The Effects of Stress on Hippocampal Place-Fields in a Fixed Environment: A Comparison of Physical Restraint or Dexamethasone to Noise Exposure

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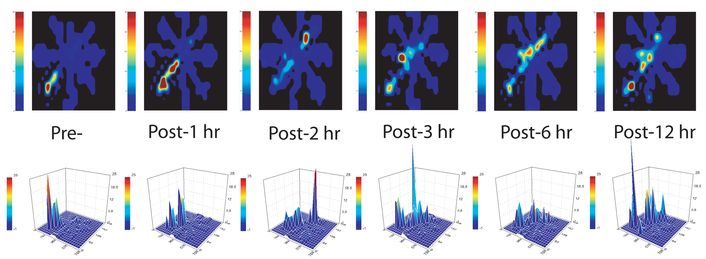
Fig. 1: Acute noise-exposure induces plasticity in CA1 place-cell place-field firing and location-specificity



Introduction

Tinnitus can be induced experientially or experimentally by brief exposure to high-intensity noise, inducing plasticity in ascending auditory pathways, classical and non-classical, including the hippocampus (Goble *et al*., 2004, 2005).

Hippocampal neurons have been replicably shown to exhibit stable place-field activity (i.e. location-specific firing) in fixed environments. Manipulations of environmental stimuli in multiple sensory modalities can alter place-field activity. Our initial novel findings (Goble *et al*., 2004) indicated that experience outside the test environment can also have a prolonged effect on place-field activity (Fig. 1).

Relatively little attention has been focused on the role of stress and stress hormones on place-field activity, although hippocampal pyramidal neurons express abundant CST receptors. It has been suggested that high-intensity noise exposure might induce a stress response, and noise-induced place-field plasticity might be due to stress instead of specific to noise-exposure.

The current study assessed hippocampal place-field activity before and after: intense noise exposure sufficient to induce tinnitus; injection of dexamethasone (a synthetic CST); physical restraint stress; or non-treated controls.

Hypotheses

- Acute noise exposure will alter location-specific (place-field) firing
- Acute treatment with CSTs will alter location-specific firing
- Acute physical restraint will alter location-specific firing

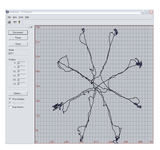


Fig.2: Screenshot from Plexon's VideoTracker Viewer displaying a rats's completed session on an eight-arm radial-arm maze

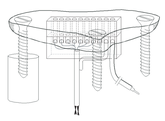


Fig.3: Micro-driveable dual- or four tetrode electrode (adapted from Kubie, 1984) using a 50-mm 10-pin Omnetics connector

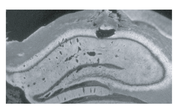


Fig.4: Histology of electrode recording site by frequency lesion

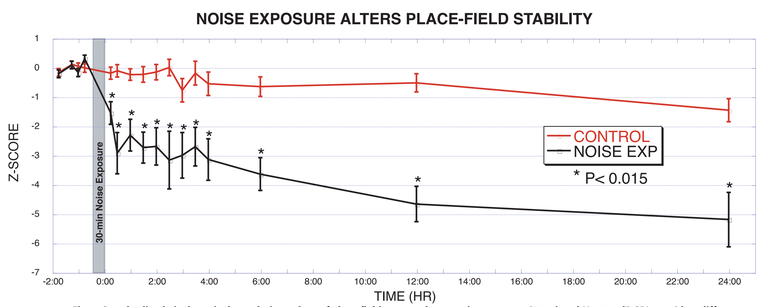


Fig.5: Standardized pixel-to-pixel correlation values of place-fields pre- and post-noise exposure (30 min, 4 kHz, 104 dB SPL tone) in a different environment. Place-cells exhibited significantly altered place-fields post-noise exposure in a stable environment.

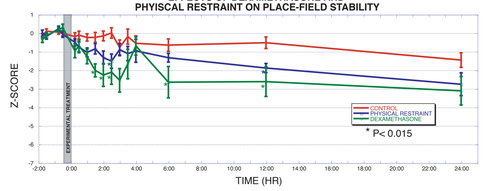


Fig.6: Effects of dexamethasone (60 µg/kg subq) or of physical restraint on place-field stability.

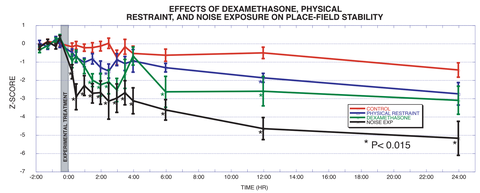


Fig.7: Effects of dexamethasone or physical restraint compared to that of acute noise-exposure on place-field stability.

Methods

Chronic implants: Two or four tetrodes (2 or 4 twisted bundles of four 25 µm Formvar-insulated nichrome wires) were threaded through a 27 ga. thin wall stainless steel cannula, attached to a vertically drivable connector assembly (adapted from Kubie, 1983), and implanted stereotaxically over the dorsal CA1 field and cemented to skull screws along with a 24 ga. reference wire. Removable red-green LEDs were affixed to a source-follower preamp. head stage for each session to facilitate monitoring of the rats' location. Shielded fine wire cables were connected via a 24-channel commutator to the MAP system (below).

Subjects & Behavioral Task: Male Long-Evans rats (350-450 g) were food deprived to 85% of pre-surgical weight over 3 d. Electrodes were implanted, 72 hr allowed for recovery, and unit activity was surveyed. Electrodes were advanced up to 50 µm daily and allowed to settle 4 hr before the next survey. Rats with well isolated single-unit activity were trained on an 8-arm radial-arm maze to visit all arms for drops of chocolate milk at the ends of arms. Each session required a rat to travel to the end of all 8 arms (taking ~2.5-4 min/session).

Pre- and Experimental Sessions: Five pre-sessions were recorded to evaluate stability of place fields, at intervals >15 min between sessions. Only single-units with stable place fields [i.e. having stable amplitude and waveform characteristics (physiological stability) and stable location-specificity (field stability, pixel-to-pixel cross-correlation average > 0.40) across all pre-experimental sessions] were then tested experimentally.

-Noise Exposure: a 4 kHz, 104 dB SPL tone was presented for 30 min through an overhead speaker in a sound chamber in a separate environment.

-Dexamethasone: 60 ug/kg in 1% ethanol in 0.9% saline was administered subcutaneously at the scruff of the neck.

-Physical Restraint: 10 min placed in bag immoblizer, then snuggly wrapped in acoustic foam (head and chronic implant unrestrained).

-Controls: 30 min in same sound chamber as noise exposure group with no tone presented.

Immediately after treatment, rats were placed on the maze and unit activity was assessed for post-treatment effects, under identical conditions. Testing occurred at 15 min intervals for the first 2 hr, every 30 min up to the 4th hr, then at the 6th and 12th hour.

Recording & Data Analysis: Amplification and filtering of multi-unit signals was performed using a Multichannel Acquisition Processor (MAP) System (Plexon Inc., Dallas, TX) and spike waveforms recorded and template sorted using Plexon's RASPUTIN and OffLine Sorter software. Spatial location was recorded using Plexon's VideoTracker System (Fig. 2). Place-fields were analyzed with NeuroExplorer and NeXScript (Nex Technologies, Littleton, MA). Pixel-to-pixel correlation anaylsis was performed in MatLab (MathWorks, Natick, MA) and was used to compare place field stability between sessions. Monte Carlo statistics were used to determine the best-fit pre-treatment session. The average correlation and standard deviation were used to determine the standardized value of all post-treatment correlation changes. Data for early post-treatment intervals are collapsed above for graphic analysis. Nonparametric Wilcoxin t-tests of two related samples were performed on all data (SPSS, Chicago, IL).

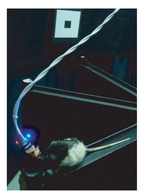


Fig. 8: Picture of rat drinking chocolate milk reward at the end of a radial-arm and of square extramaze cue.

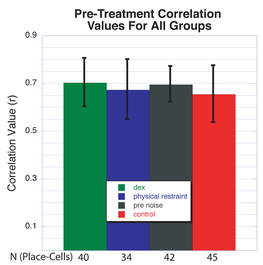


Fig. 9: Correlation values of all 5 pre-treatment place-field sessions correlated to the best-fit place-field session for all place-cells in the study

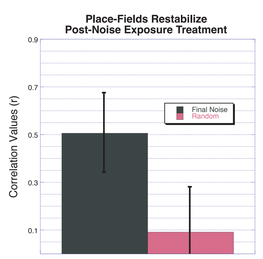


Fig. 10: Correlation values of the final 5 post-noise exposure treatment place-field sessions correlated to the best-fit place-field of those sessions compared to

Results

Fourteen rats with stable place-field activity (Fig. 9) were selected for study (8 underwent noise exposure, 3 physical restraint, 4 dexamethasone injection, 5 controls). The place-fields of 160 stable CA1 single-units were studied (42, 34, 40 & 46 per treatment, respectively). A total of 3,231 place-field sessions were analyzed for spatial location (892, 731, 752 & 856 per treatment, respectively).

Place-fields significantly changed location-specific firing post-noise exposure at all time periods tested (Fig. 5, $p<0.015$). Monte Carlo analysis of the final 5 sessions for place-fields after noise exposure treatment showed a new stable place-field emerged, $r = 0.51$ (Fig. 10). These new fields were located in significantly different places from the initial field, compared to random place-field correlations, $r = 0.09$.

Place-fields also significantly changed location-specific firing post-dexamethasone treatment (Fig. 6) and, to a lesser degree, post-physical restraint treatment (DEX at 1.5, 2, 2.5, 6, 12-hr; physical restraint at 2.5 and 12-hr only ($p<0.015$)). The magnitude and duration of these changes were less than those observed after noise exposure.

Summary

- Noise exposure alters stability of hippocampal place-fields in a stable environment
- Place-cells activity post-noise exposure re-stabilizes with altered firing fields
- Dexamethasone and physical restraint alter hippocampal place-fields less than tinnitus-inducing acute noise exposure
- Auditory system plasticity alters hippocampal location-specific firing; the effects of stress cannot account for the changes observed in this paradigm
- Noise (tinnitus) and stress (DEX, physical restraint) induce novel forms of hippocampal neuronal plasticity, unrelated to the configuration of stimuli within a stable environment

Acknowledgements

This work was supported by a grant from the American Tinnitus Association to A.R.M. and L.T.T., BBS and DARPA funding to L.T.T., and a predoctoral research fellowship from Jeffrey H. Owen to T.J.G.