NMR Microscopy Analysis of the Zebra Finch Neural Pathway Following Exposure to Nicotine or Ecstasy

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ABSTRACT: Male Zebra Finches were given differing amounts of nicotine and ecstasy to determine if there are any permanent changes in the brain structures that involve the learning and vocalization of song. NMR microscopy was used to acquire brain scans, both *in vivo* and *ex vivo*, to analyze regions of interest suspected of structural changes.

Why Nicotine and Ecstasy?

About 4 million American adolescents are smokers. Despite the major health risk for this age group, a large gap in research on the behavioral and neurobiological effects of nicotine still exists. Nicotine is a common stimulant and a potent neurotoxin. It acts on the nicotinic acetylcholine (ACh) receptors, and leads to an increasing flow of adrenaline, a stimulating hormone. In addition, it increases dopamine levels in the reward circuits of the brain. Nicotine enhances concentration, learning, and memory due to the increase of Ach.

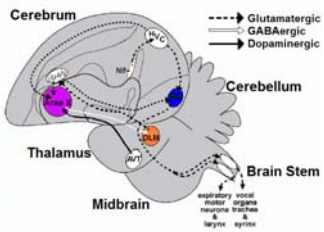
Preclinical research examining the long-term effects of ecstasy (3,4 methylenedioxymethamphetamine; MDMA) has predominately been conducted in mature subjects, but there has been increasing interest in adolescent exposure. MDMA acts as both a stimulant and psychedelic, producing an energizing effect, as well as distortions in time and perception and enhanced enjoyment from tactile experiences. Research on animals indicates that MDMA is a neurotoxin that has adverse effects on neurotransmitters used in the brain for communication. Exposure is linked to long-term neuron damage involved in mood, thinking, and judgment. Chronic use of MDMA causes reduced performance on cognitive or memory tests.

Nicotine Dosing (subcutaneous)	*Equivalent Human Dose*	*Ecstasy Dosing (intraperitoneal)*	*Equivalent Human Dose*
Low (.054mg/kg)	3 cigarettes	Low (10 mg/kg)	3 pills
Mid (0.18mg/kg)	10 cigarettes	Mid (20 mg/kg)	6 pills
High (0.54mg/kg)	1.5 packs of cig.	High (30 mg/kg)	9 pills

Why Finches?

Instead of the usually preferred mammals, the Zebra Finch songbird is used due to its complex and analogous brain structure to humans. Songbirds possess virtually all basal ganglia structures (including Area X, RA, Fascii, and DLM, seen below). Humans and songbirds both learn speech at a young age by imitation of adults. As these behaviors are learned and practiced, permanent neural pathways are created in the brain's vocal and auditory regions.

Neural Pathways



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NMR SPECTROSCOPY

Nuclear magnetic resonance (NMR) spectroscopy is a technique used by scientists to obtain physical, chemical, electronic and structural information about the molecules that make up a material.

The nucleus of an atom, composed of protons and neutrons, can behave like a small magnet due to a property known as "spin". Hydrogen atoms are a good example as their nuclei have single protons. Their spins normally are randomly oriented so their magnetic fields act in all different directions, canceling each other's effect. When placed in a magnetic field, the proton spins align and can be measured through the NMR effect. The interaction of these nuclei with each other and with the larger external magnetic field is the basis of NMR spectroscopy.

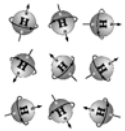
Below is a simplified explanation of NMR.

NMR/MRI Timeline

1946	MR phenomenon - Bloch & Purcell
1952	Nobel Prize - Bloch & Purcell
1950	
1960	NMR developed as analytical tool
1970	
1972	Computerized Tomography
1973	Backprojection MRI - Lauterbur
1975	Fourier Imaging - Ernst
1977	Echo-planar imaging - Mansfield
1980	FT MRI demonstrated - Edelstein
1986	Gradient Echo Imaging
	NMR Microscope
1987	MR Angiography - Dumoulin
1991	Nobel Prize - Ernst
1992	Functional MRI
1994	Hyperpolarized ^{129}Xe Imaging
2003	Nobel Prize - Lauterbur & Mansfield

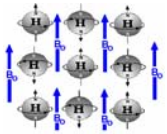
Step 1:

Hydrogen nuclei in the tissue sample have one spinning proton. Initially, these nuclei spin in different directions. The different spins create different magnetic fields in a variety of directions that have zero net magnetic effect.



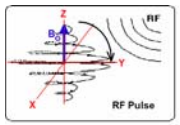
Step 2:

The sample is placed in a powerful magnetic field (B_o). Once inside the magnetic field, the spinning protons will align with the magnetic field. Although some protons may become aligned against the field, a slight excess will point in the direction of the field, as shown below.



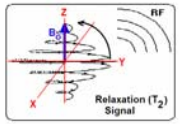
Step 3:

A Radio Frequency (RF) pulse is then applied to the sample for a short period of time to tip the orientation of the magnetic spins from +z-direction (of applied B_o) to the x-y plane. In conserving angular momentum, the nuclei will precess about the +z-axis at the Larmor frequency and will slowly decay to alignment with B_o.

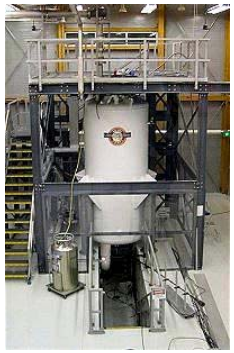


Step 4:

Following the RF pulse, the nucleus releases energy as it returns to its alignment with B_o. The photon of energy released from the nucleus is in the RF range. The frequency of this RF wave is used to identify the chemical make up of the sample. Different materials release this signal at unique frequencies.



900 MHz - 21.1T Ultra-Wide Bore Magnet



Why Large Magnetic Field?

The analytical tool, Magnetic Resonance Imaging (MRI), utilizes Nuclear Magnetic Resonance (NMR) techniques, but imparts spatial information. Whereas clinical MRI uses a super-conducting magnet with a field strength of 1 to 3 Tesla, the Zebra Finch experiments were performed using an NMR superconducting magnet with a field strength of 21.1 Tesla (1 T = 20,000 times Earth's magnetic field). This unique magnet is the first 21.1 T system capable of accommodating living animals. The advantage to having a high field strength is that it produces much greater image resolution needed for pioneering research.

NMR MICROSCOPY

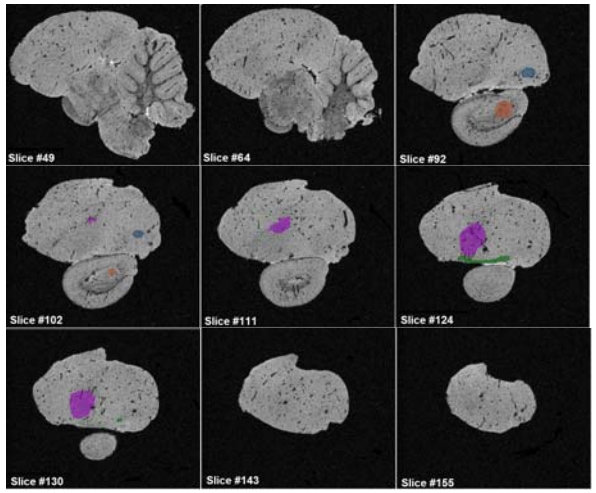
Amira 3D Isosurface Left Brain Views of Zebra Finch Using The 900 MHz Ultra-Wide Bore Magnet

Frontal View

Sagittal Side View

Amira Orthoslice Segmentation

The following are a selection from a series of sagittal slices starting from the midbrain outward toward the left side of an adult finch brain. The colored areas are the same structures shown in the 3D images above. Manual segmentation of these structures was performed using the Amira 4.1 software, including calculations of structural volumes.



The data acquired by each NMR scan, such as the data above, required 3 hours of imaging time at a resolution of 40x40x40 µm. The repetition time (T_R) and echo time (T_E) were set to 100 and 7.5 ms, respectively, to provide the image contrast necessary to identify brain structures. These times can be manipulated in order to provide optimal contrast and resolution for segmentation of structures.

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