Measuring the functional effects of BOTOX on the brainstem using MR Spectroscopy (MRS) and fMRI

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Abstract

The injection of BOTOX into peripheral muscles has been shown to have therapeutic effects in a growing number of indications including disorders involving skeletal muscle (e.g., strabismus, blepharospasm, cervical dystonia, spasticity, cosmetic), smooth muscle (e.g., bladder), glands (axillary hyperhidrosis) and nociceptive pain (e.g. migraine) (Brin. 2014). The mechanism of action on peripheral muscle is established, and the effects on peripheral sensory nociceptors continue under active investigation (Burstein et al., 2014; Zhang et al., 2016). Recently, several studies have suggested that peripheral BOTOX injections in the region of the glabellar lines (corrugator and proceris muscles) may be effective in treating major depression. In addition, peripheral cranio-facial injections have been found effective in treating chronic migraine (Whitcup et al, 2014). It is established that peripheral interventions can modulate functional CNS connectivity in motor and sensory disorders (Cook et al., 2014; DeGiorgio et al., 2013). In addition, functional imaging has demonstrated that modifying eyebrow height or corrugator contraction activity can have functional changes at the level of the amygdala (Heller et al., 2011; 2014; Hennenlotter et al., 2009; Kim et al., 2014). The mechanism underlying the effect of peripheral BOTOX injections on afferent systems is not well understood. Nevertheless, brainstem pathways are integral in this setting as the VIIth cranial nerve provides efferent innervation to the target muscles and sensation is mediated by the Vth cranial nerve. We propose to further explore the functional effects of BOTOX injections on brain in healthy controls. Our approach will involve the measurement of brain metabolites (precursors of metabolism) in the brainstem using MRS as well as functional connectivity (resting state MRI) and functional activation from sensory motor tasks using high resolution fMRI. Examining fMRI of the brainstem and the functional connectivity of the brainstem and connections to cortex, may provide additional insight into the effects of BOTOX on neurologic and psychiatric disorders.

Overview of fMRI

Functional MRI (fMRI) allows the non-invasive visualization and mapping of human brain function (Friston et al., 1999, Francis et al., 2000, Jancke et al., 2000, Muller et al., 2009). Neural activity in functioning areas of the brain reduces local oxygen concentration, resulting in subsequent increase in blood flow (to replenish the cells with oxygen). This oxygenated blood creates a blood oxygen level dependence (BOLD) effect on the MR signal. By this BOLD effect, one can indirectly detect where cerebral metabolism is taking place.

There are few fMRI studies investigating the effects of BOTOX that have demonstrated relationships between facial feedback and emotional stimuli in the amygdala (Kim et al. 2014, Hennenlotter et al. 2008). These results suggest that motor input from facial expressions plays a role in perception of emotion. In fact, there are numerous current theories that peripheral motor function is intrinsic to all cognitive processing. By modulating peripheral input into the brainstem by relaxing facial muscles innervated by the brainstem nuclei, it is possible that reduced tonic activation (from the facial muscles) will affect other neural network thresholds as well, promoting functional plasticity, and leading to quantifiable differences in the fMRI signal.

Overview of MRS

Magnetic resonance imaging spectroscopy (MSI/MRS) is used to quantify the level of various metabolites (intermediary and byproducts of metabolism) in the brain. Many of these metabolites can be measured using conventional proton (1H) imaging or with specialized Phosphorous (31P) techniques. MRS yields data from an arbitrary-sized voxel in the form of a spectrum, from which metabolic concentrations can be determined (Figure 1). These concentrations can then be compared across groups or related to individual differences in behavior, treatment response, etc.

For example, a number of metabolites *index neural activity and can be used to dissociate excitatory from inhibitory activity*. Total Creatine (**tCr**) signal in MRS is a combination of both phosphocreatine (**PCr**) and creatine (**Cr**). Both act as ATP regulators and their concentration in the brain is reflective of the high-energy phosphate buffer system. Glutamate (**Glu**) is the most abundant metabolite in the brain and a dominant excitatory neurotransmitter, released during neuronal excitation and converted to Glutamine (**Gln**). The Glutamate-Glutamine cycle is highly energy demanding and readily observed. **GABA** is the most important inhibitory neurotransmitter in the brain and, while more difficult to measure with MRS, can be accurately quantified with specialized protocols. **GABA** levels have been shown to change during motor learning (Floyer-Lee et al, 2006). In addition, elevated **GABA** levels have been detected in migraine (Aguila et al. 2015), and depression (Licata et al. 2014) making this metabolite well suited for this work.

Other metabolites that can be measured *index cellular energetics* in the form of phospholipid precursors, metabolites, and high energy phosphates. A 31P MRS scan, for example, can measure the phosphocreatine (**PCr**) and **ATP** (two doublets and a triplet) resonances with their ratio frequently used as a tissue-energy status indicator. Similarly, the phosphomonoester (**PME**) and phosphodiesters (**PDE**) peaks represent precursors of membrane synthesis and breakdown products.

Finally, several of our *target metabolites are linked to cellular structure and, as such, are often found to be markers of pathology*. N- Acetyl-Aspartate (**NAA**) in the brain is localized to the neuronal bodies, axons and dendrites and is hence considered a marker for neuronal integrity and function. Studies of disease involving loss of neurons (stroke, epilepsy, tumors, multiple sclerosis) have shown a consistent decrease of NAA. Additionally, the choline peak in the brain consists of Choline (**Cho**), Phosphocholine (**PCho**) and Glycerophosphocholine (**GlPCho**) and reflects cellular density and membrane breakdown.

Our proposed work uses cutting edge techniques to investigate whether effects of BOTOX can be observed in the CNS. The techniques will maximize the number of detectable metabolites by including not only traditional 1H MRS, but eventually also including 31P-based MRS.

Preliminary Data

Our research group has significant experience in a wide range of MRI techniques, including MRS. As we have only recently begun MRS scanning on the 3T Philips magnet at UC Irvine, we present here sample data from this scanner to demonstrate its viability. Demonstrations

of functional, structural, and diffusion-based MRI on the UCI scanner are available in our published work (e.g., Bennett and Stark, 2015, Stark et al. 2015, Mattfield and Stark 2015, Yunshkevich et al. 2015, Huffman and Stark 2014, Lacy et al. 2012).

Sample 1H MRS data

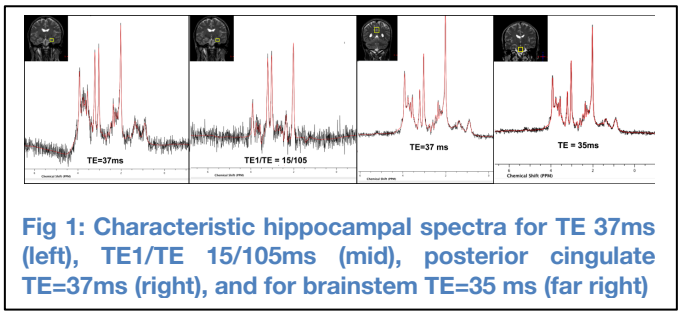

Fig 1: Characteristic hippocampal spectra for TE 37ms (left), TE1/TE 15/105ms (mid), posterior cingulate TE=37ms (right), and for brainstem TE=35 ms (far right)

To demonstrate our ability for 1H MRS in the hippocampus, brain stem and posterior cingulate, we have collected pilot data using our 3T Philips MRI in combination with a 32-channel head coil. 1H spectroscopy data were acquired using the PRESS localization sequence (Bottomley, 1984; TR/TE = 2000/37 ms), from a 2.5x1.5x1 cm voxel in the left and right hippocampi of all subjects following an established protocol (Valenzuela et al, 2003). For the posterior cingulate, a larger voxel measuring 2x2x2 cm was selected. Due to its smaller location, the brainstem voxel measured 1.5x1x1 cm All spectra were processed using the TARQUIN software (v.4.3.6) for NMR/MRS spectra quantitation (Wilson et al., 2011) and a sample is shown in Figure 1.

Preliminary 1H MRS results are shown in Figure 2 and are remarkably similar to prior work (Bartha et al., 2008). This measurement is shown in the hippocampus but can be done in any other brain structure, including the brainstem and the posterior cingulate.

Sample SV-PRESS (1H) data for GABA

In addition to the standard metabolites measured with short TE (37ms) PRESS, we now have the capability for improved GABA measurements by choosing the combination of TE1/TE=15/105 ms results in most optimized parameters to resolve the 1.89 and 2.28 ppm peak of the GABA spectrum. We are currently testing and tuning its parameters.

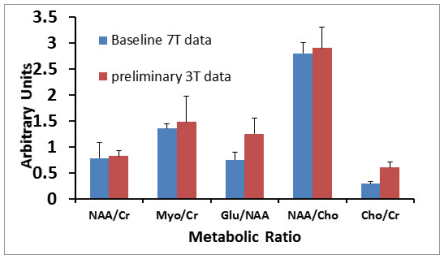

Fig 2: Our hippocampal SV PRESS (N=9, red) and from Bartha et al. (2008, blue)

High resolution fMRI of the Brainstem

Our laboratory routinely collects high resolution fMRI of the medial temporal lobe for memory and aging studies. Coincidentally, our protocol is also ideally oriented to observe fMRI of the brainstem. Initial results with high resolution (1.5mm) fMRI of the brain stem are shown in Figure 3.

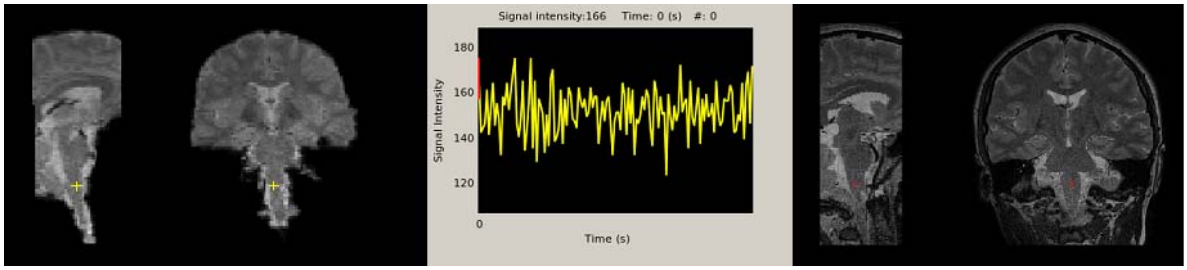

Fig 3. (Left) high resolution fMRI image, before spatial smoothing with corresponding time series (TR=2). On right, high resolution T2 image of brainstem for region of interest registration.

Hypotheses

There are two goals of the proposed preliminary work. The first is to determine whether there are metabolic differences in the brain stem in healthy individuals as a result of peripheral administration of BOTOX into the glabellar muscles. This is an open question that can be addressed with MRS. While the doses used in cosmetic treatments are low, an effect via the trigeminal nerve is certainly possible and knowing whether such an effect exists in this cohort would be highly relevant for attempts to understand the effects of BOTOX on disorders such as migraine or depression. Importantly, we proposed to diverge from typical MRS here and to employ "functional MRS" by collecting MRS data during the performance of an emotionally-arousing task. If BOTOX is altering emotional processing in the brain via a "facial feedback" mechanism, altered metabolite levels in the brainstem might be resolved.

The second goal is to better understand the effect of BOTOX on functional activity (measured using fMRI) in the brain and in the brainstem. Consistent with prior work, we propose that BOTOX will alter emotional-related activity in the amygdala (Kim J, 2014). We propose to extend this work in several ways by measuring fMRI activity in the brainstem as well and understanding its relationship to activity in structures such as the amygdala. Again, if BOTOX is altering emotional processing via an alteration of the facial feedback mechanism, this could be resolved either as an alteration of fMRI activity levels or as an alteration in "functional connectivity" (correlation of activity over time) between the brainstem and the amygdala.

Proposed Protocol: Pilot Study on Healthy Controls

Our aim is to be able to examine the effects of BOTOX on the CNS to determine potential mechanisms for the effects of BOTOX on disorders such as migraine and depression. To date, there are no MRS studies of BOTOX to guide the investigation. We therefore propose a staged design with an initial pilot study in healthy controls undergoing BOTOX treatment for cosmetic purposes, potentially followed by an expanded study in patients being treated for migraine or depression. Additionally, in this initial pilot, we will explore the feasibility of brainstem fMRI in those two groups of participants. The connectivity between regions in the brainstem will be studied in the presence of angry/happy faces paradigm and at rest. Possible connections with metabolite changes will be explored as well.

Participants: We propose to scan 10 healthy, right handed females. Because the sample size is so small, we will be constraining the inclusion/exclusion criteria to minimize variability. Participants will be naïve BOTOX users (e.g. no previous use of BOTOX), ages 30-40 years old. Participants will be excluded for the presence of neurological or psychiatric conditions or the presence of any risk factors for MRI. Participants will be recruited from UCI and/or the surrounding community via e-mail blasts and word-of-mouth. They will receive $50 compensation per MRI scan and the cosmetic BOTOX will be provided by Allergan.

Design: We propose a pre- and post-design. One scan will be collected prior to BOTOX injection and the second will be collected 2-4 weeks post-injection. BOTOX injections will be limited to 20 units in the glabellar area, as approved by the FDA and administered by a

certified clinician at the UC Irvine Medical Center (ENT Satellite Office in Irvine). Each scanning session will last approximately one hour and include:

1) T_1 MPRAGE (3D whole-brain structural image) = 5 min
2) T_2 localizers (axial, coronal and sagittal) = 6 minutes
3) Resting state fMRI = 7 minutes
4) fMRI (reduced TR and FOV) =15 min
5) MRS–SV of brain stem = 7 min

MPRAGE: This is a standard 3D structural image of the whole brain that will allow us to localize all fMRI findings as well as to determine whether there are any pre-existing cortical abnormalities in any participants. While we do not expect to observe any pre- vs. post-structural changes regional brain shape or size, we will be able to assess these factors.

Resting-state fMRI: We will collect whole-brain fMRI data for 7 min of eyes-open resting state fMRI. This data is commonly used to reveal patterns of network-level connectivity (and group-level changes in connectivity) in the brain (Power et al., 2011). Changes in resting states between groups (with and without BOTOX) would likely be observed. We will examine the frequency of switching of modes (unique waveforms from multi-component analysis) between groups. We will also explore the functional connectivity between dura-sensitive neurons and their projections to the hypothalamus, thalamus and mid-brain, as shown by Burstein (Burstein et al.,1998).

fMRI: The volunteers will participate in an fMRI scanning session following the protocol of Kim et al. 2014. Photos of series of angry, happy and surprised facial expressions (consisting of 9 male and 9 female identities) will be presented for 17, 50 and 1000 ms followed by black and white pattern, presented for 250 ms (serving as a retinal wipe). During each trial, participants will be asked to report using a button box whether they thought each of the faces they saw represent a positive or a negative emotion. We will analyze fMRI data of the brainstem following a modified method used by Beissner et al. 2011.

MRS-1H This will serve as an exploratory study to quantify a series of standard metabolites (described above) from a voxel located in the brainstem. If BOTOX injections invoke plasticity in the afferent motor pathways, the MRS data acquired may detect altered Glutamate or GABA concentrations, as GABAergic systems relate to short term motor learning and behavior (Stagg et al. 2011, Floyer-Lea et al. 2006). Differences in metabolite concentrations in brainstem following the fMRI task may show a link between this region and the amygdala, where those activations have been shown to occur (Kim et al. 2014). These concentrations may be further modulated in the presence of BOTOX injection.

Problems and Alternatives:

Our preliminary data of 1H-MRS of the hippocampus, brainstem and posterior cingulate have yielded results comparable with other published studies. However, the small voxel sizes used in our preliminary data yield spectra with moderate noise. This can be improved by increasing the voxel sizes in line with other literature. Recent advances have provided the means to

measure hard to resolve peaks (such as GABA) through the use of multi echo PRESS parameter selection should mitigate the need for new and expensive hardware and software.

Additionally, fMRI of the brainstem is not common and some claim it poses some additional difficulty due to increased physiological noise (Beissner et al. 2011). Our high resolution fMRI data of the hippocampus/brainstem do not seem to be suffering from these issues, but it is possible that the thicker slices and larger voxel sizes used in whole brain (standard-resolution) scans will. We will conduct tests on several existing datasets in our lab and others to determine the magnitude of any such effects and will adjust to higher-resolution oblique coronal images (covering the brainstem and amygdala, but potentially eliminating portions of either frontal or occipital cortex) if needed.

Finally, we have not proposed to include 31P MRS in this initial, pilot study. We are currently developing a 31P protocol and building the coil that will subsequently be used to acquire the 31P MRS data. This part of the protocol would be added to the preliminary study provided that the coil is ready in the timeframe for initiation of this project.

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