



Research papers

Acute high-intensity sound exposure alters responses of place cells in hippocampus

T.J. Goble¹, A.R. Møller, L.T. Thompson*

School of Behavioral and Brain Sciences, The University of Texas at Dallas, 800 W. Campbell Rd, Richardson, Dallas, TX 75080, USA

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ABSTRACT

Overstimulation is known to activate neural plasticity in the auditory nervous system causing changes in function and re-organization. It has been shown earlier that overstimulation using high-intensity noise or tones can induce signs of tinnitus. Here we show in studies in rats that overstimulation causes changes in the way place cells of the hippocampus respond as rats search for rewards in a spatial maze. In familiar environments, a subset of hippocampal pyramidal neurons, known as place cells, respond when the animal moves through specific locations but are relatively silent in others. This place-field activity (i.e. location-specific firing) is stable in a fixed environment. The present study shows that activation of neural plasticity through overstimulation by sound can alter the response of these place cells. Rats implanted with chronic drivable dorsal hippocampal tetrodes (four microelectrodes) were assessed for stable single-unit place-field responses that were extracted from multiunit responses using NeuroExplorer computer spike-sorting software. Rats then underwent either 30 min exposure to a 4 kHz tone at 104 dB SPL or a control period in the same sound chamber. The place-field activity was significantly altered after sound exposure showing that plastic changes induced by overstimulation are not limited to the auditory nervous system but extend to other parts of the CNS, in this case to the hippocampus, a brain region often studied in the context of plasticity.

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1. Introduction

There is considerable evidence that both overstimulation and deprivation of sensory stimulation can cause re-organization of different structures in the CNS through activation of neural plasticity (Wall, 1977; Kaas, 1991; Kaas et al., 1990; Popelar et al., 1994; Nicolelis et al., 1991, 1993) (for a review, see Møller (2007)). Sound exposure has also been shown to cause a decrease in inhibition in the cochlear nucleus (Henderson and Møller, 1975), changes in the tonotopic map of the dorsal cochlear nucleus (Kaltenbach et al., 1992), an increase in acoustically evoked activity in inferior colliculus (IC) neurons (Willott and Lu, 1982), an increase in the amplitude of auditory evoked potentials recorded from the IC (Popelar et al., 1994; Salvi et al., 1990), a decrease in GABAergic inhibition on IC neurons (Szczepaniak and Møller, 1995), and changes in temporal inte-

gration in IC neurons (Gerken et al., 1991; Szczepaniak and Møller, 1996).

The cause of many forms of severe tinnitus is abnormal neural activity in the central nervous system. It has been hypothesized that altered neural synchrony in central neural circuits (Eggermont, 2007a,b) and perhaps in the auditory nerve (Møller, 1984), plays an important role in causing tinnitus. Other studies have found signs of re-organization of the auditory cortex in individuals with tinnitus (Mühlhnickel et al., 1998).

Evidence of abnormal organization of the ascending auditory pathways comes from studies of individuals with tinnitus. Thus the fact that electrical stimulation of the median nerve at the wrist can alter the perception of tinnitus in some individuals with tinnitus (Møller et al., 1992) was taken as an indication that the non-classical (polysensory, non-specific or extralemnisal) auditory pathways were activated in these individuals thus signs of re-routing of auditory information (for a review, see Møller (2007)). The non-classical auditory pathways are normally not active in adults (but there are signs that the non-classical pathways are active in children (Møller and Rolins, 2002)). Activation of the non-classical auditory pathways open up subcortical connections to limbic structures via the dorsal and medial thalamus, and that may explain why some individuals with tinnitus have phonophobia and other affective disorders.

Functional imaging studies have shown signs of activation of limbic structures in some individuals with tinnitus (Lockwood

Abbreviations: dB, decibels; SPL, sound pressure level; CNS, central nervous system; IC, inferior colliculus; PET, positron emission tomography; CA1, Cornu Ammonis field 1; μ A, microAmperes; sec, second(s); m, meter(s); cd, candela; cm, centimeter(s); ml, milliliter(s); mm, millimeter(s); Hz, Hertz; min, minute(s); hr, hour(s); DC, direct current; MAP, Multichannel Acquisition Processor; μ m, micrometer(s); SEM, standard error of the mean

* Corresponding author.

E-mail address: tres@utdallas.edu (L.T. Thompson).¹ Present address: Sentient Medical Systems, 10151 York Road, Suite 120, Cockeysville, MD 21030, USA.

et al., 1998). PET studies have found increased activation in secondary auditory cortex, pre-frontal cortex, and limbic structures in individuals after induced tinnitus-like perception (Mirz et al., 1999, 2000). Other studies in humans have found signs that the amygdalo-hippocampal complex is involved in some forms of tinnitus (De Ridder et al., 2006). Animal studies have not previously examined whether plasticity occurs in the hippocampal region in noise-exposure models of tinnitus, nor what form that plasticity might take.

In the present study in rats, we examined whether location-specific place cell activity in the hippocampus is altered after animals have been exposed to high-intensity tones. Plasticity in this well-characterized functional correlate of hippocampal activity (O'Keefe, 1999) would indicate that the hippocampus is altered by noise exposure, strengthening the hypothesis that brain regions that are not part of classical auditory pathways are involved in the pathophysiology of tinnitus.

Place cells are pyramidal cells in the CA1 region of the dorsal hippocampus which exhibit location-specific (place-field) activity, i.e. increased firing frequency when an animal is in a particular area of the environment, particularly when it is moving through that area. The firing rate of a place cell typically increases by an order of magnitude or more from a basal firing rate of <1 Hz (Muller et al., 1987; O'Keefe and Burgess, 1996; Thompson and Best, 1989) when the animal enters the cell's place-field. These cells are thus "tuned" to spatial locations. Place cell firing is affected by cognitive and memory functions, with spatial cues present in the physical environment largely governing place cell responses (O'Keefe and Burgess, 1996; Eichenbaum et al., 1999; Shapiro et al., 1999; Pavlides and Winson, 1989). The place-specific firing of place cells can be altered by moving cues between recording sessions (O'Keefe and Conway, 1978; Olton et al., 1978; Shapiro et al., 1997) but in a fixed environment, place-fields remain stable and they can persist in the same place for as long as 5 months or even more (Kentros et al., 2004; Muller et al., 1987; Save et al., 2000; Thompson and Best, 1990). Although some place cell studies (Hill and Best, 1981; Rossier et al., 2000) have been interpreted to indicate relative insensitivity of place-fields to auditory stimuli, other evidence (Best and Hill, 1982; Russell et al., 2003; Stackman et al., 2002) indicates a significant contribution not only of auditory but also of inner ear vestibular input in determining place cell firing and spatial navigation.

Here we present evidence that sound exposure (4 kHz tones at 104 dB SPL presented for 30 min) that has been shown to produce tinnitus (Szczeplaniak and Møller, 1995, 1996) when presented in one environment alters the place-specific firing position of rat hippocampal CA1 place cells in a different environment in the absence of any continued sound stimulus. In other words, intrinsic plasticity is expressed in terms of altered hippocampal place cell activity, both shortly after and well after the spatially irrelevant sound exposure has ended.

2. Materials and methods

2.1. Chronic implants

Thirteen Long-Evans rats (Harlan Inc., Indianapolis, IN) weighing 300–450 g were prepared for implantation with chronic recording electrode assemblies (see following section for a full description of these assemblies). Rats were housed one per cage on a 12 hr/12 hr light–dark cycle in a temperature controlled room with *ad libitum* access to water. Rats were trained over a one week period to traverse spatial mazes (see maze environments description below). After this initial training, rats were anesthetized with isoflurane and treated with atropine sulphate (0.25 mg/kg, i.p.). Microelectrodes were implanted stereotaxically over the right dorsal CA1 pyramidal layer of the hippocampus using the coordinates

4.0 mm posterior and 2.5 mm lateral to bregma, and 1.6 mm ventral to the pial surface. The drivable microelectrode assemblies were secured to skull screws with dental acrylic and sterile petroleum jelly was applied to the exposed brain surface to provide a flexible seal for future electrode driving. The animals were allowed 72 hr to recover after surgery before electrode advancement and recordings were made. This type of implant in our hands has been demonstrated to yield stable recordings over intervals from days to months in duration (Thompson and Best, 1990).

2.2. Chronic electrodes

The electrodes used were made from 25 μ m Formvar-insulated nichrome wire threaded through a 27-gauge thin-wall stainless steel cannula, in the form of four bundles of tetrodes (each tetrode consisting of four microwires closely twisted together to enhance the reliability of single-unit isolation and identification; see Gray et al., 1995; Quirk and Wilson, 1999). The electrodes were electrically connected to a 10-pin Omnetics (Minneapolis, MN) connector with colloidal silver paint (Ted Pella, Redding, CA). An insulated 24-gauge wire attached to a skull screw was used as a reference. The recording electrode was assembled into a chronic vertical-axis microdrive as previously described (Kubie, 1984).

At the completion of all experiments, rats were deeply anesthetized with urethane and perfused intracardially with formaldehyde. During perfusion, positive current (10 μ A for 10 sec) was passed through the implanted microwires to deposit iron ions at the recording site. The brain was removed, and coronal sections were processed for histological verification of electrode placements within the CA1 pyramidal cell layer of dorsal hippocampus.

2.3. Maze environment

The studies were performed in a 3 \times 3 m spatial testing room using a black eight-arm 1.4 m diameter radial-arm maze located in the center of the room, elevated 1.22 m from the floor and surrounded by a black curtain that reached from the floor to a height of 2.44 m. Dim overhead light (0.5 cd) was on during recordings to allow visibility of the explicit spatial cues located by the curtain. Three vertically oriented white visual spatial cues (approximately 2.5 cm wide and 25 cm high) visible from the maze (see Simpson and Gaffan, 1999) were placed 15 cm beyond the ends of the maze arms.

2.4. Behavioral procedures

Rats were trained over a one week period to traverse all arms of the eight-arm radial-arm maze, obtaining small rewards (<0.1 ml) of chocolate milk at the end of each arm. The eight-arm radial-maze provided a limited and well-defined pathway for exploration with explicitly defined reward goal locations (see Olton et al., 1978). This limited the behavioral repertoire of rats on this particular maze, and ensured relative uniformity in spatial exploration over repeated sessions. A behavioral session was completed when the rat had traversed all eight arms of the radial-arm maze. Chocolate milk was baited at the end of each arm in a hidden cup before each session. These relatively brief sessions were used to maximize repeated sampling while maintaining high levels of activity over the course of the day. Average recording sessions were 188 \pm 13.6 sec in duration.

The rat's spatial location was monitored by video camera in the maze environment by encoding the coordinate occupied by a dual LED attached to the head stage. A Video Tracker Processing Unit (Plexon Instruments, Dallas, TX) digitized and recorded spatial coordinates at a sampling rate of 100 Hz, which were used in subsequent place-field analyses (see description below).

2.5. Sound exposure

After 5 separate stable baseline recording sessions on the radial-arm maze were obtained for individual place cells, rats were removed from the maze environment and placed in a $55 \times 73 \times 55$ cm sound chamber environment in another location either for 30 min exposure to a 4 kHz tone at 104 dB SPL or for 30 min confinement in the same sound chamber without any sounds presented. The intensity of the tone was measured using a sound-level meter (Radio Shack, Fort Worth, TX). The sound-level meter was placed in the bottom of the sound chamber without the animal present and the sound intensity was adjusted to 104 dB SPL.

Immediately after sound exposure or control confinement, rats were placed back in the radial-arm maze and place-field activity recorded every 15 min for the first 2 hr, then every 30 min for the next 2 hr, then at intervals 6, 12, and 24 hr post-treatment.

2.6. Data acquisition and analysis

Multiunit signals from the tetrodes were fed to a DC powered unity-gain preamplifier (Plexon Instruments, Dallas, TX) plugged on top of the microdrive electrode assembly. A low-drag multi-channel recording cable and a 32-channel commutator (Dragonfly,

Ridgeley, WV) connected the recorded signals to AC coupled differential amplifiers (gain of 10) followed by step-programmable gain control amplifiers (up to 32x gain; Plexon Instruments, Dallas, TX). The recorded multiunit activity was high- and low-pass filtered at 250 Hz and 8 kHz using a Multichannel Acquisition Processor (MAP, Plexon Inc., Dallas, TX).

Single-unit potentials were isolated from these multiunit signals and examined in real-time (during acquisition) using NeuroExplorer (Nex Technologies, Littleton, MA) sorting software and further post-processed using Off-Line Sorter (Plexon Instruments, Dallas, TX). Conservative approaches were used to discriminate single-unit activity (see Gray et al., 1995; Emondi et al., 2005). Waveforms that had a signal-to-noise ratio less than 3:1 on a tetraode channel were not accepted for further analysis. If no unit potentials meeting these criteria were present on any of the four tetrodes, the electrode assembly was advanced up to $40 \mu\text{m}$ and allowed to settle 2–3 hr before the potentials were examined again. Once single-units were isolated using principle-component analyses of the low- and high-pass filtered signal, templates of the four waveforms characteristic of that unit (i.e. the single-unit tetraode waveforms) were saved and used for discrimination of that unit in all subsequent experimental sessions. Examples of these tetraode waveforms thus characterized are shown in Figs. 1 and 2 (from

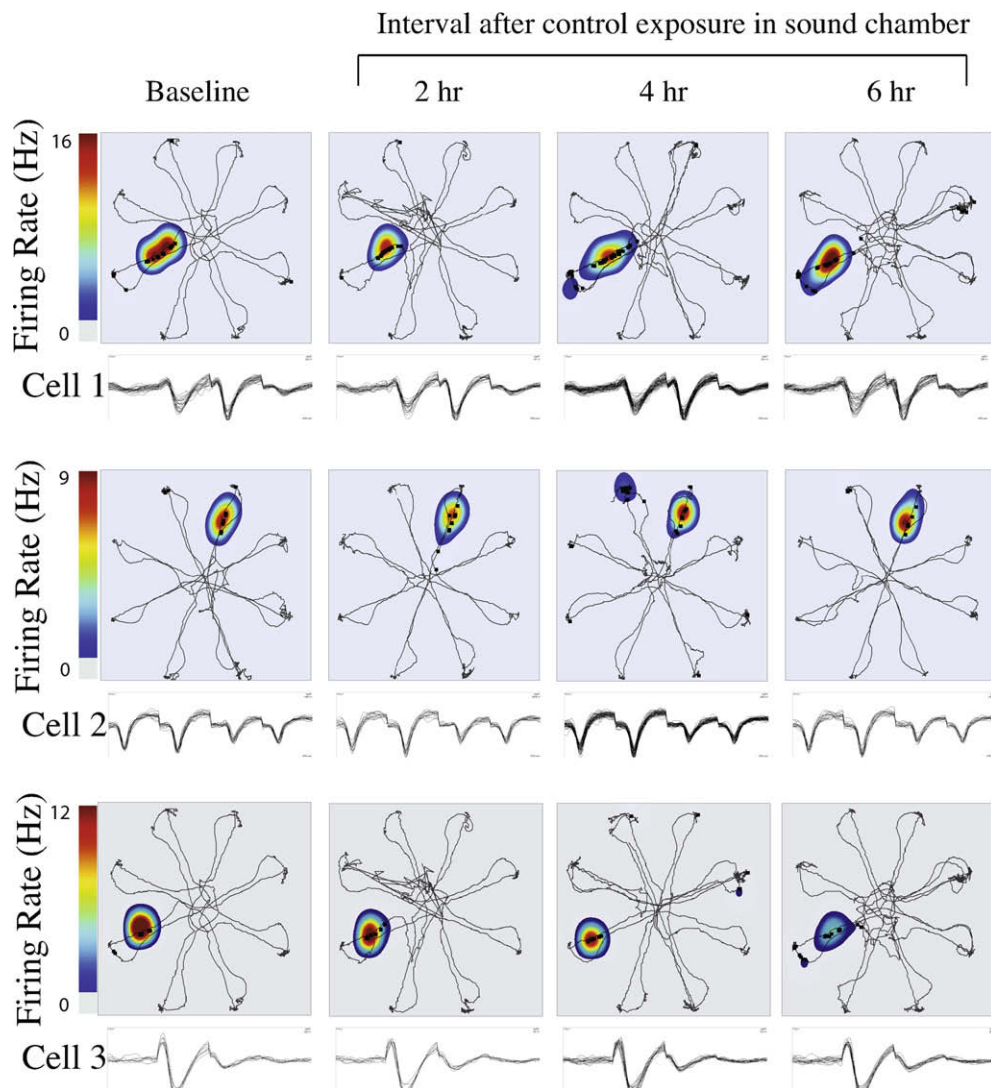


Fig. 1. Typical examples of results of recordings from place cells in control rats showing stable place-fields. Overlay map of the behavioral path (thin line), single-unit firing positions (black squares), place-fields (colored contours), and below each map the tetraode waveforms for 3 units from control condition rats (no-sound exposure). The firing rate scale of each unit is displayed at left. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

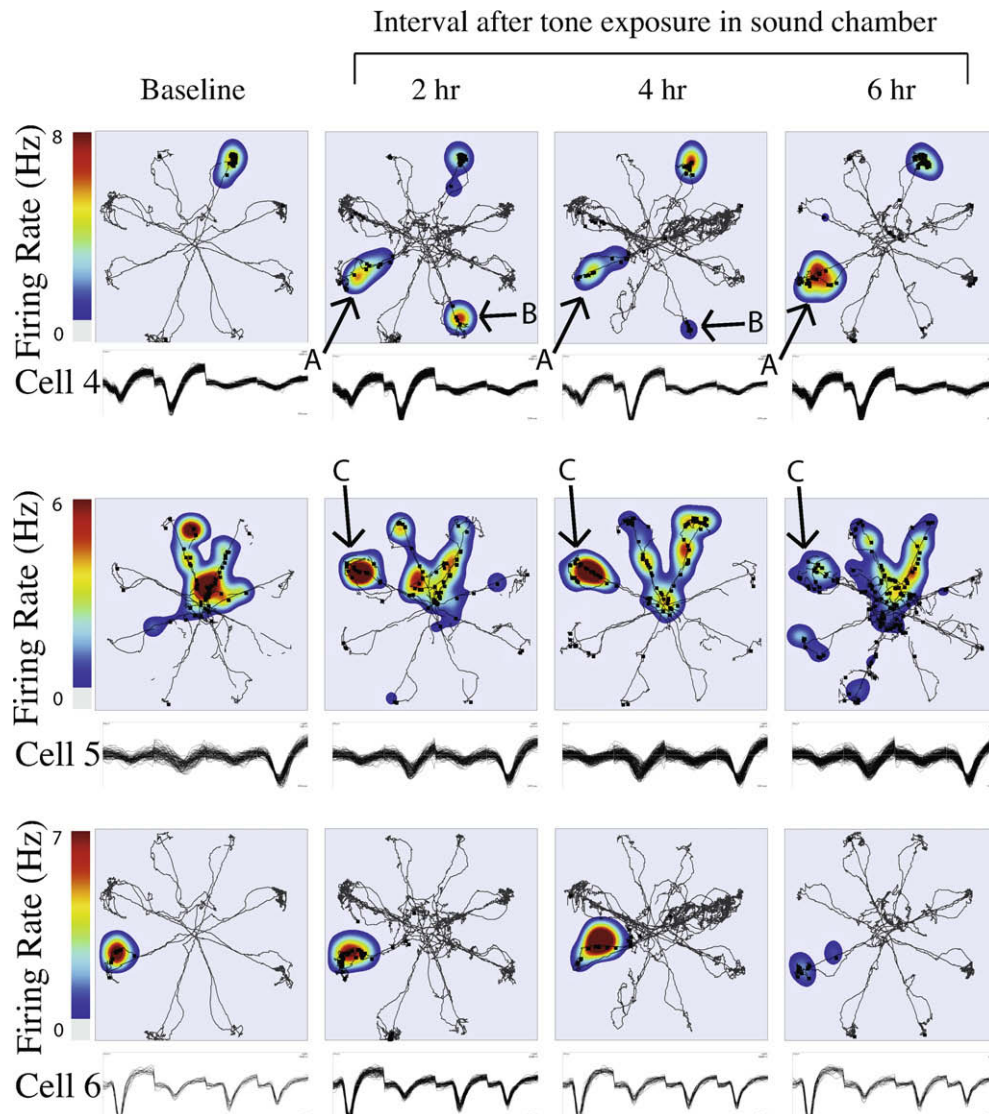


Fig. 2. Typical examples of results of recordings from place cells in rats before and after sound exposure showing how place-fields changed after sound exposure. Overlay map of the animal's behavioral path (thin line), single-unit firing position (black squares), place-fields (colored contours), and below each map the tetraode waveforms for 3 units from rats that underwent sound exposure (4 kHz tone, 104 dB SPL, 30 min). The firing rate scale of each unit is displayed at left. (For interpretation of color mentioned in this figure legend the reader is referred to the web version of the article.)

left-to-right in each example are the signals recorded on each of the four electrodes making up the tetraode). These illustrate the physiological stability of the neuronal firing activity recorded hours apart, below each firing map.

2.7. Statistical analyses of place-field stability

Location-specific firing-rate activity during each session was analyzed for the stability of place-fields across multiple baseline sessions (i.e. for a minimum of 2 hr, but typically over more than 12 hr) and then compared with later place-field activity after either sound exposure or control exposure to the same environment. The Cartesian coordinate firing position of each place cell event was used to calculate firing-rate maps on a session-by-session basis for each individual place cell, with a pixel size of approximately 1.25 mm. Individual place-fields were operationally defined as a set of at least 9 contiguous pixels with firing rates more than 2 standard deviations above the grand mean (i.e. the mean firing for the entire session) of that single-unit (cf. Kentros et al., 2004; Muller et al., 1987; Save et al., 2000; Thompson and Best, 1989).

Since each session consisted of a relatively small sample of unit firing activity (typically, each session was encoded by <200 firing events per place cell), for statistical purposes the firing of each place cell on the maze was binned into 5×5 cm grids for analysis, with the spatial environment each rat explored thus encompassed into 18×18 grids.

Once individual place cell firing rate maps per session were constructed, each was filtered using a Gaussian filter (using NeuroExplorer), i.e. the raw single-unit firing rate functions were smoothed using an iterative weighted distance algorithm. For each pixel, the value of the raw firing rate was smoothed by a weighted sum of values from pixels on all sides and corners. The sum of the values across all neighbors was added to the firing rate of the assigned grid and that sum was divided by the total weight accumulated in each grid to reduce variance introduced by sampling errors (see Hetherington and Shapiro, 1997; O'Keefe and Burgess, 1996; Skaggs and McNaughton, 1998 for more extensive discussions of sampling issues and of the algorithms used in analyses of place-field stability).

Pearson r correlations between firing-rate maps (i.e. between bins with firing activity above the defined threshold to constitute

a place-field) were then computed across sessions for each individual place cell. Monte Carlo statistics were used to compare the best-fit baseline session to all other baseline sessions, and generated a Z-score representing the average variance for each place grid analyzed for each place cell. For each place cell, correlations between these baseline sessions and all subsequent firing-rate map sessions (either after control or tone-exposure) were calculated and expressed in terms of Z-scores. A Z-score < 1 thus indicated that an individual place cell exhibited a stable place-field compared to the place-cells baseline firing rate or location, while a Z-score > 1 indicated plasticity in the place-field (i.e. a decrease in stability of this location-specific firing).

2.8. Analyses of discharge rate data

The following place-field characteristics were analyzed: (1) Grand-mean firing rate – the mean firing rate of each single-unit during each maze session recorded; this measure is independent of location. (2) Peak rate – the maximum firing rate in the highest firing rate bin within a defined place-field; (3) Mean in-field firing rate – the mean firing rate within the place-field calculated by dividing the total spikes fired within the field by the total amount of time the rat spent within the field; (4) Mean out-of-field firing rate – the mean firing rate in all areas located outside defined place-fields, calculated by dividing the total spikes fired out-of-field by the total time spent out-of-field; (5) Place-field size – the total area of the pixels within the place-field; (6) Centroid displacement – the centroid was calculated as the weighted Cartesian coordinate center of the place-field, based on firing rate distribution within the place-field. The centroid displacement was then calculated as the Euclidean distance between the centroid of a defined place-field on two separate sessions (i.e. a low centroid displacement value between two recording sessions indicates the weighted geometric center of the place-fields in the two sessions are similar).

Each of these measures were standardized using the means and variance observed within the five baseline sessions in order to allow comparisons of data from individual place cells across sessions, while recognizing that each cell exhibited unique firing rates and differing characteristic variance on these respective variables. Z-scores on each measure from different place cells could then be directly compared because each represents standardized variance from mean baseline data for each place cell. Z-scores for firing rates were computed as follows, using each session's place cell discharge rate (Y), the baseline mean rate (M_y) and the standard deviation of the baseline rate (S_y).

$$Z = \frac{Y - M_y}{S_y}$$

Session by session Z-scores and their standard error of the mean (SEM) were used to graphically present the results of this study. These data were analyzed using *t*-tests of two independent samples, using Welch's correction to protect against cases of unequal distribution of variances in the samples collected.

3. Results

A total of 193 hippocampal CA1 single-units were studied, 63 units from 5 control rats (not exposed to high-intensity noise) and 130 units from 8 experimental rats pre- and post-sound exposure. Sixty-one of these neurons (32% of the initial sample) exhibited very stable place-fields for five sequential baseline sessions ($n = 31$ control; $n = 30$ tone exposed) when studied for at least 24 hr prior to experimental manipulation (in multiple cases in excess of 72 hr) and were used for the analyses reported here. On the

basis of established neurophysiological criteria (Fox and Ranck, 1975, 1981) and histological verification of their location within *s. pyramidale* of CA1, these stable place cells were identified as complex-spiking pyramidal neurons, and met a criteria of low (< 3 Hz; typically less than 1 Hz) basal mean firing rates and clear location-specific baseline place-fields (analyzed between-sessions by comparisons of pixel-by-pixel firing rate cross-correlation coefficients (R) > 0.50 between baseline sessions). Most of the single-units that failed to meet these criteria for inclusion because they did not exhibit stable place-fields during baseline recordings were identified as theta cells (Fox and Ranck, 1975, 1981), based on their higher (>5 Hz) basal firing rates, shorter duration action potentials, and lack of spatial-specificity. A total of 1715 place cell sessions were analyzed during repeated maze testing to assess and characterize stability or plasticity after high-intensity sound exposure.

During baseline sessions, the correlation coefficient of the pixels representing the place-fields for all neurons from session-to-session was very high for all place cells ($r = 0.72 \pm 0.01$). The grand-mean firing rate (averaged across the entire spatial environment) for all place cells during baseline sessions was 0.91 ± 0.05 Hz, well within the published normative firing range for hippocampal pyramidal neurons in freely-behaving rats (Fox and Ranck, 1975, 1981; Thompson and Best, 1989, 1990). The mean out-of-field (i.e. outside the pixels defining the place-field) firing rate was 0.55 ± 0.04 Hz. The mean in-field firing rate was 5.54 ± 0.29 Hz, a 6-fold ratio between in-field and grand-mean firing rates and at least a 10-fold ratio between in- and out-of-field firing rates. Notably, the mean observed peak within-field firing rate for all stable place cells studied was 21.97 ± 1.24 Hz, a 24-fold ratio between peak-firing and grand-mean firing rates and a 40-fold ratio from maximal in-field to mean out-of-field firing, with a 4-fold ratio between peak- and mean- in-field firing rates. The average place-field size was 17.37 pixels square (86.85 mm^2).

Fig. 1 illustrates stable place-fields of three different place-cells from control rats exploring the radial-arm maze environment. The path traveled by the rats as tracked by the Plexon system is shown as a line following the approximate dimensions of the maze, while place-field maps (color-coded for intensity of firing) are superimposed over these behavioral maps. Most neurons (82% of stable place cells) exhibited single well-defined place-fields, while a smaller number exhibited more than one place-field per single-unit. In these typical examples of place-fields illustrated in Fig. 1, neurons exhibited place-fields that were confined to small discrete regions on a single radial-arm of the maze. Notice that the fields remained stable at all time intervals tested in control rats. Analyses of the neuronal firing activity from control rats showed that the physiological as well as the location-specific measures remained stable for up to 24 hr (i.e. remained within ± 1 Z-score) (see Table 1, which summarizes the stable firing characteristics of CA1 place cells from control rats, expressed as Z-score differences from baseline).

Fig. 2 illustrates the plasticity observed for many place cells in sound-exposed rats. Thirty seven percent of all place cells in noise-exposed rats exhibited significant changes in location-specificity (most showing shifts in excess of 2 Z-scores), while less than 10% of place cells in control rats exhibited any notable drift in location-specific firing; none of the neurons in control rats developed new or multiple place-fields over time. As shown in the top panels of Fig. 2, many neurons that (during baseline sessions prior to sound exposure) exhibited single distinct place-fields developed new or multiple place-fields very rapidly after sound exposure, with one or more of those fields stabilizing in new locations that persisted over successive testing sessions. This plasticity took the form not only of changes in mean firing rates, but also significantly decreased firing rates within the former place-field and increases in firing (shifting from relative silence during baseline to new

Table 1

Stability of place-field firing characteristics of CA1 complex-spike cells from control treated rats (no-sound exposure). Values listed indicate the variance from baseline measure (expressed as Z-scores).

	1 hr Post	2 hr Post	3 hr Post	4 hr Post	6 hr Post
Grand-mean firing rate (Hz)	0.17 ± 0.39	-0.63 ± 0.18	-0.57 ± 0.30	-0.87 ± 0.21	-0.89 ± 0.41
Peak in-field firing rate (Hz)	0.06 ± 0.17	-0.01 ± 0.17	-0.31 ± 0.13	-0.50 ± 0.12	-0.46 ± 0.24
Mean in-field firing rate (Hz)	-0.13 ± 0.15	-0.30 ± 0.15	-0.46 ± 0.21	-0.68 ± 0.18	-0.97 ± 0.31
Mean out-of-field firing rate (Hz)	-0.37 ± 0.16	-0.47 ± 0.11	-0.04 ± 0.31	-0.28 ± 0.22	-0.52 ± 0.16
Place-field size (cm)	-0.21 ± 0.13	0.09 ± 0.23	0.36 ± 0.23	0.02 ± 0.20	-0.42 ± 0.24
Place-field location correlation	-0.21 ± 0.20	-0.58 ± 0.20	-0.28 ± 0.31	-0.60 ± 0.35	-0.80 ± 0.44

Table 2

Changes in place-field firing characteristics of CA1 complex-spike cells from rats after sound exposure. Values listed indicate the variance from baseline measure (expressed as Z-scores).

	1 hr Post	2 hr Post	3 hr Post	4 hr Post	6 hr Post	12 hr Post
Grand-mean firing rate (Hz)	0.36 ± 0.31	1.78 ± 0.59	1.61 ± 0.72	3.38 ± 1.35 ^a	0.77 ± 1.00	-2.01 ± 0.44
Peak in-field firing rate (Hz)	0.59 ± 0.22	0.57 ± 0.23	0.27 ± 0.28	1.04 ± 0.55	0.26 ± 0.40	-0.77 ± 0.35
Mean in-field firing rate (Hz)	-0.17 ± 0.14	0.06 ± 0.18	-0.37 ± 0.17	0.16 ± 0.23	-0.40 ± 0.26	-1.56 ± 0.24
Mean out-of-field firing rate (Hz)	0.22 ± 0.20	1.42 ± 0.40	1.67 ± 0.64	2.01 ± 0.66	0.97 ± 0.78	-0.03 ± 0.66
Place-field size (cm)	0.25 ± 0.16	0.05 ± 0.19	0.34 ± 0.36	0.29 ± 0.27	0.07 ± 0.31	-0.58 ± 0.29
Place-field location correlation	-1.99 ± 0.34	-2.44 ± 0.38	-3.57 ± 0.70 ^a	-3.49 ± 0.50 ^a	-4.16 ± 0.70 ^a	-5.49 ± 0.73 ^a

^a Normalized values ± Z-scores.

sustained high firing rates) in new locations within the maze, resulting in formation of new location-specific place-fields. Table 2 summarizes the observed plasticity in the firing characteristics of CA1 place cells after experimental sound exposure, expressed as Z-score differences from baseline. The smaller number of place cells that exhibited multiple place-fields during baseline also exhibited significant plasticity in the location of these fields post-noise exposure. As seen in the bottom row of panels in Fig. 2, even place cells in sound-exposed rats that did not show significant changes in the Cartesian centroid of their place-field locations showed expansions of the field area and changes in firing rates both within and outside the place-field. After sound exposure, normalized spatial location correlation values decreased significantly, and grand-mean and out-of-field firing rate increased compared to the control confinement condition.

Sound exposure significantly altered place-field location correlations with the baseline values for the entire 24 hr period post-sound exposure (±3 Z-scores; see Fig. 3). Data were assessed for the five blocks of recordings for baseline and for intervals from 1 hr through 24 hr after sound exposure, with up to five recordings included at each time interval graphed. Analyses of place-fields

from sound-exposed rats showed that while some measures remained stable (values were within ±1 Z-score), many others changed significantly. A total of 18 measurements from sound-exposed rats had changes in excess of 1 Z-score, compared to measurements during baseline.

The location of place-fields changed significantly at each time period after sound-exposure ($n = 30$, $p < 0.015$) but not after control treatment ($n = 31$, $p > 0.3$), as shown in Fig. 3. In this figure, the normalized spatial location correlation (expressed in Z-scores) compared to the baseline values is shown for units from both control and sound-exposed rats (black bar represents the 30 min treatment time period, with data presented as the mean Z-score ± SEM for each group). Sound-exposed correlation values decreased immediately after sound exposure and plateaued around 4 hr post-sound exposure while all control rat measures remained stable (i.e. within ±1 Z-score).

During baseline, place-fields had an average centroid displacement of 11.17 ± 0.13 cm (see Fig. 4). This distribution was unaltered by control treatment, with average displacements of 11.73 ± 0.24 cm ($t = -1.50$; $df = 206$, $p = 0.07$). However, the centroids of place-fields from sound-exposed units 2 hr post-treatment

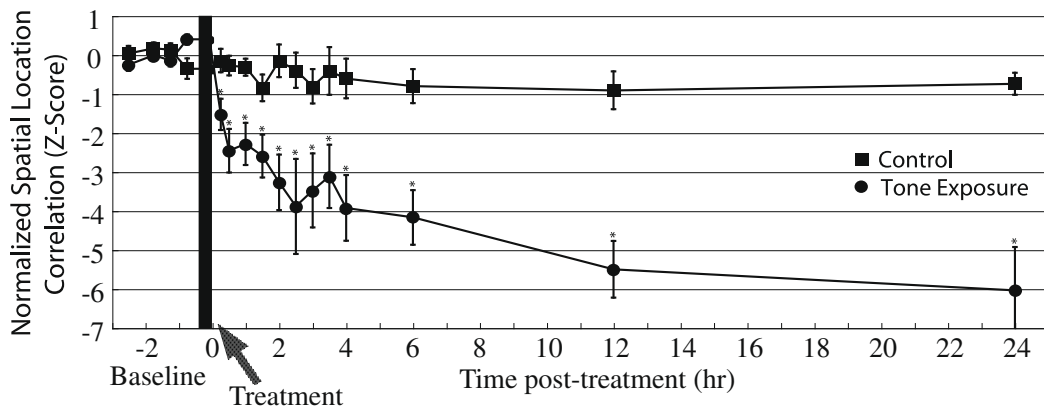


Fig. 3. Illustration of how sound-exposure altered place-field location-specific stability as a function of time after tone exposure (filled circles) and control (filled squares). The normalized spatial location correlation (expressed in Z-score) compared to overall baseline norms is shown for units from both control and sound-exposed rats (black bar is 30 min treatment condition). Means ± SEM are shown.

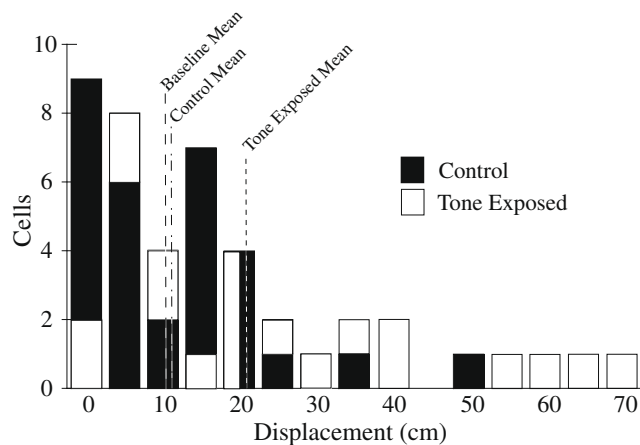


Fig. 4. Sound exposure increased displacement of place-field geometric center 2 hr after sound exposure. The average of the five sequential baseline geometric centroids was used for comparison of the Euclidean distance to each post-treatment centroid.

were significantly different from baseline: average, 21.44 ± 0.34 cm ($t = -4.44$; $df = 167$, $p = 0.00002$). For comparison, only 3 of the 31 control condition cells (10%) had place-field centroid displacement greater than one standard deviation from the combined baseline, compared to 11 of the 30 (37%) of the sound-exposed place cells.

4. Discussion

The main finding of the present study is that exposure to a 4 kHz tone at 104 dB SPL for 30 min alters previously stable responses of hippocampal place cells. Place cells in rats normally have a high degree of stability over periods of hours and days, in some cases up to 5 months time (Thompson and Best, 1990). After high-intensity sound exposure in the present experiments, many place cells exhibited grossly altered place-field firing properties: changes in place-field position (centroid displacement, see Figs. 2 and 3); changes in normalized spatial location correlation values (see Figs. 3 and 4); and changes in normalized grand-mean firing rates, in in-field and out-of-field firing rates, and in peak-firing rates compared to controls (see Tables 1 and 2). These changes were exhibited in the separate and distinctive spatial maze environment, an environment independent of the noise exposure, with the noise stimulus absent from and never directly associated with this spatial environment.

It has been shown earlier that sound stimulation using the same sound exposure used in this study creates signs of hyperactivity in the inferior colliculus in rats that resemble conditions associated with tinnitus (Szczepaniak and Møller, 1996). The results of the present study support the hypothesis that plasticity caused by exposure to intense sounds extends to the hippocampus. In neuroimaging studies of individuals with tinnitus, de Ridder showed evidence that some forms of tinnitus are associated with functional changes in the hippocampus (De Ridder et al., 2006). It has earlier been shown that some individuals with tinnitus have signs of involvement of the non-classical ascending auditory pathways (Møller et al., 1992), thus providing a subcortical route to limbic structures from the dorsal-medial thalamic auditory nucleus, from where there are connections to the amygdala (Herry et al., 2007; Ostlund and Balleine, 2008). The current findings corroborate and add to these earlier studies. The results of the present study confirm that neural plasticity induced by sound exposure can affect not only classical auditory but also non-lemniscal brain regions, in a way that may help explain

symptoms that often occur together with tinnitus. Activation of subcortical connections to limbic system structures may begin to explain affective symptoms that many patients with tinnitus have, including depression and phonophobia. These findings suggest that additional work on tinnitus-related plasticity in limbic regions is needed.

The hippocampus normally integrates recent sensory information from all sensory modalities with mnemonic and non-sensory information, so place cell location-specific firing is not dependent on any single sensory modality but rather governed by multiple contextually relevant modalities. It has been shown that rotation of visual cues change firing position of place cells (Fenton et al., 2000), while rotation of auditory cues or a change to different auditory stimuli can also change firing properties of hippocampal neurons (Tamura et al., 1992). The present findings indicate that sound exposure experiences in a separate, contextually unrelated environment can affect location-specific firing of hippocampal neurons during normal spatial exploration, and suggest that hippocampal plasticity may play a significant role in the pathophysiology of tinnitus.

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