MICROGLIA IN THE RAT COCHLEAR NUCLEI: A PLAYER IN TINNITUS-RELATED CIRCUIT REORGANIZATION?

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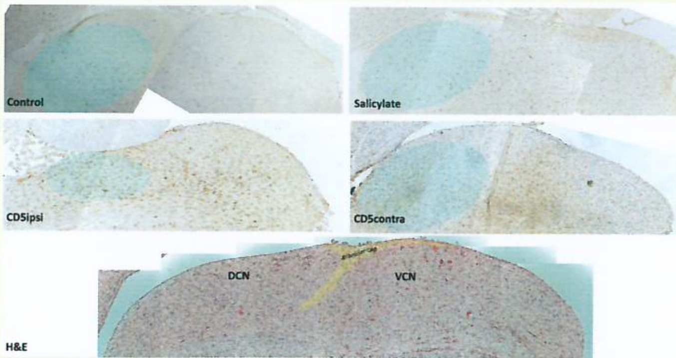
INTRODUCTION

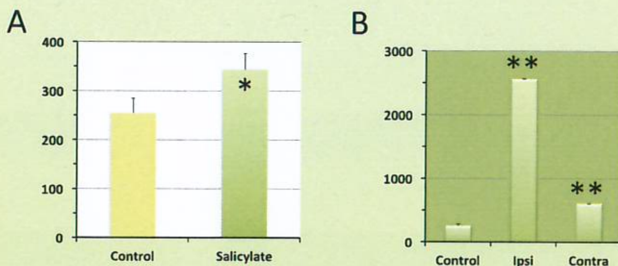
Although tinnitus origins are still elusive, hyperactivation of cochlear nuclei (especially DCN) appears necessary in its early stages, presumably as a response to signals coming from an unbalanced/damaged periphery (Tsounopoulos 2008). DCN hyperactivation results from changes in the interneuronal inhibitory network (Middleton et al. 2011) that increase the discharge of projection cells. A similar disinhibition of projection cells is observed in the spinal cord during the onset of chronic pain, where it is associated to a microglia-dependent remodeling of the first central station following peripheral damage (Ferrini et al. 2013).

Microglia, the resident macrophage of nervous tissue, has been found to contribute to synaptic plasticity in healthy brains ((Wake et al. 2012), and to modify its population density and activation state in response to inflammation and tissue damage, regulating the excitability of surrounding neural circuits in several pathological states (Eyo and Dailey 2013). Activation of microglia can proceed along several pathways producing cytotoxic/proinflammatory and phagocytic forms; usually, the activation of microglial cells is accompanied by shape changes from a "resting" state characterized by a small soma and several thin and ramified processes, to "activated" states with thicker processes or, in more extreme cases, to a rounded, macrophage-like form without processes (Kettenmann et al. 2013). On the other hand, physiological plasticity phenomena may be accompanied by a variation of microglial density without changes in its activation (Wake et al. 2013). In this work we measured microglia density and activation in cochlear nuclei following treatments that are known to induce tinnitus.

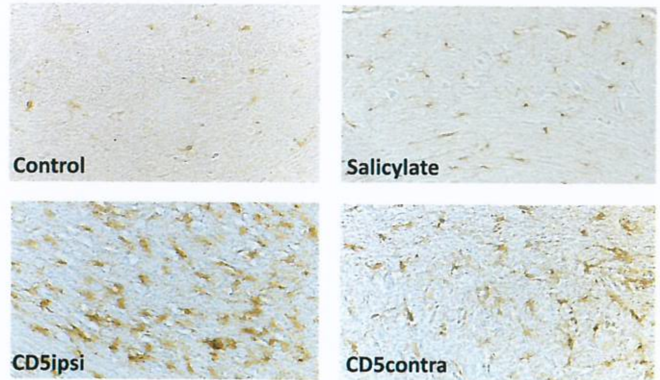
RESULTS



Coronal brainstem slices immunolabeled for Iba-1 show microglia distribution in cochlear nuclei (10x). In all panels, nuclei were oriented with DCN on the left and nerve root on the right. Bottom panel shows a section colored with H&E, to evidence the granular cap (highlighted in yellow), which served as a reference structure for marking nucleus boundaries. Approximate DCN boundaries are indicated for each reconstruction with a blue shadow. In control conditions, microglial cells displayed a small soma and few thin processes. Salicylate treatment increased the number of microglial cells, although their morphology remained similar to resting. 5 days after unilateral cochlear destruction, clear microgliosis was observed, both in the ipsilateral (CD5ipsi) and contralateral nuclei (CD5contra). In the latter, a density increase was also observed in a region presumably corresponding to the granular cell domain (asterisk). Noise trauma produced less obvious effects on microglia density (not shown).



Effect of tinnitogenic treatments on microglial density in the rat DCN. Data are expressed as number of processes/mm^2, since in thin sections the whole cell arborization was not always preserved. This measure also privileges thicker processes, since thin processes are often missed, and so it enhances detection of microglia activation. A: After treatment with salicylate (n=7) a significant increase in DCN microglia density was observed vs. controls (n=6). B: 5 days after unilateral cochlear destruction, a strong and highly significant ($p<0.01$) increase in microglial density was observed in the DCN on both sides. * = $p<0.05$, **=$p<0.01$



Microglia morphology was observed at higher magnification. In control conditions (A), microglial cells displayed a small soma and few thin processes. After salicylate treatment, microglial cells did not display a clear activated morphology, although processes seemed slightly thicker than control. After cochlear destruction, on the other side, clear morphological changes were observed. In acute preparations (5d after destruction) microglial cells were roundish and larger in the ipsilateral CN (CD5ipsi) and displayed thicker processes in the contralateral side (CD5contra). A clear increase in number was also seen.

CONCLUSIONS

Microglia density in rat DCN after salicylate treatment was higher than in controls, consistently with the plasticity observed in extralemniscal auditory pathways following treatment with this drug (Stolzberg et al. 2012). The increase in density was lower, and the morphological change more subtle, when compared to cochlear destruction. The latter condition, which also dramatically increases density and activation of microglia in the ventral cochlear nuclei (Santamaria et al. 2012) is also associated to the onset of tinnitus. However, tinnitus evoked by salicylate is reversible, whereas that deriving from peripheral damage is usually irreversible. Since resting microglia is able to prune synapses but not to destroy neurons, whereas activated microglia has a strong phagocytic activity, it is possible that circuits during salicylate-evoked tinnitus are transiently imbalanced, but subsequent homeostatic mechanisms (Turrigiano 2012) are able to bring the activity back to normal; on the other hand, after removal of nerve fibers or whole neurons by phagocytic microglia, reversal is no longer possible.

METHODS

Experiments were performed on Wistar rats, 25 days old at the time of treatment. Acute tinnitus was induced with intraperitoneal injection of high doses of Na-salicylate (300 mg/kg), one injection/die for 3 days. Rats were sacrificed 2, 24 and 48 hours after the last injection. As a condition inducing chronic tinnitus we performed unilateral surgical destruction of the cochlea.

For the surgery, animals were anesthetized with ether (10 ml/kg) and diazepam (20 mg/kg IP). The skin behind the ear was shaved, and a retroauricular incision was made to identify the external auditory canal. After the bulla was exposed, the cochlea was completely destroyed using a fine drill.

After sacrifice, the rat head was placed in carbogen-bubbled Krebs solution, brain was removed and placed in 4% PFA, where it remained at least 24h. Care was taken not to exceed 6 min between sacrifice and fixation because times longer than 10 min have been reported to induce tissue damage and microglia activation (Nimmerjahn et al. 2005). After fixation, samples were dehydrated and included in low-melting paraffin. Samples were sliced at 10 μm, deparaffinized, rehydrated and immunostained for Iba-1 (WAKO, expressed by microglia and macrophages) with streptavidin-biotin DAB (DAKO) secondary staining.

Microglia cell numbers and DCN areas were measured using imageJ (cell counter and measure Area plugin), with a blind procedure where experimenters were not aware of the nature of the samples observed. DCN limits were defined by analizing hematoxylin-eosin sections for the position of the granular cap indent within cochlear nuclei. Statistical significance was obtained from one-tailed t-test for homoscedastic samples.

REFERENCES

- Eyo UB, Dailey ME. Microglia: Key Elements in Neural Development, Plasticity, and Pathology. J Neuroimmune Pharmacol. 2013 Jan 27.
- Ferrini F, Trang T, Mattioli TA, Laffray S, Del'Guidice T, Lorenzo LE, Castonguay A, Doyon N, Zhang W, Godin AG, Mohr D, Beggs S, Vandal K, Beaulieu JM, Cahill CM, Salter MW, De Koninck Y. Morphine hyperalgesia gated through microglia-mediated disruption of neuronal Cl^- homeostasis. Nat Neurosci. 2013 Feb;16(2):183-92.
- Fuentes-Santamaría V, Alvarado JC, Juiz JM. Long-term interaction between microglial cells and cochlear nucleus neurons after bilateral cochlear ablation. J Comp Neurol. 2012 Sep 1;520(13):2974-90.
- Kettenmann H, Kirchhoff F, Verkhratsky A. Microglia: new roles for the synaptic stripper. Neuron. 2013 Jan 9;77(1):10-8.
- Middleton JW, Kiritani T, Pedersen C, Turner JG, Shepherd GM, Tzounopoulos T. Mice with behavioral evidence of tinnitus exhibit dorsal cochlear nucleus hyperactivity because of decreased GABAergic inhibition. PNAS 2011 May 3;108(18):7601-6.
- Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science. 2005 May 27; 308(5726):1314-8.
- Stolzberg D, Salvi RJ, Allman BL. Salicylate toxicity model of tinnitus. Front Syst Neurosci. 2012;6:28.
- Turrigiano G. Too many cooks? Intrinsic and synaptic homeostatic mechanisms in cortical circuit refinement. Annu Rev Neurosci. 2011;34:89-103.
- Tzounopoulos T. Mechanisms of synaptic plasticity in the dorsal cochlear nucleus: plasticity-induced changes that could underlie tinnitus. Am J Audiol. 2008 Dec;17(2):S170-5.
- Wake H, Moorhouse AJ, Miyamoto A, Nabekura J. Microglia: actively surveying and shaping neuronal circuit structure and function. Trends Neurosci. 2013 Apr;36(4):209-17.

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