Neuroprotective Effects of Alpha Lipoic Acid on Ecstasy-exposed Zebra Finches: An *in* and *ex vivo* volumetric analysis

P. Foroutan[1,2], C. P. Perreault[3,4], S. L. Cappendijk[3,4], and S. C. Grant[1,2]

[1]The National High Magnetic Field Laboratory, The Florida State University, Tallahassee, FL, United States, [2]Chemical & Biomedical Engineering, FAMU-FSU College of Engineering, Tallahassee, FL, United States, [3]Biomedical Sciences, The Florida State University, Tallahassee, FL, United States, [4]College of Medicine, The Florida State University, Tallahassee, FL, United States

Introduction - The amphetamine derivative 3,4-methylenedioxymethamphetamine (MDMA), more commonly known as Ecstasy, is a popular recreational drug mainly used by young people aged 12-30 years. The National Institute on Drug Abuse (NIDA) estimates that approximately 470,000 people in the USA age 12 and older have used MDMA in the past 30 days. In humans and animal models, MDMA induces hyperthermia, with body temperatures reportedly as high as 109° F [3], which has been linked with the process of oxidative stress [1]. Oxidative stress can result in premature cell senescence and the increased release of free radicals [2]. Because young MDMA users may be more prone to develop neurodegenerative diseases, a potential treatment to retard neurodegeneration is to inactivate free radicals. Antioxidants scavenge free radicals, and may prevent DNA damage and apoptosis. The natural occurring antioxidant alpha lipoic acid (ALA) has been shown to improve memory and to delay structural mitochondrial decay in rats [4]. The aim of this study is to find a correlation between the oxidative stress induced by MDMA and the potential beneficial attributes of ALA in the neuronal network of the zebra finch. High resolution MR imaging and biochemical measures have been used to evaluate the effects of MDMA exposure and ALA treatment in the zebra finch, an accepted model pertinent to the study of cognitive processes related to the naturally learned process of vocal communication [5].

Method – ***Animals and Experimental Setup***: 6 adult male zebra finches were used in this study. All animals were housed singly in custom built recording cages, under a 14:10 D/L regimen (lights on at 08:00). To mimic human MDMA intake, MDMA was administered in a binge pattern (10 mg/kg, i.p., 3 consecutive days, 30 min before 08:00). ALA (30 mg/kg, i.p.) was administered for 7 consecutive days at 14:00. Following an injection habituation period, three animals were treated with ALA followed by MDMA (ALA+MDMA, preventative). The other three animals were treated with MDMA followed by ALA (MDMA+ALA, regenerative). On a daily basis, song patterns and physiological parameters were recorded.

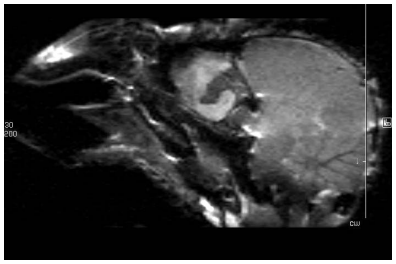

Fig 1. 2D FSE of *in vivo* Finch brain acquired at 21.1 T

Imaging protocol: All MR data were acquired using a 21.1-T vertical magnet equipped with a Bruker Avance Console and Micro2.5 gradient system. Five days before the start of the habituation period, an *in vivo* pre-scan was performed. Immediately after the last drug administration, a post-scan (drugs onboard) was performed. For *in vivo* scans, a 30-mm birdcage transmit/receive coil was used. The zebra finches were anesthetized with Equithesin (0.03 mL/30 g animal, i.m.) and immobilized within the coil. Conventional 2D fast spin-echo (FSE) scans with fat suppression (TE/TR=6.5/2500 ms) were acquired during a period of <55 min. Following the post-scan, brains were excised. For *ex vivo* scans, the brain was immersed in Fluorinert (3M, Corp), loaded into a 10-mm NMR tube and imaged with a birdcage coil. *Ex vivo* analysis included the acquisition of 2D FSE scans together with 3D gradient-recalled echo (GRE) scans (TE/TR=7.5/150 ms). 3D GRE datasets were acquired at an isotropic resolution of 40 µm. Following completion of the *ex vivo* scans, a histological analysis using luxol fast blue was performed on the brain tissue.

Results and Discussion – Volumetric analysis of the song nuclei show that the volume of area X was decreased in the preventative group (ALA+MDMA) compared to regenerative animals (MDMA + ALA). A similar tendency of diminished volume, although not as pronounced as in Area X, was observed in the DLM. Area X is the first relay of the anterior forebrain pathway and monitors adult song. In a preliminary study performed in our laboratory, it was shown that a single administration of MDMA (10mg/kg i.p) induced a significant decrease of song production in adult male zebra finches. The diminished size of the Area X may correlate with this decrease is song production because previous studies have shown that the volume of song nuclei depends on the amount of song produced [6]. However, in our histological analysis of the brain tissue, a higher cell density in the anterior forebrain was observed in the preventative group compared to the regenerative group. It has been shown that antioxidants can affect cell adhesion [7] and, therefore, could induce morphological changes in the zebra finch brain song nuclei.

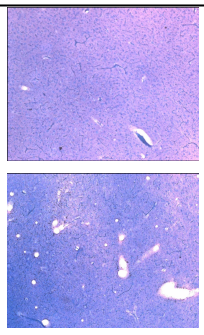

Fig 2. H&E sections of ALA+MDMA (top) & MDMA+ALA (bottom) zebra finch brain tissue

Volumes of the song nuclei [Area X, Medial nucleus of the dorsolateral thalamus (DLM) & robustus archialis (RA)] and Telencephalon

A=ALA+MDMA (preventative) B=MDMA+ALA (regenerative)

Animal	Treatment	$V_{exterior}$ (10^8 µm^3)	$V_{Area\ X}$ (10^8 µm^3)	V_{DLM} (10^8 µm^3)	V_{RA} (10^8 µm^3)	$V_{Telenc.}$ (10^8 µm^3)
1	A	9164.6	23.0	5.5	3.9	1667.2
2	A	10507.5	15.7	3.2	-	1676.8
3	A	10829.9	17.4	4.3	3.4	1686.5
4	B	11819.8	21.5	4.4	-	1639.4
5	B	12023.1	23.6	4.3	4.0	1594.2
6	B	10547.4	23.1	6.1	4.7	1684.7

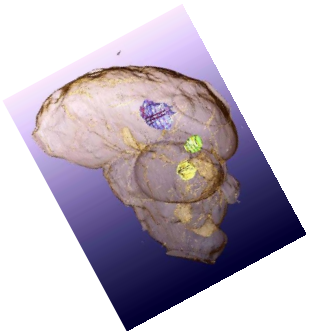

Fig 3 Volume rendering of segmented song nuclei generated from an *ex vivo* 3D GRE dataset (40-µm isotropic resolution)

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

1. Carvalho M *et al. Arch. Toxicol.* **76**:66-72, 2002
2. Chen HM *et al. Biochem. & Biophys. Research Comm.* **354**:96-101, 2007
3. Harris G *et al. Univ of Missouri, Columbia,* pages 1-20, January 2002
4. Liu JK *et al. Ann. N.Y Acad. Sci.*, **959**:133-166, 2002
5. Nottebohm F. *Ann. N.Y. Acad Sci.* **1016:** 628-658, 2004
6. Dloniak SM *et al. Hormones and Behaviour,* **39**:95-105, 2001