A Volumetric Analysis of the Adult Zebra Finch Brain after Single and Repetitive Ecstasy Exposure

P. Foroutan[1,2], S. L. T. Cappendijk[3], and S. C. Grant[1,2]

[1]The National High Magnetic Field Laboratory, Tallahassee, Florida, United States, [2]Chemical & Biomedical Engineering, Florida State University, Tallahassee, Florida, United States, [3]Biomedical Sciences, College of Medicine, The Florida State University, Tallahassee, Florida, United States

Introduction – The use of the recreational drug 3,4-methylenedioxymethamphetamine (MDMA, or more commonly, Ecstasy) has increased worldwide in recent years, especially among young adolescents. The drug is mainly used by people aged 18-30 years—though users include pre-teen adolescents—and most often in a binge pattern. Unlike most other drugs of abuse, MDMA is not physically addictive, and many people inaccurately consider the drug to be harmless. However, research has shown that users may be more prone to develop neurodegenerative disorders (*i.e.* Alzheimer's and Parkinson's Disease) as they age. Previous human studies have shown that some MDMA users experience long lasting depression and impairment in learning and memory [1,2], which is supported by fMRI studies that display altered activation in regions of cognition, emotion and motor function with MDMA use [3,4]. Although morbidity and mortality are relatively low [5], the large number of MDMA users and the unknown chronic effects of the drug remain a crucial public health issue. The aim of this pilot study was to investigate possible MDMA-induced neurodegeneration in the neuronal network of the adult zebra finch, an accepted model for studies of high-order cognitive processes and neuronal plasticity. As zebra finches exhibit postnatal neurogenesis similar to humans, this animal model is extremely suitable to use in longitudinal studies. By monitoring the neuronal circuitry underlying the song and auditory pathways, it is possible to evaluate cognitive impairments following MDMA exposure. To assess morphological changes in the anatomy of the excised finch brain, high-resolution magnetic resonance imaging at 11.75 T was performed for volumetric analysis.

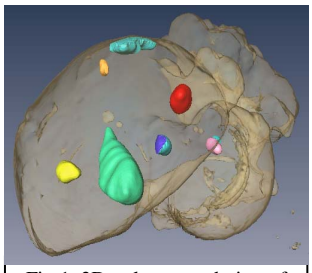

Fig 1. 3D volume rendering of song nuclei. Red=RA; Blue=DLM; Light Blue=HVC.

Animal Protocol – Eighteen adult male zebra finches were used in this study. The birds were divided into three MDMA treated groups and three age-matched control groups. MDMA (10 mg/kg, i.p.) was given 1x/day as a single, double or triple treatment where the second and third injects were given on consecutive days. Control animals received 0.03 mL/10 g animal saline, i.p. Six hours following each final drug administration, brains were excised and fixed in 4% paraformaldehyde.

Imaging Protocol – All MR data were acquired using an 11.75-T vertical magnet equipped with a Bruker Avance Console and Micro2.5 gradients. The brain tissue was washed in phosphate buffered saline (PBS) prior to imaging and immersed in Fluorinert (3M, Corp). Using a 10-mm birdcage coil, three-dimensional gradient-recalled echo (GRE) scans (TE/TR=15/200 ms) were acquired at an isotropic resolution of 40 µm. Acquisition time per scan ranged between 3-5 hours depending on the geometry of the excised tissue. Based on a preliminary study investigating the effects of temperature on generated contrast, the acquisition temperature was set to 5˚C. Following scanning, histological analysis using Luxol Fast Blue (LFB) staining was performed on 18-µm thick brain sections. Segmentation of song nuclei and telencephalon was performed in all three planes (x, y & z) utilizing a 3D volume rendering program (AMIRA 4.1.1) in order to obtain tissue statistics (Fig 1).

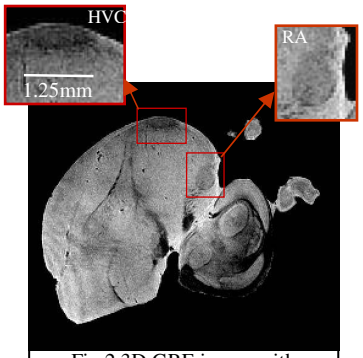

Fig 2.3D GRE image with isotropic resolution 40µm, showing the HVC.

Results and Discussion – In this study, we were able to identify and segment numerous structures (including song nuclei) in the excised zebra finch brain without the use of exogenous contrast enhancement, as was necessary in previous studies [6]. Although a pilot study, volumetric data (Table 1) demonstrated certain trends. A comparison between MDMA and saline-treated animals displays an overall decrease in the sizes of the telencephalon and most song nuclei, including the robust nucleus of the arcopallium (RA) and the medial nucleus of the dorsolateral thalamus (DLM). The HVC (formerly called the high vocal center), however, showed the opposite trend, namely an increase in volume, when comparing the treatment groups with their corresponding controls. In addition, the volume of the HVC may increase with the number of MDMA treatments, which could be attributed to MDMA-induced auditory input. In one of our preliminary studies, we showed that a single administration of MDMA induced a significant decrease of song production in adult male zebra finches. Previous studies also have shown that the amount of produced song depends on the volume of song nuclei [7, 8]; therefore, decreases in the RA and DLM may be consistent with reduced song production. Histological sections displayed demyelination with increased number of MDMA treatments (Fig 3). This demyelination may have impacted our ability to identify and quantify volumetric changes in certain song nuclei, particularly the HVC (Fig 3 & 4). Current data suggests that the adult zebra finch brain did not display statistically significant MRI volumetric changes in the song nuclei in response to acute MDMA exposure, although other alterations (*i.e.* demyelination) of the song nuclei may have been identified. Future studies will analyze song nuclei volume in juvenile birds to investigate the impact of acute and chronic MDMA exposure during development.

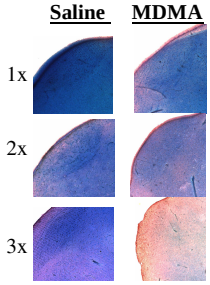

Fig 3. Comparison of LFB stained brain tissue showing the HVC.

Volumes of the song nuclei [HVC, medial nucleus of the dorsolateral thalamus (DLM), the robust nucleus of the arcopallium (RA)] and Telencephalon

	V_{RA}		V_{HVC}		V_{DLM}		$V_{telenc.}$	
	Saline	MDMA	Saline	MDMA	Saline	MDMA	Saline	MDMA
1x	3.2±0.8	2.9±0.4	2.4 (n=2)	1.0 (n=1)	2.4±0.9	2.0±0.5	1425±38	1278±113
2x	3.8±0.1	3.2±0.7	1.9 (n=2)	3.2 (n=2)	2.7±0.5	2.1±0.4	1309±32	1213±133
3x	2.9±0.7	2.4±0.1	1.4±0.4	3.5 (n=1)	1.9±0.9	1.5±0.4	1188±60	1218±54

Table 1. Average volumes and standard deviations of some of the major song nuclei & telencephalon. All volumes are $10^8 \mu m^3$

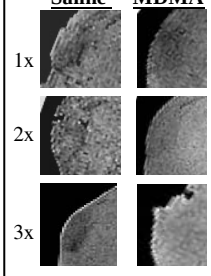

Fig 4. Comparison of 3D GRE sections (40-µm isotropic) visualizing the HVC.

Acknowledgements & References– This research was supported by The National High Magnetic Field Laboratory (NSF DMR-0084173), The Florida State University and the State of Florida. All MRI data were acquired at the NHMFL, Tallahassee, FL.

1. Gouzoulis-Mayfrank E & Daumann J (2006), Review, *Addiction,* 101:348-61
2. Morgan MJ (2000), Review, *Psychopharmacology (Berl)*, 152:230-48
3. Daumann J *et al.* (2004), *Biological Psychiatry*, 56:349-355
4. Lingford-Hughes A (2005), *Current Opinion in Pharmacology*, 5:42-46
5. Proceedings of the Community Epidemiology Work Group (2007), *Epidemiologic Trends In Drug Abuse*, National Inst. On Drug Abuse (NIDA)
6. Poirier C *et al.* (2008), *NeuroImage*, 41:1-6
7. Nottebohm F *et al.* (1986) *Behav. & Neural Biol.* 46:445-471
8. Van Meir V *et al.* (2006) *NeuroImage*, 31:981-92