Evidence for Multisystem Plasticity in Non-Classical Auditory Regions in Early Stages of Tinnitus

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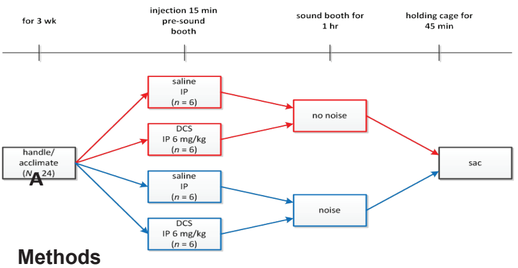
Introduction

Chronic tinnitus is a currently incurable and inadequately understood pathological condition affecting 10-15% of people, with severe life-altering effects in 1-2%. Current therapeutic strategies largely focus on classical auditory pathways from cochlea to cortex. Tinnitus develops over time, and new evidence indicates interactions of multiple plastic neural systems including, but not limited to, both canonical and non-canonical auditory paths of the brain. For example, our lab and others have demonstrated hippocampal plasticity in early and later stages of tinnitus (Goble et al., 2009; deRidder et al., 2006), have shown strong causal linkages between amygdala and hippocampus in auditory-mediated plasticity (Donzis & Thompson, 2013; Farmer & Thompson, 2012), and have shown the excitatory biomarker Arc (activity-related cytoskeletal protein) is up-regulated in hippocampus within 1 hr after noise trauma exposure (Kapolowicz et al., 2013, 2014).

For the current study, male rats were exposed to acute bilateral high-intensity noise (16 kHz, 115 dB, for 1 hr). Western blot analyses were used to assess neural plasticity in multiple brain regions 30 min later. Expression of biomarkers of plasticity included: cGMP-dependent protein kinase (PKG), which has been linked to memory consolidation in the hippocampus (Hc), amygdala (Amy), and cerebellum (Cb); calcium-calmodulin-dependent kinase 4 (CaMK4), which has been linked to activation of transcription factor cAMP response element binding protein (CREB) in the Hc, Amy, and nucleus accumbens (nAC); and calcium-calmodulin-dependent kinase 2 beta (CAMK2b), which has been linked to long-term-depression in the Hc and Cb a priori identified as responding to stressful stimuli. Our results confirmed a multiple-system modulation in response to the stressful noise-trauma event, including the regions Cb, nAC, Hc, and vertical diagonal band among others with respect to these biomarkers. One region consistently exhibiting plasticity in multiple biomarkers was the medial entorhinal cortex, a critical bridge between cortical and limbic structures. These data indicate that additional regions and additional biomarkers should be strongly considered in the design of new therapeutic interventions.

Behavioral Paradigm



Methods

Subjects: All animal uses were approved by the UTD IACUC. Male Long-Evans rats were housed on a 12 hr light/dark cycle in a temperature-controlled room, with food and water available ad libitum.

Behavior: Rats were handled daily for 3 wk while maintaining a constant environment to minimize confounds and to assure that observed changes in protein expression were due to noise trauma. The rats were either exposed to noise trauma or no noise trauma in a sound controlled chamber.

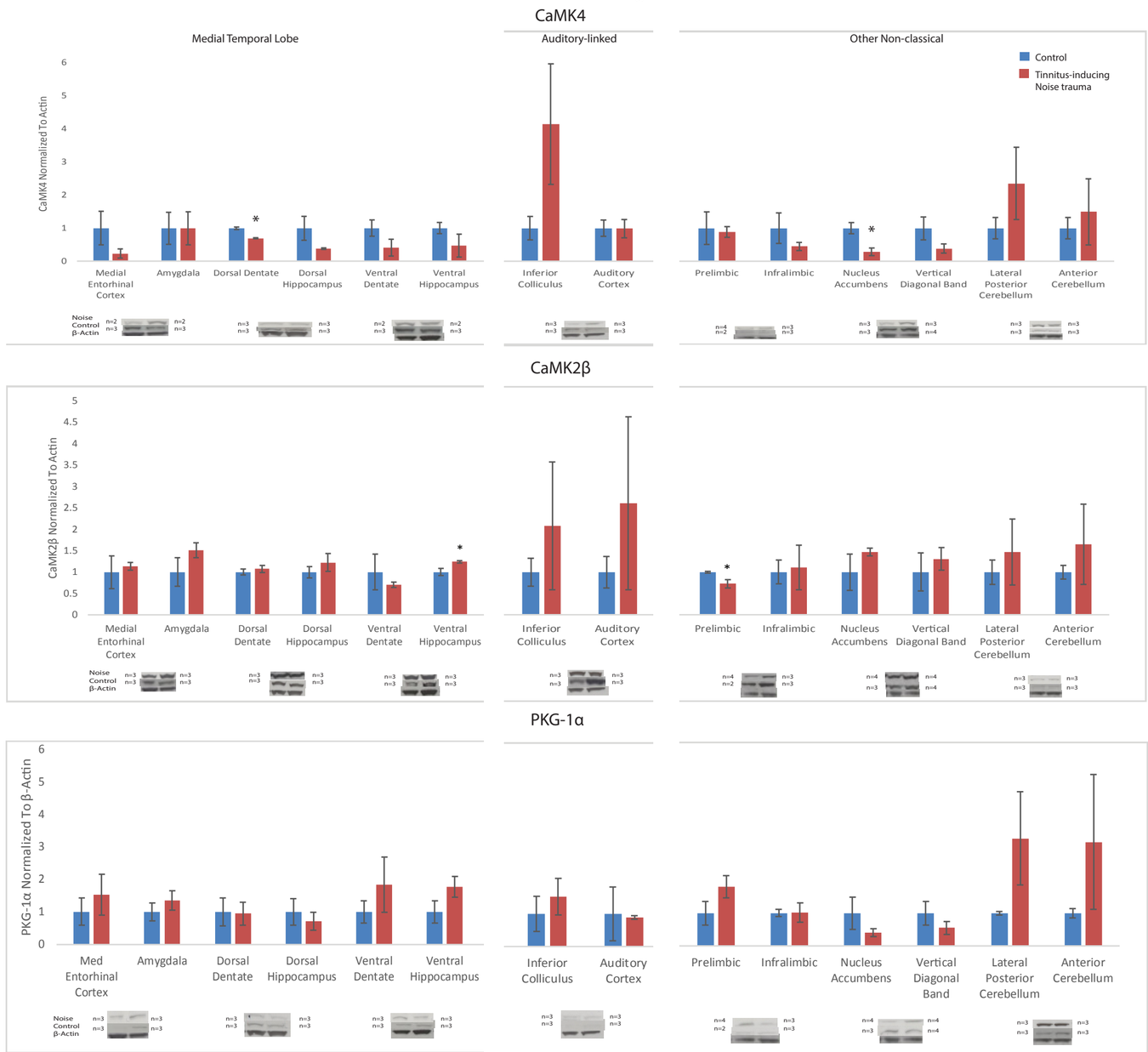
Noise Exposure: Acute noise trauma was induced bilaterally (16 kHz, 115 dB) for 1 hr.

Western Immunoblotting and Analysis for CaMK4, CaMK2β, and PKG-1α: Rats were sacrificed 30 min after time in the sound booth ended, a time point that is important for HPA-induced glucocorticoid and noradrengergic interactions that promote memory (Makara and Haller, 2001; Roozendaal, 2003). Brains were flash frozen in 2-methylbutane submerged in a mixture of dry ice and ethanol and stored at -80° C until processed. 500 µm sections were taken and 1.22 mm diameter tissue punches collected from 15 regions identified using a rat atlas (Paxinos & Watson, 1998). Punches were homogenized in a 0.1 M phosphate buffer containing a protease inhibitor cocktail. A Qubit fluorometer and protein assay kit was used to determine the concentration of protein in each sample. 15 µg tissue samples were heated in a sample buffer with reducing agent and loaded into wells and run on an SDS-PAGE gel for ~55 min at 200 V and 200 mA per gel. Gels were transferred onto a nitrocellulose membrane via the iBlot dry blotting system (LifeSciences) for 7 min. The membrane was washed with Tris-buffered saline, and blocked with 5% skim milk for 1-24 hr on a rotator. The membrane was probed overnight with the primary antibodies of interest (CaMK4, CaMK2 β, PKG-1α, and Actin) diluted in 5% skim milk. The membrane was incubated in a secondary HRP-linked antibody for 1 hr followed by an ECL chemiluminescent detection of immunoreactivity. Membranes were placed on films with 3 min exposure times, developed, and scanned into a computer for analysis. Actin was used as a loading control for normalizing CaMK4, CaMK2β, and PKG-1α expression. Western blot densitometry was analyzed using ImageJ 1.46r software, and statistical comparisons were made using Excel software. A series of planned comparisons were made using a between groups, two-tailed Student's *t*-test to assess how CaMK4, CaMK2β, and PKG-1α varied between each of the 2 groups. A probability level of $p < 0.05$ was considered significant.

Hypotheses

A noise trauma event will induce an upregulation of enzymatic activity in CaMK4, CaMK2β, and PKG-1α at stress-associated cell populations not typically considered to be part of the classical auditory circuit.

Western Immunoblotting Results



Above Figures. Evidence of rapid plasticity at a 30 min time point in CaMK4 (top), CaMK2β (middle), and PKG-1α (bottom) protein expression among the medial temporal lobe, auditory-linked, and non-classical auditory cell populations. There was considerable regional variability, indicating that a simplistic interpretation (e.g. noise trauma induces rapid plasticity throughout the circuit) is not well supported. A statistically significant downregulation of CaMK4 was seen in both the dorsal dentate nucleus and nucleus accumbens. Additionally, there was an upregulation of CaMK2β in ventral hippocampus, but a downregulation in prelimbic prefrontal cortex. Normalized data are presented as means ±SEM.

Conclusions

Effects of Noise Trauma on CaMK4 expression

Medial temporal lobe: Acute noise trauma significantly down-regulated protein expression in the dorsal dentate compared to controls. There was a trend for down-regulation of protein expression in the dorsal hippocampus and the medial entorhinal cortex.

Auditory-linked regions: Acute noise trauma showed a trend for up-regulation in the central nucleus of the inferior colliculus, but not the auditory cortex.

Other non-classical regions: Acute noise trauma significantly down-regulated protein expression in the nucleus accumbens compared to controls.

Effects of Noise Trauma on CaMK2β expression

Medial temporal lobe: Acute noise trauma significantly up-regulated protein expression in the ventral hippocampus compared to controls.

Auditory-linked regions: Acute noise trauma had no immediately significant effect on these regions.

Other non-classical regions: Acute noise trauma significantly down-regulated protein expression in the prelimbic area of the prefrontal cortex.

Effects of Noise Trauma on PKG-1α

Medial temporal lobe: Acute noise trauma caused a trend for up-regulation of protein expression in the ventral hippocampus.

Auditory-linked regions: Acute noise trauma had no immediately significant effect on these regions.

Future Directions

- Follow cortico-striatal circuit's role into tinnitus development.
- Differentiate cells expressing plasticty products with immunohistochemistry.
- Follow time course of place cell disruption and stabilization as potential time points for further molecular assays involving cytokines and trophic factors.

References

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