison of the
601 ESRs to the first and second cycles of amplitude-modulated white noise showed that the
602 (forward) suppression of the response to the second cycle was much stronger after 18 months
603 than after three months of exposure ($F_{1,11295}=92.6$, $p<1e-10$, t -tests in Fig. 4b). We reasoned
604 that this increase in forward suppression might affect the detection of short transients, such as
605 gaps, by decreasing the post-gap neural peak response but it was not the case (Supp. Fig. 4b).

606 However, the increase in response duration affected the depth modulation transfer
607 functions: after a lifetime of exposure, the ability of cortical neurons to distinguish 40%, 85%
608 and 100% amplitude modulation in white noise ($F_{1,7571}=9.5$, $p=2e-3$, t -tests in Fig. 4c) was lower
609 than that of unexposed animals. Could the changes in modulation depth processing be due to
610 a decrease in the dynamic range of neurons, with cortical sites able to respond to a smaller
611 range of intensity levels before reaching saturation? More non-monotonic rate-intensity
612 functions were observed after a lifetime of exposure, but the overall slope and dynamic range
613 extracted from these functions were not modified by exposure, irrespective of its duration
614 (Supp. Fig. 4ci,cii,ciii,civ).

615 We then measured temporal modulation transfer functions, which track the phase-
616 locking properties of neurons to various rates of amplitude modulation (Fig. 4d). We observed
617 a decrease in the phase-locking abilities of neurons for fast modulation rates, between 14 and
618 24 Hz, after 18 months of exposure ($F_{1,14847}=12.1$, $p=5e-4$, t -tests in Fig. 4d). Consistent with
619 our previous results on depth modulation processing (Fig. 4c) and forward suppression (Fig.
620 4b), this result suggests that the ability of neurons to follow temporal patterns in complex
621 sounds is degraded by noise exposure. We addressed this hypothesis by computing, for each
622 cortical site, the spectral coherence between spiking activity and the spectrogram of a complex

623 sound (the random double sweep, RDS, Gourévitch et al., 2015) taken at the BF of the cortical
 624 site (Fig. 4e). After a lifetime of exposure (but not after three months of exposure), we observed
 625 a massive decrease in the coherence between the RDS stimulus and the neural response, for
 626 most of the temporal modulation rates present in the stimulus ($F_{1,107315}=470.4$, $p<1e-10$, t -tests
 627 in Fig. 4e).

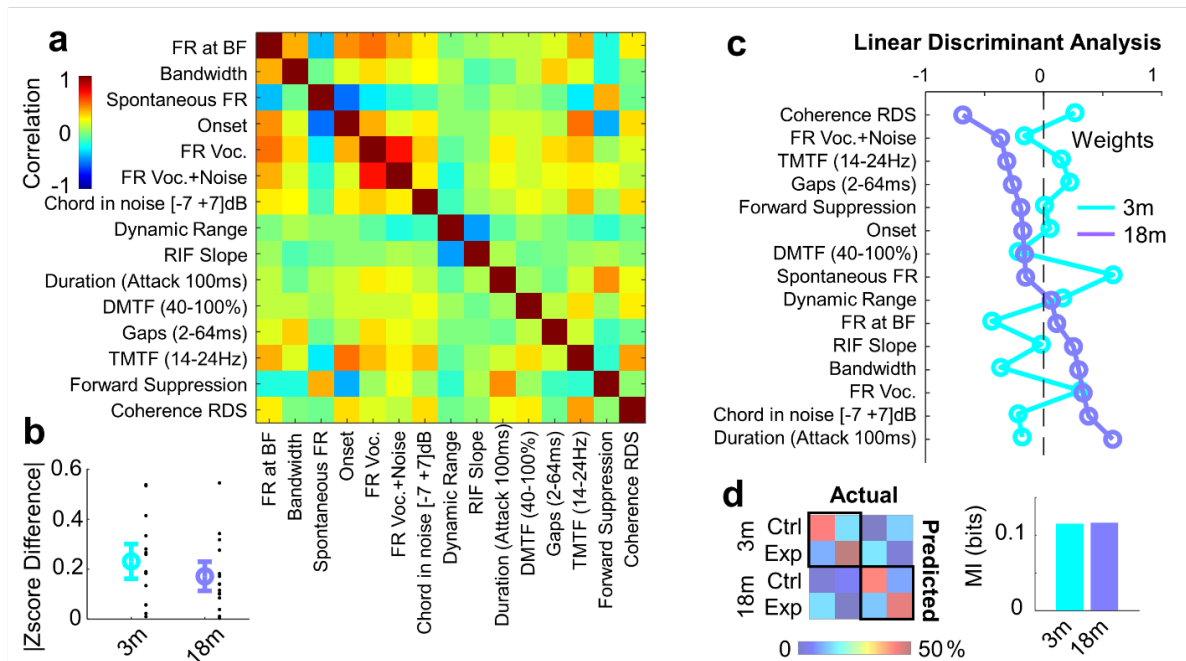


628
 629 **Fig. 4: Lifetime exposure to moderate noise affects the temporal response of the auditory cortex.** *ai* (left) Average
 630 response of all cortical sites to a 250 ms broadband white noise burst (125 ms rising time). (right) Quantification
 631 of the duration of the response evoked by a white noise stimulus lasting 250 ms. Post-hoc t -test: $*p<0.05$ as in all
 632 subsequent plots. *aii* As in *ai* for a 100 ms white noise stimulus. *b* (left) Average response of all cortical sites to two
 633 cycles of 250 ms broadband white noise bursts. (right) Quantification of forward suppression, estimated by
 634 calculating the ratio of the maximum firing rate evoked by the second cycle to that evoked by the first cycle. *c* (left)
 635 Average response of all cortical sites to four cycles of 250 ms broadband white noise bursts with a depth modulation
 636 of 70%. (right) Quantification of phase-locking, as measured by determining vector strength (VS) as a function of
 637 amplitude modulation depth. *d* (left) Average response of all cortical sites to white noise bursts repeated at a rate
 638 of 14 Hz. (right) Quantification of phase-locking, as measured by determining vector strength as a function of
 639 modulation rate, i.e. the neural temporal modulation transfer function (TMTF). *e* (left) A one-second excerpt of the
 640 response of 16 cortical sites to the random double sweep stimulus (RDS), the energy of which is represented in the
 641 time-frequency domain (kHz vs ms) in light gray. Each point is a spike and each cortical site is displayed at the
 642 ordinate corresponding to its best frequency, in its own color. (right) Percentage of neurons displaying significant
 643 coherence between the response of a cortical site and the spectrogram of the RDS taken at the best frequency of
 644 the cortical site, as a function of the temporal modulation rate of the stimulus. The green line indicates a significant
 645 difference between the two groups $*p<0.05$, t -test.

646 These results indicate that, after a lifetime of noise exposure, cortical degradation affects the
 647 temporal aspects of neuronal responses and differs from the rate-related effects observed after
 648 three months of exposure.

649 Multidimensional analysis

650 The above findings were objectively quantified in a multidimensional analysis. We extracted 15
651 variables from previous analyses and standardized them (Fig. 5a). Exposure had an effect of
652 similar magnitude on these variables in animals exposed to noise for three or 18 months (Fig.
653 5b). We also used this matrix of weakly correlated variables to build linear discriminant
654 functions for distinguishing between our four experimental groups (3 months of exposure, 18
655 months of exposure, and unexposed animals of the same ages, Fig. 5c). Such discrimination was
656 possible, as the rate of successful cortical site classification into the correct original group
657 ranged from 40 to 50% (Fig. 5d). We then computed the mutual information (Fig. 5d, right) of
658 each submatrix (black squares, Fig. 5d, left) associated with a duration of exposure. The mutual
659 information was similar for both durations of exposure, implying that it was no easier to
660 separate the cortical sites of exposed and unexposed animals after an 18-month exposure
661 period than after a three-month exposure period, at least on the basis of our characterization
662 of cortical activity. The linear discriminant function for distinguishing between the cortical sites
663 of exposed and unexposed animals in the 18-month group (Fig. 5c) did not correlate with that
664 of the three-month group ($\text{corr}=-0.39$, $p=0.15$). Overall, these results suggest that the cortical
665 effects observed after 18 months of exposure were no stronger than or related to those
666 observed after three months of exposure.



667

668 **Fig. 5: The effects of lifetime and short-term exposure on the auditory cortex are orthogonal.** **a** Matrix of the
669 correlation between variables, summarizing the previous results (description at the end of the caption). **b** Z-score
670 difference in absolute values between exposed and unexposed animals for the 3-month and 18-month exposure
671 groups (t-test, $T=-6.33$, $p=0.1$). **c** Linear discriminant function: linear combination of the variables best separating
672 the cortical sites of exposed and unexposed animals from the three-month and 18-month exposure groups. Weights
673 are displayed and were sorted according to their contribution to the 18-month exposure function. **d**(left) Confusion
674 matrix obtained for the linear discriminant analysis displaying the classification of neurons achieved with the
675 parameters displayed in **a**. (right) Mutual information quantification of 2x2 submatrices (outlined in black) for each
676 duration of exposure. The variables were as follows: firing rate (FR) at BF: normalized FR at the BF (Fig. 3a_{ii});
677 bandwidth: as in Fig. 3a_{iii}; spontaneous FR: as in Fig. 3a_{iii}; onset: as in Supp. Fig. 3f_{ii} averaged across all values of
678 rising slope; FR voc: FR response to vocalization as in Fig. 3 b_{ii}, 0dB noise; FR Voc. + Noise: onset response as in Fig.
679 3 b_{ii}, averaged between 60 and 70 dB of noise; chord in noise [-7 +7]dB: as in Fig. 3c_{ii}, averaged across SNR levels
680 between -7 and +7 dB, i.e. those with significant differences between exposed and unexposed cortical sites; dynamic
681 range: as in Supp. Fig. 4c_{iii}; RIF slope: slope of rate-intensity function as in Supp. Fig. 4c_{iv}; duration (rising slope
682 100 ms): as in Fig. 4a_{ii}; DMTF (40-100%): depth modulation transfer function as in Fig. 4c, averaged across
683 modulation depth values between 40 and 100%; gaps (2-64 ms): as in Supp. Fig. 4b, averaged across gap durations
684 between 2 and 64 ms; TMTF (14-24 Hz): temporal modulation transfer function as in Fig. 4d, averaged across
685 modulation rates between 14 and 24 Hz; forward suppression: as in Fig. 4b, averaged across repetition rates
686 between 4 and 16 Hz; coherence RDS: as in Fig. 4e, averaged across frequencies between 1.5 and 16 Hz.

687

688 Lifetime noise exposure does not degrade behavioral performance in a depth

689 modulation task

690 During the initial design of the protocol for this study, it was not possible to predict the

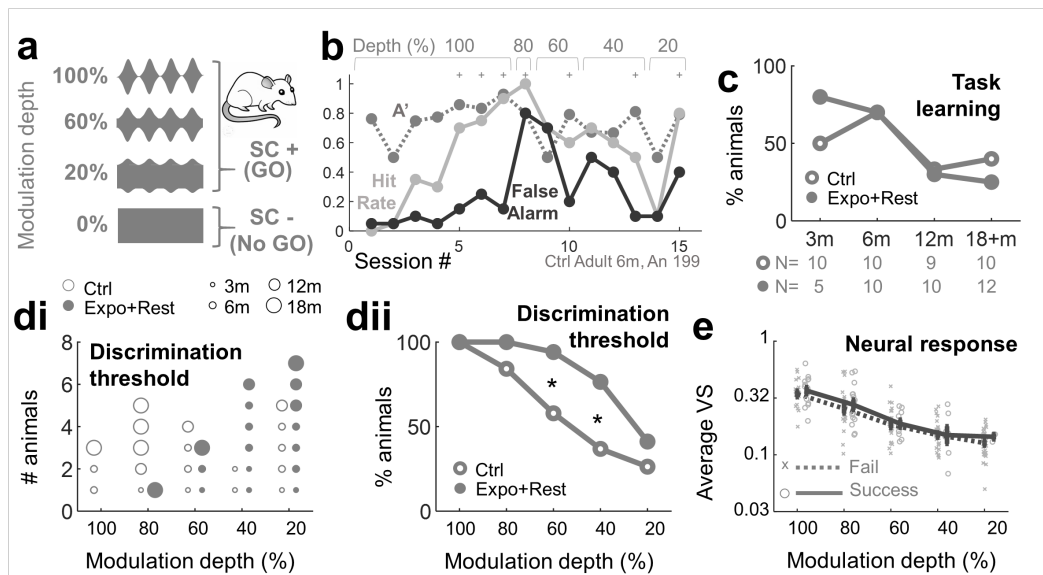
691 perceptive aspects potentially altered by lifetime exposure. We suspected that the prolonged

692 auditory masking undergone by animals due to noise exposure might affect their ability to

693 detect subtle changes in amplitude modulation. We thus decided to test the ability of animals
694 to detect low levels of depth modulation during a Go/NoGo task (Fig. 6a-b), using white noise
695 with the same amplitude modulations as in Fig. 4c. The findings reported above suggest that
696 the neural representation of modulation depth in the core auditory cortex is significantly
697 degraded by lifetime exposure to noise (see Fig 4c).

698 Behavioral experiments generated two results inconsistent with this view. First, the ability to
699 learn the task with a modulation depth of 100% was unaffected by exposure to noise,
700 regardless of its duration (Fig. 6c, exposure effect, $F_{1,68}=0.06$, $p=0.8$; interaction exposure and
701 duration of exposure, $F_{3,68}=0.6$, $p=0.62$). We detected an age-related deficit in learning ability
702 in both groups (duration of exposure, $F_{3,68}=3.3$, $p=0.025$; see also our previous study Ocelli et
703 al. 2019). Second, although the number of animals that successfully learned the task (with our
704 criteria, see Methods) was limited (19/39 in the control group and 17/37 in the exposed group),
705 these animals had slightly better performances for discriminating small variations of depth
706 modulation after 3, 6, 12 and 18 months of exposure to noise (Fig. 6d, Supp. Fig. 6b). This
707 slightly better performance did not seem to be related to motor abilities, as the latency of
708 motor responses to the CS+ stimulus was similar in exposed and unexposed animals (Supp. Fig.
709 6c). This result contrasts sharply with those for electrophysiology. Actually, we found that the
710 behavioral output of a given animal (success or failure) was not dependent on the mean phase-
711 locking level measured in the neural population for the animal concerned, regardless of
712 amplitude modulation depth or noise exposure status (Fig. 6e).

713 The above results suggest that either a few months or a lifetime of noise exposure does not
714 adversely affect the detection and discrimination of amplitude modulation depth, despite its
715 degraded neural representation in the auditory cortex.



716
 717 **Fig. 6: Depth modulation perception is not damaged by long-term noise exposure.** **a** The behavioral task was an
 718 aversive Go-NoGo protocol in a shuttle box. The animal had to discriminate between noise with (SC+, Go) and
 719 without (SC-, NoGo) amplitude modulation at 4 Hz, the level of depth modulation varying between 20 and 100%
 720 (the same stimulus as that used to test neuronal responses, see Fig. 4c). **b** Individual example of a learning curve:
 721 first sessions include only a depth modulation of 100% to enable the animal to learn the task, a condition considered
 722 to be achieved after three successive sessions with an A' value >0.75. Depth modulation was then progressively
 723 decreased until A'>0.75 for a session. Failure in three successive sessions was the criterion for stopping the task.
 724 The threshold was defined as the last depth modulation successfully achieved. **c** Percentage of animals that were
 725 able to learn the task. **di** For the animals that successfully learned the task at a modulation depth of 100%, the
 726 threshold of depth modulation for each animal is shown as a circle, the diameter of which is proportional to
 727 exposure duration. For animals able to learn the task, noise-exposed animals (filled circles) had slightly better
 728 discrimination thresholds than those unexposed to noise. **dii** Percentage of animals achieving correct
 729 discrimination. Exposure facilitated discrimination, whatever its duration (three-way ANOVA: exposure, exposure
 730 duration, modulation depth, $F_{1,152}=14.9$, $p=2e-4$). The hypothesis of a normal distribution of the residuals for this
 731 model could not be rejected (Supp. Fig. 6a). Data for each exposure duration are shown in Supp. Fig. 6b. The
 732 percentage difference between exposed and unexposed animals was significant for modulation depths of 60% and
 733 40% (proportion test, $p=0.012$, $p=0.017$ respectively, "*" in the plot). **e** Average VS of neurons as in Fig. 4c for all
 734 groups and for animals that failed (dotted curve, round markers) or succeeded (plain curve, cross markers) in the
 735 behavioral task for each amplitude modulation depth. No relationship was found between the behavioral output
 736 of the animal and the mean VS of neurons from the auditory cortex (four-way ANOVA: exposure, exposure duration,
 737 modulation depth, and behavioral output; behavioral output effect, $F_{1,4952}=2.2$, $p=0.14$; interaction exposure and
 738 behavior, $F_{1,4952}=1.1$, $p=0.3$; interaction modulation depth and behavior, $F_{4,4952}=1.2$, $p=0.31$).

739

740 Discussion

741 Prolonged or lifetime exposure to a daily noise dose of 80 dB SPL has long been considered to
 742 result in a negligible or no permanent threshold shift in humans (ISO 1990; Lawton 2001) and
 743 animals (Canlon and Fransson 1995; Noreña et al. 2006; Liu et al. 2020). Consistent with
 744 expectations, we detected no permanent threshold shift (greater than expected for age) in rats,
 745 even after a lifetime of exposure. As levels known to lead to noise-induced hearing loss are

746 similar in rats and humans (Chen et al. 2014), this result may be considered to validate current
747 employment regulations in industrial countries, which recommend the use of hearing
748 protection at noise levels above 80 dB SPL.

749 Nonetheless, the level of exposure tested here, 80 dB SPL daily, may induce a negligible TTS
750 (0-5 dB) in humans (Ward et al. 1976). It has been suggested that noise below a particular SPL
751 will produce no TTS, no matter how long an individual is exposed; this SPL, known as “effective
752 quiet” (Ward et al. 1976) has been estimated at between 76 and 78 dB(A) in humans (Ward et
753 al. 1976; Stephenson et al. 1980; Mills et al. 1981) and 77 dB SPL in rats (Chen et al. 2014). In
754 both humans and rats, 80 dB(A) or SPL, respectively, generates a very small TTS that rapidly
755 disappears. In addition, the TTS is thought to reach an asymptote after a few hours to days of
756 exposure at moderate levels (Viall and Melnick 1977; Woodford 1977). Contrary to these
757 hypotheses, we found here that a TTS can emerge (even after more than three months of
758 exposure without TTS), then increase between 6 and 12 months of exposure, and subsequently
759 becoming masked by the age-related threshold shift after 18 months of exposure (Fig. 2a).
760 Protective mechanisms, such as the medial olivocochlear reflex, may become weaker, or the
761 mechanisms associated with “ear toughening” (Niu and Canlon 2002) may become less efficient
762 over time. Alternatively, a TTS associated with moderate noise exposure may trigger damage
763 to the auditory system that accumulates over time. Indeed, mechanisms underlying TTS
764 observed with louder sounds (>100 dB SPL) involve several inner ear sensorineural structures,
765 including hair cells and their stereocilia, supporting cells within the organ of Corti, endothelial
766 cells and fibrocytes within the stria vascularis and spiral ligament, as well as dendritic processes
767 of the auditory nerve, through mechanical overstimulation, excitotoxicity and inflammatory
768 processes (Mulroy et al. 1990; Puel et al. 1998; Nordmann et al. 2000; Henderson et al. 2006;
769 Kujawa and Liberman 2009), all potentially contributing to so-called “hidden hearing loss” or

770 synaptopathy (Kujawa and Liberman 2015). It is, thus, crucial to determine whether moderate
771 noise-induced and synaptopathic noise-induced TTS have functional and anatomical
772 phenotypes in common.

773 The main phenotype (and definition) of synaptopathy is a loss of synapses between the IHCs
774 and spiral ganglion neurons (Kobel et al. 2017). Several studies have demonstrated
775 synaptopathy in rat models (Singer et al. 2013; Altschuler et al. 2016, 2019; Hickox et al. 2017),
776 including the Sprague-Dawley strain. However, we observed no such loss of synapses at any
777 cochlea location in our animals exposed to noise for 6 or 18 months (Fig. 2dii). It has been
778 suggested that a fine line at about 90 dB SPL separates neuropathic and non-neuropathic TTS
779 (Fernandez et al. 2015; Jensen et al. 2015). Consistent with this absence of synaptopathy, the
780 aging-related auditory threshold shift was not accelerated by lifetime exposure in the same way
781 as it is after synaptopathic events or early traumatic exposure (Kujawa and Liberman 2006;
782 Fernandez et al. 2015). If similar mechanisms are at work in rats and humans, our results
783 suggest that the workers in industrial countries protected by regulations limiting noise
784 exposure are unlikely to suffer from synaptopathy. Our results also suggest that the wave I
785 amplitude assay does not necessarily provide a reflection of underlying synaptic health
786 (Fernandez et al. 2015) or massive TTS (Lobarinas et al. 2017): a permanent decrease in CAP
787 amplitude (Fig. 2ciii) was observed in the presence of moderate TTS and absence of
788 synaptopathy.

789 DPOAEs were not affected by lifetime exposure in our conditions, suggesting that outer hair
790 cell functioning was intact or that there was a only a transient decrease in DPOAE amplitude
791 after noise exposure (Zhao et al. 2018). Nevertheless, they do not seem to be associated with
792 synaptopathic phenotypes (Kujawa and Liberman 2015).

793 With the notable exception of the CAP decrease in magnitude, the peripheral auditory system
794 was remarkably robust to lifetime noise exposure. Note, however, that female rodents are
795 slightly less prone to acoustic trauma than males, possibly due to the role of estrogen signaling
796 (Milon et al. 2018; Shuster et al. 2019; Lin et al. 2021). We investigated whether a similar
797 robustness applied at the cortical level. The adult brain is much more resistant to experience-
798 dependent plasticity than the juvenile brain (Keuroghlian and Knudsen 2007), but several
799 studies have shown that prolonged exposures at non-traumatic levels can trigger massive,
800 albeit reversible, plastic changes at the cortical level (Noreña et al. 2006; Pienkowski et al. 2011;
801 Zheng 2012; Zhou and Merzenich 2012; Lau et al. 2015; Thomas, Guercio, et al. 2019; Thomas
802 et al. 2020). Unlike Zheng (2012), we found no signs of tonotopy disruption in our animals,
803 whatever the duration of exposure (Supp. Fig. 3c). Any tonotopy disruption is unlikely to have
804 been completely reversed by the three-week rest period, as the time constants for such
805 reversals are typically longer (Pienkowski and Eggermont 2012). Functional reorganizations
806 may be absent because synaptic weight distributions were not “unbalanced” by our stimulus,
807 which should elicit excitation patterns similar to those for which the network has long been
808 shaped, unlike unmodulated white noise (Zheng 2012; Thomas, Friedman, et al. 2019),
809 narrowband sounds (Noreña et al. 2006) or fast noise bursts (Zhou and Merzenich 2012; Lau et
810 al. 2015). For instance, narrowband sounds may introduce an unbalanced excitation/inhibition
811 pattern, resulting in a decrease in evoked activity within the stimulation band, closely
812 associated with an increase in evoked activity outside that band (Noreña et al. 2006; Pienkowski
813 et al. 2013).

814 It has been suggested that prolonged passive exposure can also alter GABAergic expression
815 (Zhou et al. 2011; Zhou and Merzenich 2012), leading to a re-opening of the critical period
816 favoring functional reorganization (Zhou et al. 2011; Thomas, Friedman, et al. 2019). In

817 particular, this unbalanced excitation/inhibition state can elicit maladaptive cortical plasticity,
818 leading to auditory disorders, including tinnitus and hyperacusis, an unusual intolerance to
819 moderate sound intensities (Thomas, Guercio, et al. 2019). Correlates for such disorders may
820 involve decreases in the ratio of the mean and spontaneous firing rates (for tinnitus) or of the
821 mean and maximum firing rates (for hyperacusis (Pienkowski Martin et al. 2014)). We found no
822 neurophysiological sign of hyperacusis, such as an increased slope, in our neural rate-intensity
823 functions (Supp. Fig. 4c). However, we observed an increase in spontaneous, but not evoked,
824 activity after three months of exposure (Fig. 3a_{ii},a_{iii}; Supp. Fig. 3a), a possible correlate of
825 tinnitus (Noreña and Eggermont 2003; Munguia et al. 2013). Previous passive exposure studies
826 obtained the opposite result (Munguia et al. 2013; Pienkowski 2018), but were based on
827 narrowband stimulation. After a lifetime of exposure, the increase in spontaneous activity was
828 no longer detectable, which may appear counterintuitive. Indeed, the 18-month exposure
829 group had been experiencing TTS for months, and the decrease in auditory input may trigger
830 central plasticity (Roberts et al. 2010), as suggested by the frequent co-occurrence of hearing
831 loss with tinnitus and hyperacusis. It is possible that any “re-opening” of a critical period (or
832 imbalance in neural activity, as indicated by abnormal levels of spontaneous activity) remains
833 limited in duration or to a particular age range. Consistent with this idea, we observed no
834 change in GABA expression in the thalamocortical system after a lifetime of exposure (Supp.
835 Fig. 7a_{ii}).

836 Beyond spontaneous activity, is the neural representation of sound modified by prolonged
837 noise exposure? The available evidence is limited to rare electroencephalographic studies
838 (Kujala et al. 2004; Brattico et al. 2005; Samelli et al. 2012). In our study, despite the limited
839 peripheral effects, long-term (3 months) and lifetime (18 months) exposures to noise clearly
840 affected sound processing in the auditory cortex in surprisingly different manners. The increase

841 in spontaneous activity in the three-month exposure group may have led to a deterioration of
842 detection thresholds in the auditory cortex (Buran et al. 2014). We observed such a
843 deterioration in experiments involving stimulation with chords inserted in increasing levels of
844 noise (Fig. 3c) as well as when measuring cortical auditory thresholds (Supp. Fig. 3e). This
845 deterioration applied to “onset” and post-onset responses, as shown by the response times to
846 vocalizations (Fig. 3b).

847 Cortical effects observed after 18 months of exposure did not correlate with those observed
848 after three months of exposure (Fig. 5). Indeed, and importantly, temporal deficits were
849 observed only after lifetime exposure: the evoked response tended to lengthen (Fig. 4a),
850 accounting for a poorer ability of neurons to respond accurately to auditory contrasts (Fig. 4b),
851 fast temporal amplitude modulations (Fig. 4d) and, more generally, complex acoustic temporal
852 variations (Fig. 4e). One possible mechanism for this would involve a decrease in GABAergic
853 inhibitory expression, but we observed no such decrease (Supp. Fig. 7a_{ii}). The occurrence of a
854 degradation of temporal representations over relatively long time scales of hundreds of
855 milliseconds (forward suppression and depth modulation transfer functions at 4 Hz, Fig. 4bc)
856 but not very short ones (gap detection, Supp. Fig. 4b) suggests that other mechanisms, such as
857 synaptic depression (Wehr and Zador 2005) and, more generally, short-term plasticity may be
858 involved. A few studies have suggested that short-term plasticity may deteriorate with aging
859 (Mostany et al. 2013; Singh et al. 2018). Lifetime exposure may have accelerated this
860 deterioration of short-term plasticity, by as yet undetermined mechanisms.

861 Does this degradation of temporal abilities translate into a deterioration of auditory
862 performance? We directly quantified the behavioral response to amplitude-modulated noise
863 with various modulation depths. The success rate of learning for our young rats was relatively

864 low relative to that observed in a previous similar study (Kelly et al. 2006). There are at least
865 two main reasons for this: the number of sessions allowed for each rat to learn the task (10)
866 was probably too small and we also underestimated the difficulty of escape-avoidance learning
867 for rats in a two-way shuttle box where the safe and dangerous places are not perceptually
868 different (Theios and Dunaway 1964; Moot et al. 1974; Denny 2010). Nevertheless, we found
869 that noise exposure had no effect on the ability to learn the task (Fig. 6c). For the animals able
870 to learn the task at a modulation depth of 100%, noise exposure generally improved
871 modulation depth behavioral thresholds (Fig. 6d). This result is surprising, because it contrasts
872 with the generally good agreement between electrophysiological (cortical and subcortical) and
873 perceptive measurements of temporal abilities (Zhou and Merzenich 2012; Bharadwaj et al.
874 2015). It is possible that other brain areas involved in task learning (e.g., hippocampus, striatum,
875 prefrontal areas, amygdala) partially account for the behavioral performance in the task we
876 used, especially during aging (Cabeza et al., 2002; Milshtein-Parush et al., 2017; Moran et al.,
877 2014), such that the behavioral performance correlates less with the neural coding performed
878 by auditory cortex neurons. It is also surprising that noise exposure could facilitate specific
879 perceptive abilities, although such a phenomenon was already reported when testing is
880 performed in a noisy environment on adult rats (Zheng 2012) or when rat pups are reared in
881 the presence of noise (Homma et al. 2020). Given the limited number of animals able to learn
882 the behavioral task at a modulation depth of 100% in this study, further investigations and
883 attempts to replicate this finding are required. However, this finding also opens up intriguing
884 possibilities that cannot currently be ruled out. For instance, long-term exposure may elicit
885 mechanisms either enhancing the sound-in-noise detection or compensating for the degraded
886 neural representation of sounds, as during aging (Parthasarathy et al. 2019; Anderson et al.
887 2020) or hearing loss (Fuglsang et al. 2020). The auditory cortex recordings made in this study

888 do not support the hypothesis of improvements in the use of circuits dedicated to modulation
889 depth coding (Ding et al. 2014; Slama and Delgutte 2015; Fuglsang et al. 2020). Compensatory
890 top-down attentional processes (Spitzer et al. 1988; Fritz et al. 2007) could not be tested here
891 under anesthesia. However, plastic changes altering gain or excitatory/inhibitory balance
892 (Eggermont 2017; Parthasarathy et al. 2019) could have occurred throughout the animal's life
893 as suggested by the recovery of ESR measurements between three and 18 months of exposure.
894 In any case, the maintenance of task-learning ability in animals exposed to noise is consistent
895 with the weak evidence (Kumar et al. 2012; Hope et al. 2013) or total lack of evidence (Stephens
896 et al. 2003; Grose et al. 2017; Prendergast et al. 2017; Guest et al. 2018; Füllgrabe et al. 2020)
897 for a degradation of temporal abilities and, more generally, an impairment of speech
898 perception in humans due to occupational or leisure exposure to noise.

899 Our study therefore reveals several new paradoxes. A lifetime of exposure to noise does not
900 lead to structural damage to the synaptic ribbons, at least in our experimental conditions.
901 However, there seems to be an impact on auditory nerve activity, and our findings indicate that
902 TTS can develop progressively after months of exposure to noise. These findings call into
903 question the view that repeated daily noise exposure, even at a moderate SPL, does not
904 damage auditory system function over a period of years. This view is implicit in the occupational
905 regulations of industrialized countries, which are based on a daily permissible noise exposure
906 limit (85 dB(A), 8 hours per day in general). In addition, lifetime exposure can progressively
907 degrade the central representation of sounds, without necessarily affecting perception
908 abilities, raising a new possibility of passive exposure-induced plasticity over very long-time
909 scales.

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918 Author contributions

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920 Writing – Review & Editing, J.B, B.G, J-M.E; Funding Acquisition, Resources B.G, J-M.E, J-L.P.

921 Competing Interests

922 The authors declare no competing interests

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1221 Supplementary materials

Exposure	Behavior	AI	ABRs	ABRs (exposed, no rest)	CAP	DPOAEs	Immunochemistry (AC + MGB)	N cortical sites
3 months	10/5	9/10	10/9	17				449/427
6 months	10/10		10/10	18	7/9	8/9		
12 months	9/9		9/10	14	8/7	9/6		
18+ months	10/12 ^a	7/11	9/11	7	5/7	5/7	6/5	309/444

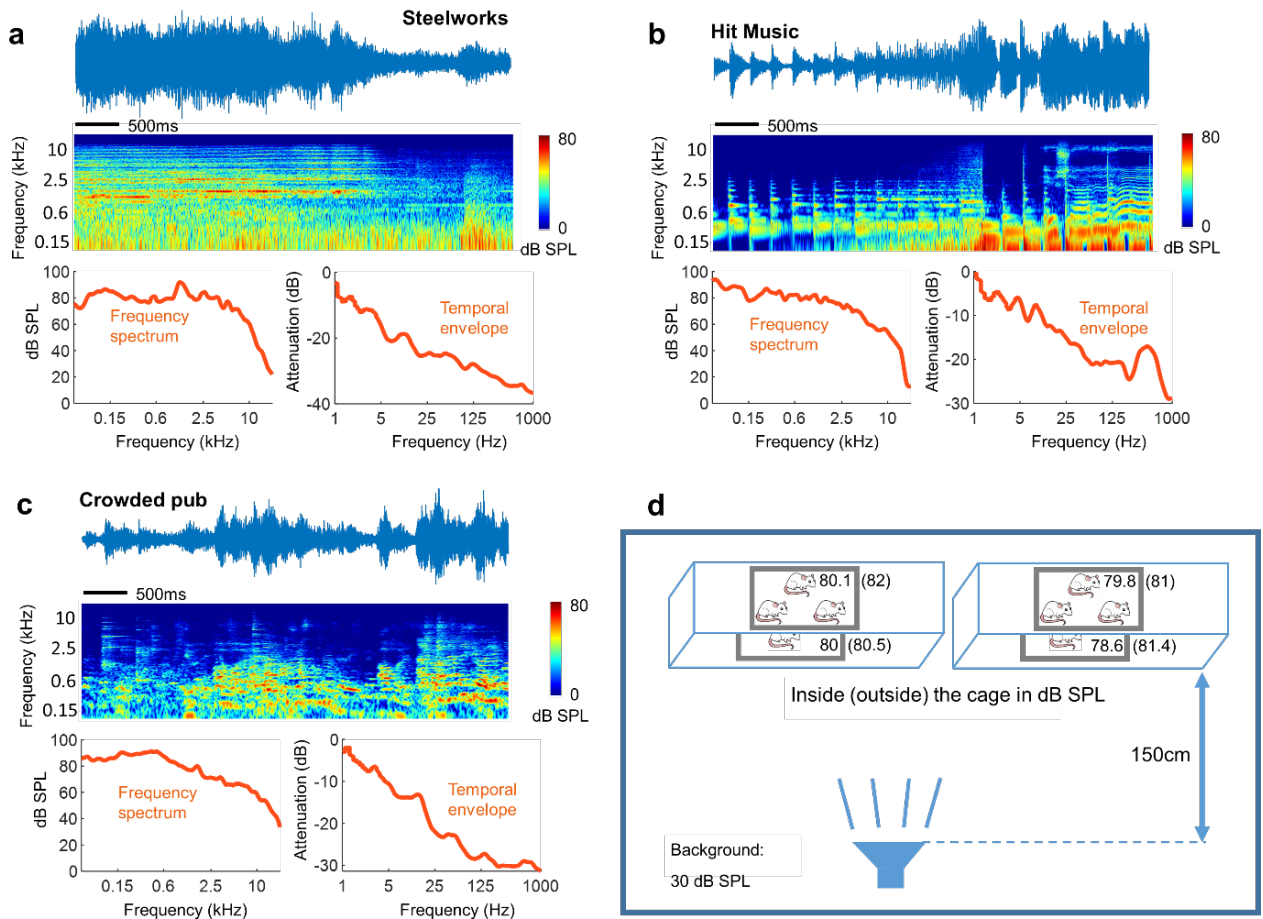
1222 **Table 1:** Number of animals (columns 2 to 8) and cortical sites (last column) for the different exposure duration
 1223 groups (control/exposed). Ten animals per group (20 per group for 18+ months) were initially planned. However, a
 1224 few animals died before, during or after surgery. Other animals were removed from the study because they
 1225 developed mammary tumors.

1226

Protocol	ABR thresholds / amplitude CAP thresholds / amplitude / latency	DPOAEs	STRFs ^{pt}	Chords in noise	BFs distribution
Corresponding figures	Fig. 1a,cii,cii,civ Supp. Fig. 2b	Fig. 1b	Fig. 3a ⁱⁱ	Fig. 3c ⁱⁱ	Supp. Fig.3b
Stimulus parameters	7	18	127	30	10
Protocol	Cortical thresholds	Attack duration	Forward Suppression	Depth Modulation Transfer Function	Temporal Modulation Transfer Function
Corresponding figures	Supp. Fig. 3e ⁱ ,e ⁱⁱ	Supp. Fig. 3f ⁱⁱ	Fig. 4b	Fig. 4c	Fig. 4d
Stimulus parameters	252	16	7	7	16
Protocol	Coherence RDS-Neural activity	Gap detection	Modulation depth		
Corresponding figures	Fig. 4e	Supp. Fig. 4b	Fig. 6e		
Stimulus parameters	129	6	5		

1227 **Table 2:** Number of stimulus parameters for the protocols used in the study.

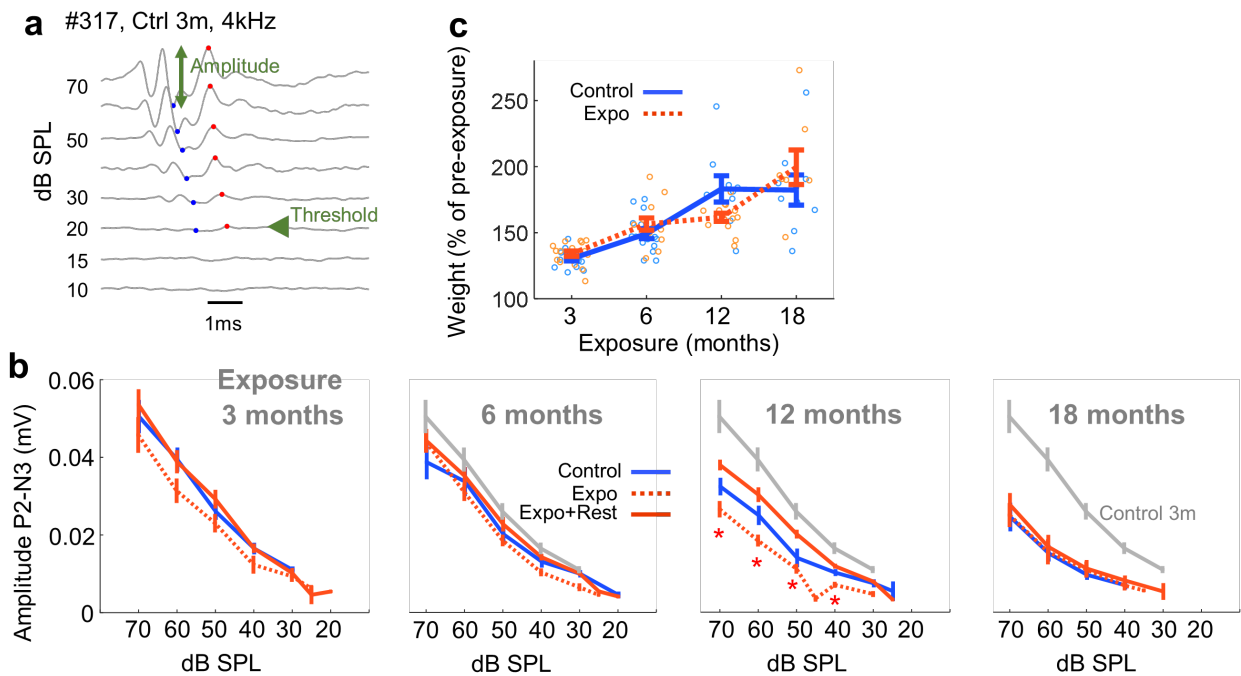
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1229

1230 **Supp. Fig. 1:** **a** As in Fig. 1a, acoustic signal (top), spectrogram (middle) and long-term frequency spectrum and
 1231 temporal envelope spectrum of the stimulus (bottom) for a recording in steelworks (personal recording). **b,c** as for
 1232 **a** but for hit music (David Guetta, Titanium) and a crowded pub (personal recording). All long-term frequency
 1233 spectra of such sounds from our working or leisure sound environments are somewhat flat or slightly low-pass up
 1234 to 10 kHz and the temporal envelope spectrum is typically low-pass with a predominance of amplitude modulations
 1235 below 5-10 Hz. **d** Rats were placed in a dedicated soundproof chamber facing a large speaker. The sound delivery
 1236 system was calibrated to achieve a flat spectrum inside the cages (which act as a natural low-pass filter) and the
 1237 SPL was also carefully checked inside and outside the cages.

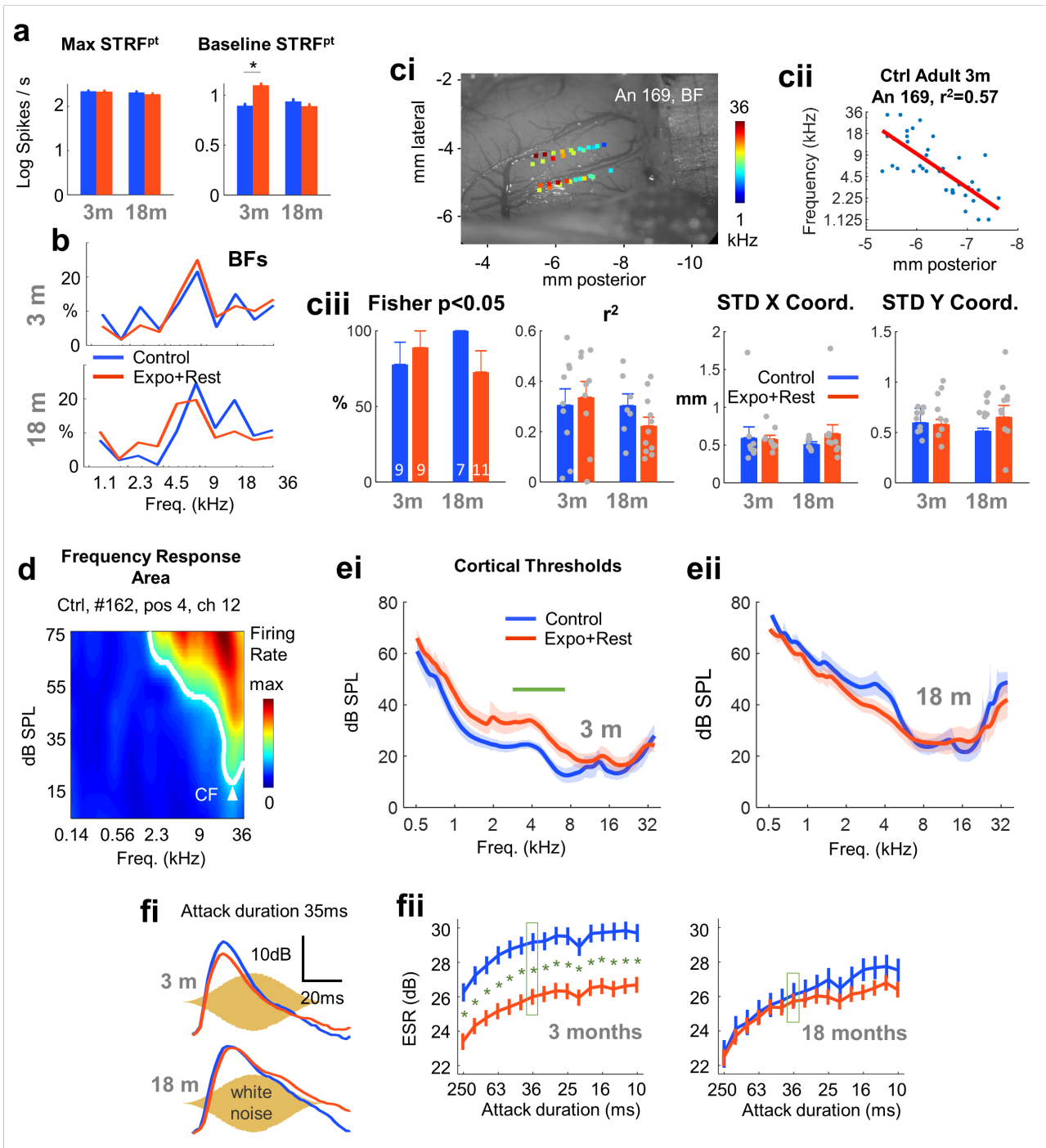
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1240 **Supp. Fig. 2:** **a** Individual example of auditory brainstem recordings (ABRs) from which the peak-to-peak amplitude
 1241 of the largest waves (P2-N3) and the auditory threshold were extracted. **b** Average P2-N3 amplitude for exposure
 1242 durations of between 3 (left) and 18 (right) months. Exposure had no significant permanent effect on amplitudes
 1243 ($F_{3,277}=0.63$, $p=0.59$). However, there was a significant temporary threshold shift after 3 and 12 months of exposure
 1244 (Expo vs. Expo+rest, exposure factor, 3 months, $F_{1,93}=6.65$, $p=0.01$, 6 months, $F_{1,102}=3.82$, $p=0.05$, 12 months,
 1245 $F_{1,81}=68.7$, $p<1e-10$) but not after 18 months ($F_{1,56}=0.45$, $p=0.50$, t -tests $*p<0.05$). **c** Follow-up of the weight of
 1246 animals in the 18-month exposure group, as a percentage of pre-exposure weight and as a function of exposure
 1247 duration. Weights obtained after 3, 6 and 12 months of exposure do not include a 3-week rest period. The global
 1248 difference between exposed and control animals was significant (exposure factor, $F_{1,91}=21.6$, $p<1e-10$), but there
 1249 was no individual difference between our animals in post-hoc tests applied to each duration of exposure.

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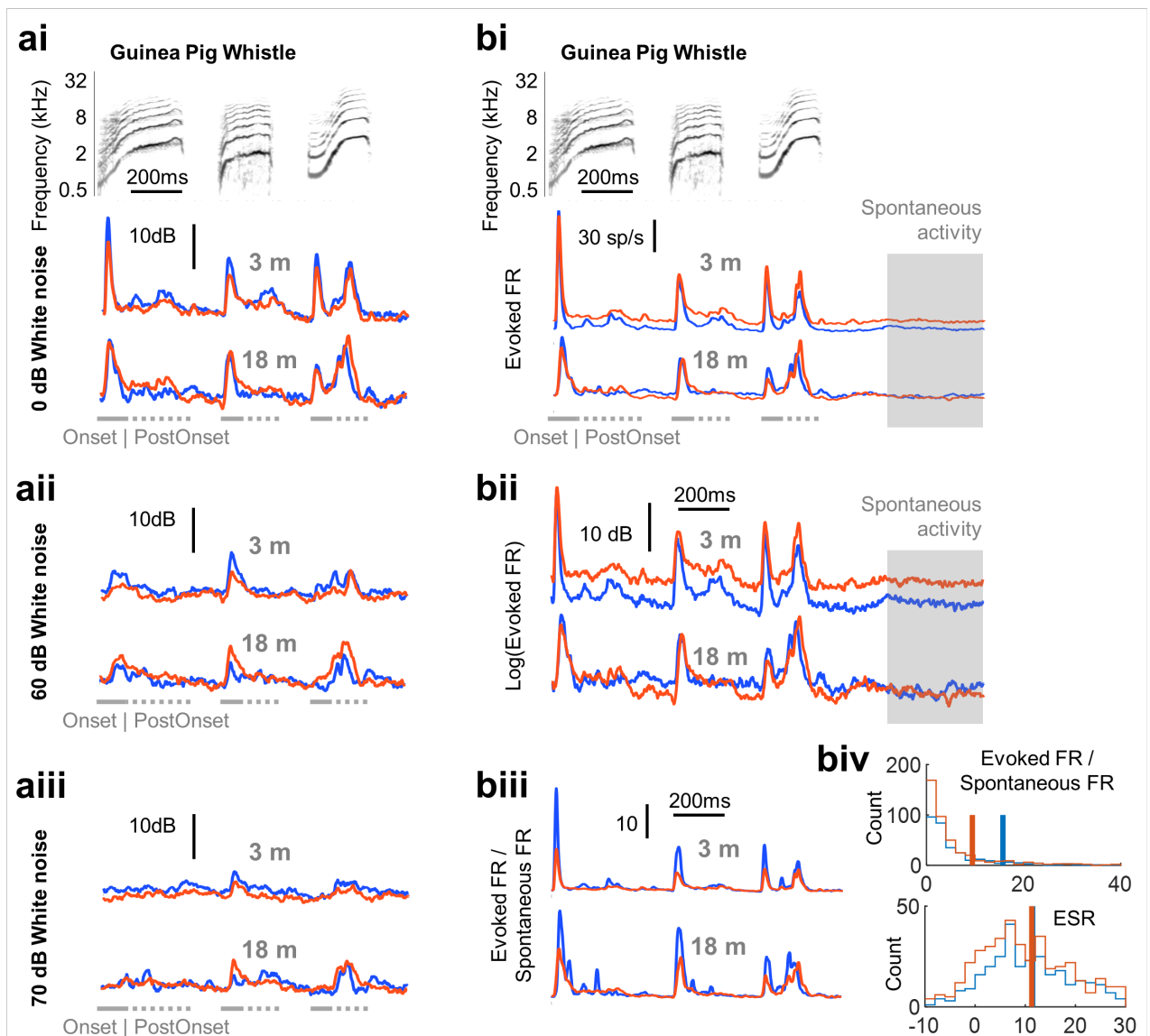


1251

1252 **Supp. Fig. 3:** a During quantification of the spectrotemporal receptive fields (STRFs^{pt}), the maximum firing rate
 1253 remained stable at 75 dB SPL after three months of exposure (left, $F_{1,1625}=2.33$, $p=0.13$) whereas the baseline firing
 1254 rate increased (right, $F_{1,1625}=54.3$, $p<1e-10$; t-tests * $p<0.05$), like the spontaneous firing rate shown in Fig. 3a.iii. b
 1255 Distribution of best frequency sampling in experimental groups. c.i Example of two microarray implantations in the
 1256 auditory cortex for an exposed animal: the best frequency is color-coded and superimposed on the picture of the
 1257 auditory cortex for this animal. c.ii Best frequency of cortical sites implanted in the same animal as a function of
 1258 anteroposterior distance to bregma. The red line is the linear regression. The topographical organization of best
 1259 frequencies, i.e. the tonotopy, is clearly visible on this example. c.iii Fisher (extreme left) and r^2 (left) statistics
 1260 extracted from a linear regression of best frequency against antero-posterior coordinates. Standard deviation of
 1261 anteroposterior coordinates (STD X, right) and mediolateral coordinates for each animal (STD Y, extreme right).
 1262 Most animals showed a topographical organization of best frequency along the anteroposterior axis as expected
 1263 (Fisher's test statistically significant) and there was no effect of exposure on r^2 , i.e. on the linear fitting of best

1264 frequency to the anteroposterior coordinate ($F_{1,32}=1.1$, $p=0.3$). This result was not dependent on spatial sampling
1265 differences, as BF distribution was approximately similar between groups (see **b**) and the dispersion of coordinates
1266 was similar between groups (STD X, ANOVA on the four groups, $F_{3,32}=0.74$, $p=0.54$; STD Y, $F_{3,32}=0.28$, $p=0.84$). **d**
1267 Frequency response area (FRA) of a cortical site in response to tones of various frequencies (abscissa) and intensities
1268 in dB SPL (ordinate). The white line is the contour at 6 standard deviations above the spontaneous activity. The
1269 characteristic frequency (CF) of the cortical site is defined as the frequency at the minimum intensity required to
1270 evoke a significant response. **ei** Average threshold values (evoked discharges 6 standard deviations above
1271 spontaneous activity) from the FRA (see **d**) after three months of exposure. **eii** as for **ei** but after 18 months of
1272 exposure. Exposure affects cortical thresholds after 3 months, but not 18 months of exposure (factor aging x
1273 exposure $F_{1,7842}=387$ $p<1e-10$, t-tests: the green line indicates the values for which $p<0.05$ for each frequency). **fi**
1274 Average response of all cortical sites to a burst of white noise with a 35 ms rising slope. A decrease in the maximum
1275 response is visible after three months of exposure but not after 18 months of exposure. **fii** Quantification of
1276 normalized neural response for various rising slope durations (the green rectangle corresponds to the individual
1277 example shown in **fi**). Results obtained after 3 months (top) or 18 months (bottom) of exposure are shown. Exposure
1278 for three months led to a decrease in the maximum ESR, whereas exposure for 18 months did not. This was the
1279 case whatever the slope of the stimulus rising time ($F_{1,14847}=85.7$, $p<1e-10$, t-tests in Supp. Fig. 3fii).

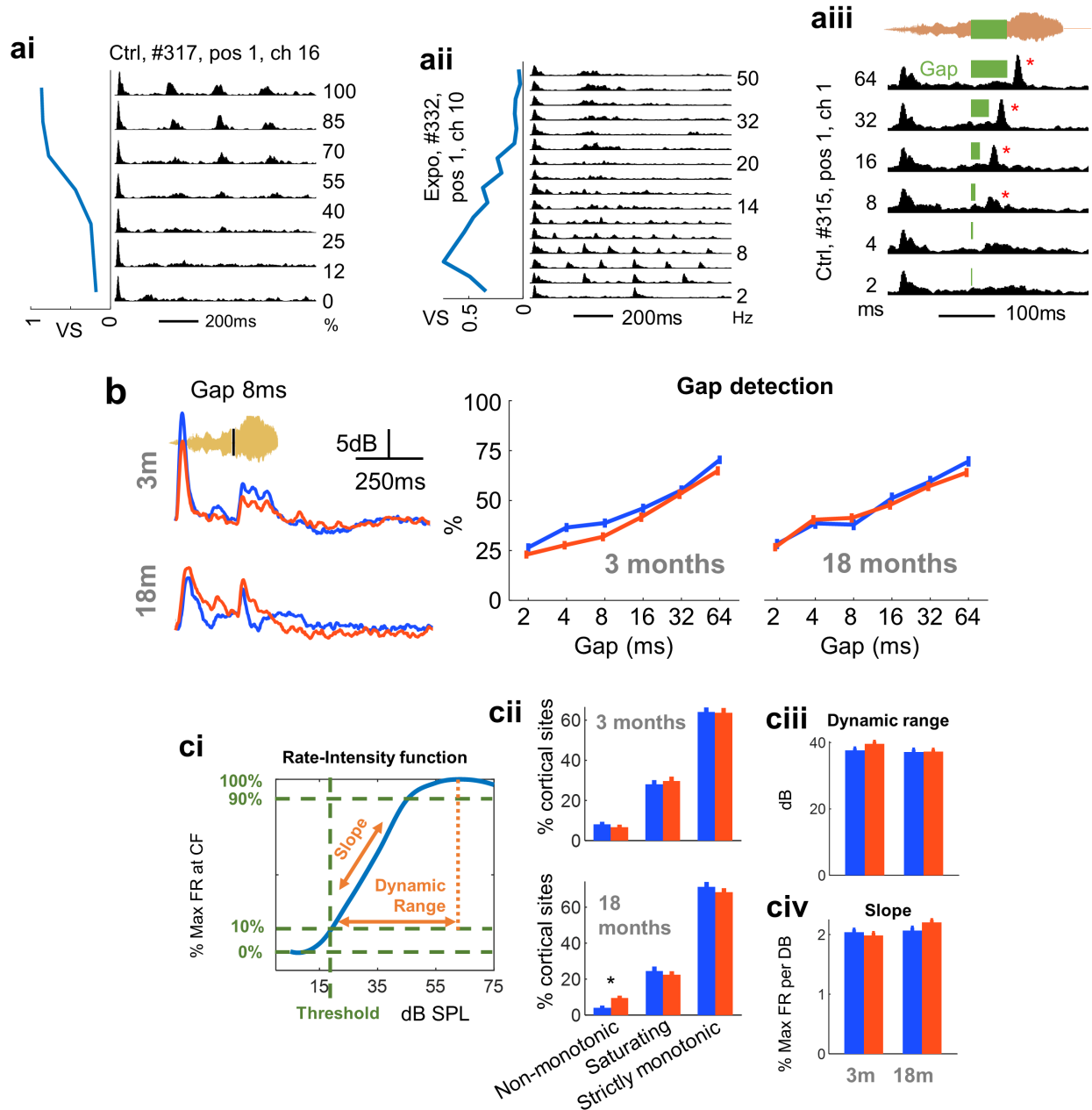
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1281

1282 **Supp. Fig. 3^{bis}:** **ai** Same plot as Fig. 3bi: Average response of all cortical sites from each group to three guinea pig
 1283 whistles (represented by the spectrograms at the top). **a ii** Same as ai with guinea pig whistles presented in 60 dB
 1284 white noise. **a iii** Same as ai with guinea pig whistles presented in 70 dB white noise. **b** Rationale for normalizing
 1285 and log-transforming the evoked firing rate **bi** Average raw evoked firing rate of cortical sites to three guinea pig
 1286 whistles (represented by the spectrograms at the top). In this protocol, the spontaneous activity is computed by the
 1287 average of evoked firing rate over the time interval [1300-1750]ms (gray area). There is a clear difference between
 1288 red and blue curves for the 3-month group which seems to stem from a difference in spontaneous activity similar
 1289 to that observed in Fig. 3c and Supp. Fig. 3a. This difference confuses the interpretation of differences in evoked
 1290 firing rate. **b ii** Average Log transform of the evoked firing rate of cortical sites to three guinea pig whistles. The use
 1291 of a Log transform more clearly emphasizes the problem raised in gi and suggests that a subtraction of the Log of
 1292 average spontaneous activity (i.e. a division of evoked firing rate by the average spontaneous firing rate) would be
 1293 helpful and pertinent. **b iii** Ratio of evoked firing rate to the average spontaneous firing rate. The baseline shift
 1294 disappeared compared to **bi**. Amplitude of red peaks appears much lower than that of blue peaks. However, this is
 1295 not the case for the 18-month group when log-transforming the same data as in Fig. 3bi. **b iv** Distribution of the
 1296 above ratio (upper panel) and of the ESR (lower panel) i.e. log-transform of the ratio, at first peak (latency 34ms).
 1297 The amplitude difference in peaks in **bi** occurs because the distribution of peak firing rates for the control group
 1298 (upper panel) is so skewed that the computation of the average is biased by extreme high values whereas the ESR
 1299 average is actually similar between the control and the exposed groups.

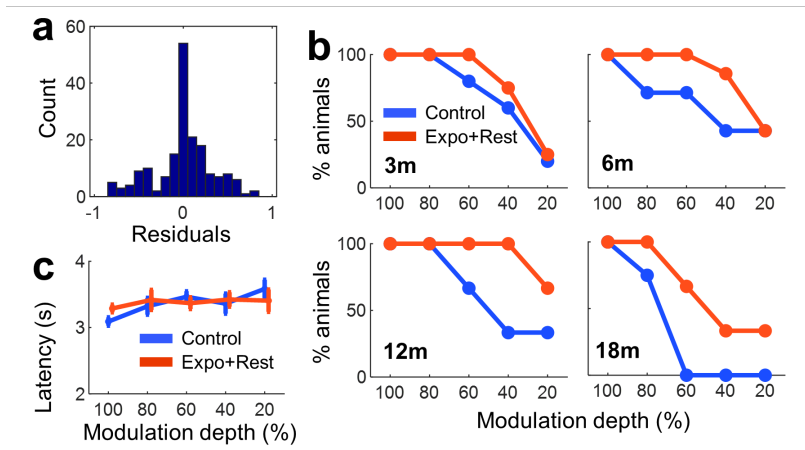
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1301

1302 **Supp. Fig. 4:** **ai** Example of cortical responses to amplitude-modulated white noise with a modulation rate of 4 Hz
 1303 and a range of modulation depths between 0 and 100%. On the left of the peri-stimulus time histograms (PSTH)
 1304 for neuronal responses, the depth modulation transfer function is quantified by vector strength (VS, abscissa)
 1305 plotted as a function of modulation depth. **aii** As for **ai** for the responses to a range of modulation rates between 2
 1306 Hz and 50 Hz at a 100% modulation depth, giving rise to the temporal modulation transfer function in blue. **aiii**
 1307 Example of PSTHs for the responses to a guinea pig whistle including gaps of 2-64 ms in duration. The temporal
 1308 envelope of the guinea pig whistle is represented at the top, with a gap symbolized by a green rectangle. A red star
 1309 indicates a significant peak in the PSTH within the 50 ms following the gap. **b** (left) Average response of all cortical
 1310 sites to a guinea pig whistle including a short (8 ms) gap. (right) Percentage of neurons for which the gap produced
 1311 a detectable peak in neural response. We were unable to identify a gap duration eliciting a significant difference
 1312 between animals exposed to noise for 18 months and unexposed animals in terms of the percentage of neurons
 1313 detecting the gap ($F_{1,9755}=4$, $p=4.7e-2$, but no significant t-test). The temporal processing of gaps was not, therefore,
 1314 altered by noise exposure. **ci** Using the responses at the CF (see Supp. Fig. 3d), we extracted the firing rate-intensity
 1315 function. **cii** Classification of rate-intensity function patterns between strictly monotonic, saturating (reaching 90%
 1316 of the maximum firing rate before 55 dB SPL) and non-monotonic. The percentage of non-monotonic patterns is

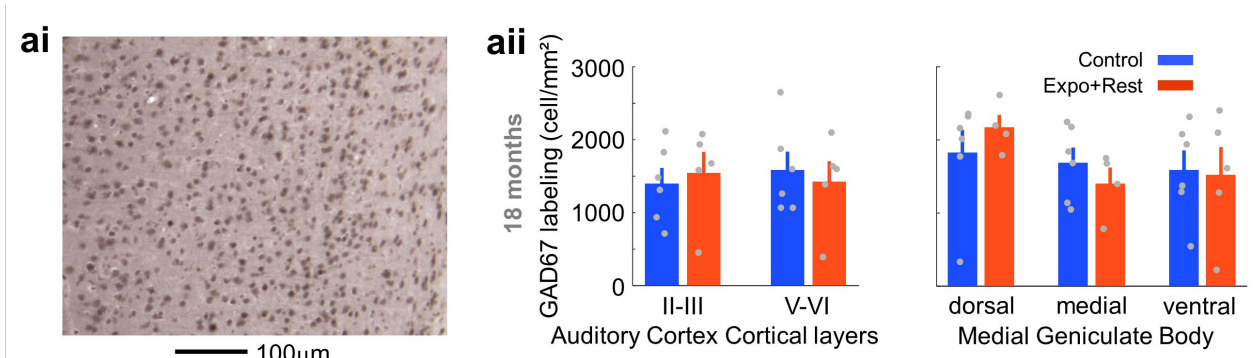
1317 significantly higher after lifetime noise exposure (proportion test, $*p < 0.05$). **ciii** Dynamic range and **civ** slope
 1318 parameters extracted from the rate-intensity function (see **ci**). Exposure had no significant effect on dynamic range
 1319 ($F_{1,1625} = 1.7$, $p = 0.19$). The ANOVA test was significant for slope ($F_{1,1625} = 3.91$, $p = 0.048$), but none of the pairwise
 1320 comparisons were significant ($p > 0.056$).



1321

1322 **Supp. Fig. 6: Behavioral performance for animals able to learn the task.** **a** Residuals for the three-way ANOVA model
 1323 of Fig. 5d. A Jarque-Berra test for normality was performed and it was not possible to reject the null hypothesis
 1324 ($Stat = 2.71$, $p = 0.2$). **b** For each exposure duration, the percentage of animals achieving correct discrimination is
 1325 shown for each modulation depth. Exposure had a positive effect on the discrimination performance of animals,
 1326 whatever its duration, but the sampling was too limited to draw firm conclusions for each exposure duration. **c**
 1327 Mean time lag to change of compartment on CS+ presentation for each modulation depth. Exposure had no effect
 1328 on this time lag (exposure effect, $F_{1,616} = 0$, $p = 0.97$; interaction exposure x duration of exposure, $F_{3,616} = 1.8$, $p = 0.14$;
 1329 interaction exposure x modulation depth, $F_{4,616} = 0.86$, $p = 0.49$).

1330



1331

1332 **Supp. Fig. 7:** *ai* Example of immunostaining for GAD67 in the primary auditory cortex (20 x magnification) of an
 1333 unexposed animal from the 18-month exposure group. *aii* Density of GAD67-positive cells in the deep and superficial
 1334 layers of the auditory cortex (left) and in three divisions of the auditory thalamus, the medial geniculate body (right).
 1335 Density is compared between exposed and unexposed animals from the 18-month group. Exposure had no
 1336 significant effect in the auditory cortex ($F_{1,18}=0.37$, $p=0.55$) or the auditory thalamus ($F_{2,25}=0.61$, $p=0.55$).

1337