In vivo imaging of nicotine-exposed adult male zebra finches

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Introduction: Research has shown that neurogenesis occurs in the songbird telencephalon during unique periods of embryonic, juvenile and adult life. This remarkable restriction of postnatal neurogenesis to the telencephalon suggests that these new cells may participate in higher associative brain functions such as perception and learning [1]. To this point, most attention has been focused on the High Vocal Center, which is part of a series of interconnected brain nuclei that control song learning and production. In addition, continual growth also occurs in the adult avian hippocampus, a process similar to the adult mammalian hippocampus. The nicotinic cholinergic system is implicated in a diversity of neurological functions such as pain modulation, reward and cognition. Studies have shown that nicotine exposure can lead to modification of cognitive functions [2,3]. In the zebra finch, nicotinic acetylcholinergic receptors are localized in several song nuclei and the hippocampus [4], and recent *in vitro* research supports the fact that the nicotinic cholinergic mechanism could play a critical role in long-term potentiation in the zebra finch brain [5]. Recent studies have used MRI and contrast enhancement to study plasticity in songbirds [6,7]. This study employs Magnetic Resonance Microscopy (MRM) acquired *in vivo* and *ex vivo* at 21.1 T (900 MHz) to examine the pharmacological impact of nicotine on zebra finch brain morphology. This unique magnetic field provides high resolutions for anatomical identification, as well as improved SNR for significantly shortened acquisition times compared to previous studies. With correlation to physiological and histological evaluations, these microimages highlight the effect of nicotine on cognitive processes in the adult male zebra finch brain.

Methods: ***Animals and Experimental Setup:*** All animal procedures are in compliance with the regulations of The Florida State University Animal Care and Use Committee. Adult male zebra finches were singly housed in recording chambers [8], where physiological parameters, such as vocalization, locomotor activity, food and water intake, were measured. After vocalization was stabilized, birds were selected randomly to undergo *in vivo* imaging. Two *in vivo* imaging sessions were held, one before the nicotine treatment (*pre-scan*) and one after (*post-scan*). Equithesin was administered 10 min prior to MRM. This anesthesia allows for *in vivo* imaging time of 60-75 min. Following *pre-scan*, animals were returned to their home cages. Nicotine treatment began after vocalization returned to baseline (±10%). Birds received subcutaneous injections of low, mid and high doses of nicotine (0.054, 0.18 and 0.54 mg/kg, respectively). An interval of 48 hrs was observed between the administrations of different doses of nicotine. Immediately following the last nicotine injection (14 days following *pre-scan*), post-scan was performed to assess morphological changes. Animals were sacrificed 24 hrs after the final nicotine treatment, and the brain was extracted to be scanned at higher resolutions. After MRM, brains were sectioned for histological purposes.

Imaging protocol: Following the administration of Equithesin, the bird was immobilized in a cotton blanket and placed within a 30-mm diameter ^{1}H linear birdcage coil for *in vivo* imaging. For ease of loading and improved survivability, the bird was inverted in this coil. A regulated flow of heated air was provided at the bottom of the coil to supply oxygen and maintain a stable environmental temperature (~26 C). All microimages were acquired on a vertical, ultra-widebore 21.1-T magnet equipped with a Bruker Avance console and Micro2.5 imaging gradients. For *in vivo* pre- and post-scan images, multi-slice 2D fast spin echo (FSE) images with fat suppression were acquired over ~5.5 min with a RARE factor = 4 and TE/TR = 6.5/2500 ms in parasagittal and coronal orientations at resolutions of 59x100x500 μm and 63x63x500 μm, respectively. For *ex vivo* imaging, finch brains were imaged individually in appropriately sized coils (e.g. 10-mm diameter) while immersed in a non-proton fluorocarbon (FC-43, 3M Corp.). Again, multi-slice 2D FSE images were acquired, both at the *in vivo* resolutions as well as at higher resolutions (40x40x150 μm). Additionally, true 3D gradient-recall echo (GRE) images were acquired from the *ex vivo* brains at a 50-μm isotropic resolution (TE/TR=10/100 ms).

Results and Discussion: The primary focus of this study was to establish a protocol for recurring *in vivo* imaging of adult male zebra finch brain. We were able to perform survival *in vivo* imaging at 21.1 T, and the quality of the *pre-* and *post-scan* is very acceptable for comparative analysis. Using these microimages, we are able to identify many of the key components of the song pathways and to assess alterations in neuroanatomy may be attributable to micotine treatment. One of the main advantages of imaging at 21.1 T was the significantly shortened imaging time per animal versus the relatively long exposure time used for previous songbird imaging efforts [6,7]. Combining these *in vivo* images with the *ex vivo* images and histological analysis advances our understanding of neurogenesis in the adult male zebra finch after exposure to nicotine. In future studies, we will be focusing not only on the adult songbird, but we will also use juvenile and senescent animals to study the process of cognitive functions after nicotine exposure. In general, this technique can contribute to a better understanding of cognitive functions not only in the zebra finch, but also in other animal models.

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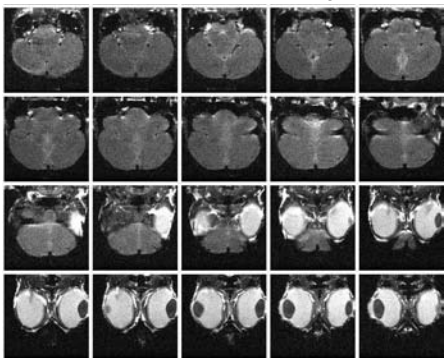

Figure 1. Coronal *in vivo* MRM (63x63x500 μm)

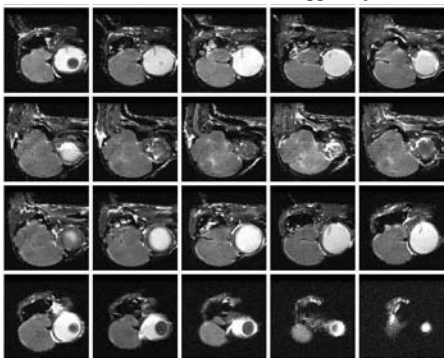

Figure 2. Parasagittal *in vivo* MRM (59x100x500 μm)

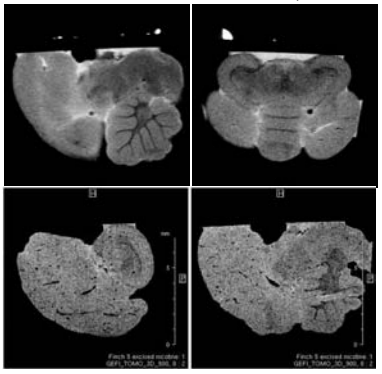

Figure 3. *Ex vivo* MRM
Top – 2D FSE (40x40x150 μm)
Bottom – 3D GRE (50 μm isotropic)