THE EFFECTS OF THETA BURST STIMULATION TO THE OCCIPITAL CORTEX ON BRAIN BIOMARKERS MEASURED BY MAGNETIC RESONANCE SPECTROSCOPY

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Abstract

Theta burst stimulation (TBS), a type of transcranial magnetic stimulation (TMS), uses repeated high-frequency bursts to induce neural noise in the cortex. An intermittent TBS (iTBS) protocol is generally considered excitatory, while continuous TBS (cTBS) is generally considered inhibitory. TBS effects are highly variable and have been primarily studied in the primary motor cortex (M1). We investigated the effects of iTBS and cTBS to the primary visual cortex (V1) on occipital Glx (glutamate and glutamine composite) and GABA+ (gamma-aminobutyric acid and macromolecules composite) concentrations compared to sham stimulation. Thirty participants received a single session of individual stereotaxically-guided TBS to the V1. Participants received either cTBS, iTBS or sham TBS. GABA+ and Glx were measured at the stimulation site using magnetic resonance spectroscopy. Baseline pre-TBS GABA+ and Glx levels were compared to those immediately post- and one hour post-TBS. The results show a trend for a decrease in GABA+ immediately following cTBS compared to one hour post-TBS and a trend for an increase in Glx immediately following iTBS compared to one hour-post TBS. There was also an increase in GABA+ from baseline to one hour-post TBS and a trend for an increase in GABA+ and Glx composite ratio levels following sham. Since there was a lack of systematic trends in the data, we suspect there is no relevant change from a single session of TBS to neurotransmitter levels in the V1. Perhaps, only repeated application of TBS may lead to substantial benefits. This indicates that a single session of TBS can be used safely in the laboratory without effects on neurotransmitter levels in the V1.

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List of Abbreviations

^{1}H	Proton
3D	Three-dimensional
3T	3 Tesla
AIC	Akaike information criterion
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANOVA	Analysis of Variance
B-I	Baseline to immediately post-TBS
B-O	Baseline to one hour post-TBS
Ca^{2+}	Calcium ion
CMRR	C2P collaboration between Siemens and the University of Minnesota
Cr	Creatine
CS	Conditioning stimulus
CSF	Cerebrospinal fluid
cTBS	Continuous theta burst stimulation
CV	Coefficient of variation
DLPFC	Dorsolateral prefrontal cortex
E-field	Electric field
fMRI	Functional magnetic resonance imaging
FoV	Field of view
GABA+	Gamma-aminobutyric acid and macromolecules composite
GABA	Gamma-aminobutyric acid
Glx	Glutamate and glutamine composite

GM	Grey matter
I-O	Immediately post to one hour-post TBS
ISI	Inter-stimulation interval
iTBS	intermittent theta burst stimulation
i.u.	Institutional units
LO	Lateral occipital
LTD	Long-term depression
LTP	Long-term potentiation
M1	Primary motor cortex
MEGA-PRESS	Mescher-Garwood point resolved spectroscopy
MEP	Motor evoked potential
Mg^{2+}	Magnesium ion
MoCA	Montreal Cognitive Assessment
MPRAGE	Magnetization-prepared rapid gradient echo
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MT	Motor threshold
Na^{+}	Sodium ion
NAA	N-acetyl aspartate
NIBS	Non-invasive brain stimulation
NMDAR	*N*-methyl-D-aspartate receptor
OCD	Obsessive-compulsive disorder
PFC	Prefrontal cortex

PPC	Posterior parietal cortex
ppm	Parts per million
pSTS	Posterior superior temporal sulcus
PT	Phosphene threshold
rTMS	Repetitive transcranial magnetic stimulation
SICI	Short-latency intracortical inhibition
SNR	Signal-to-noise ratio
SPECIAL	Spin echo full intensity acquired localised
STEAM	Stimulated echo acquisition mode
REML	Restricted maximum likelihood
REPT	Accurate and rapid estimation of phosphene threshold
TBS	Theta burst stimulation
tDCS	Transcranial direct current stimulation
TE	Echo time
TI	Inversion time
TMS	Transcranial magnetic stimulation
TR	Repetition time
TS	Test stimulus
V1	Primary visual cortex
VOI	Volume-of-interest
WM	White matter

Chapter 1:

General Introduction

Non-Invasive Brain Stimulation

Non-invasive brain stimulation (NIBS) stimulates the cortex magnetically or with a direct electric current over the scalp (Barker, Jalinous, & Freeston, 1985). In contrast, invasive stimulation requires the direct implantation of an electrode within the brain, such as for deep brain stimulation or along the cortical surface through electrocorticography-based electrodes such as for cortical surface stimulation. NIBS is safer and favourable compared to invasive procedures. The two most prevalent NIBS types are transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS). TDCS involves the application of a weak direct current to the scalp via electrodes. The current applied flows from the electrodes to the brain to complete the circuit. The electric current's direction dictates the physiological reaction (Thair, Holloway, Newport, & Smith, 2017). A negative cathodal current is inhibitory, causing hyperpolarization of neurons, while the opposite occurs for the positive anodal current (Nitsche et al., 2008; Boes et al., 2018). TDCS does not directly trigger action potentials, but rather, affects the spike timing of individual neurons, which are receiving the suprathreshold inputs (Anastassiou, Perin, Markram, & Koch, 2011; Pelletier & Cicchetti, 2015).

TMS is based on the principle of electromagnetic induction, where strong magnetic pulses modify the electric current within the brain (Oberman, Edwards, Eldaief, & Pascual-Leone, 2011; Rossi, Hallett, Rossini, Pascual-Leone, & The Safety of TMS Consensus Group, 2009). Both TMS and tDCS may induce cortical plasticity by altering the firing pattern of neurons over time. The exact neural mechanisms involved are still actively being explored. Additional research is needed to understand the underlying mechanisms involved in NIBS, including how plasticity is induced, the duration of its effect, and to resolve the inconsistencies in outcomes reported in the literature (Ridding & Ziemann, 2010).

My thesis explores the application of theta burst stimulation (TBS), a high-frequency TMS protocol, to the primary visual cortex (V1) and analyzes its effects on several biomarkers. I use magnetic resonance spectroscopy (MRS), a neuroimaging technique that measures neurotransmitter concentrations, to gain an understanding of some of the unresolved questions above.

Transcranial Magnetic Stimulation

TMS is a useful neuroscientific tool that alters cortical activity and potentially affects neuroplasticity causing subsequent changes to brain connectivity and function (Rossi et al., 2009). TMS uses Faraday's law of electromagnetic induction, where the relationship between electric and magnetic fields is reciprocal and reliant on one another (Faraday, 1832). In TMS, brief pulses of electric current move through a coil of copper wire and alter the magnetic field by producing lines of flux that pass perpendicular to the plane of the coil (Hallett, 2000). The electric field flows in loops parallel to the plane of the coil (Hallett, 2000; Eldaief, Press, & Pascual-Leone, 2013), which subsequently affects the electrical activity within underlying neural tissue likened to a virtual lesion (Harris, Clifford, & Miniussi, 2008). The precise neural mechanisms of TMS are still unclear. It is suggested that, depending on the area of stimulation, TMS exerts its effects in the gyrus or wall of the sulcus due to the proximity to the coil (Day et al., 1989; Silva, Basser, & Miranda, 2008) with the magnetic current being the strongest at the gyral crown (Opitz, Windhoff, Heidemann, Turner, & Thielscher, 2011). The induced electrical current may activate a mixture of inhibitory and excitatory neurons initiating action potentials at the axon hillock or via dendrites within these areas (Wagner, Rushmore, Eden, & Valero-Cabré, 2009; Pell, Roth, & Zangen, 2011). A change in membrane potential would cause depolarization or hyperpolarization of neurons within. The force of the electric field is determined by electrical

conductivity and permittivity (Wagner et al., 2009). If sufficiently strong, the current can propagate to peripheral regions (Pell et al., 2011) through axonal projections (Lee et al., 2003; Solomon-Harris, Rafique, & Steeves, 2016). This can cause temporary excitation or inhibition of the stimulated area and secondary regions. An alternative view is that TMS introduces random noise in neural processing. The introduction of random neural events will reduce the strength of relevant signals changing the firing pattern of neurons within the specific area for a certain amount of time (Harris et al., 2008; Allen, Pasley, Duong, & Freeman, 2007). The direction and duration of TMS's neural effects are dependent on numerous factors, including the coil type and stimulation parameters such as the waveform, intensity, frequency, duration, and the pattern of stimulation.

Coil Type

The main differences between coil types are the electrical field spatial features: depth of penetration and focus (Deng, Lisanby, & Peterchev, 2013). Coils are composed of different substances and vary in shape dependent on need. The core material contains either a magnetically inert substrate (air coils) or a solid ferromagnetically active material. Solid cores are more efficient in transferring electrical current to the magnetic field and can thus operate for longer without overheating (Salvador, Miranda, Roth, & Zangen, 2007). Regarding shape, the round coil is the original form and provides powerful stimulation (Deng et al., 2013). The figure-of-eight coil superimposes two-circular coils. Each coil has a field of current flowing in the opposite direction, which delivers more focal stimulation at their intersection (Ueno, Tashiro, & Harada, 1988; Wagner et al., 2009). The cloverleaf coil consists of four sets of nearly circular windings and provides more efficient focal stimulation to long fibres compared to the figure-of-eight coil (Deng et al., 2013). These coils are generally meant for targeted stimulation and are

used for superficial cortical targets that vary from 1-3 cm in depth (Zangen, Roth, Voller, & Hallett, 2005). However, the figure-of-eight coil has shown the potential to reach deeper stimulation areas such as the visual cortex, which is demonstrated by the perception of a flash of light in the visual field after stimulation. (Marg & Rudiak, 1994; Kammer & Baumann, 2010). A double-cone coil or H-coil is generally used to reach deeper targets. The coil conforms more to the head's shape and allows deeper magnetic penetration ranging from 4-8 cm (Zangen et al., 2005). There is commonly a trade-off between focus and penetration depth as the electric field is always more substantial and more fixed at the surface. Stimulation coils that reach deeper cortical regions typically lack focussed stimulation as the electric field in the center of a uniform conduction sphere is zero, which means there is a greater spread of the magnetic field (Heller & van Hulsteyn, 1992; Deng et al., 2013). The figure-of-eight air coil is the most widely used due to the balance between the focus of stimulation and the ability to affect deeper targets, whether directly or indirectly.

Waveform

There are two different waveforms of stimulation: monophasic or biphasic. Each waveform is differentiated by the sinusoidal curvature and the number of current oscillations (Delvendahl et al., 2014; Pell et al., 2011). Monophasic waveforms occur when the pulse current terminates after one full cycle of oscillation. This results in a monodirectional current flow (Kammer, Beck, Thielscher, Laubis-Herrmann, & Topka, 2001b). Single-pulse studies were initially performed with monophasic waveforms, mostly used for determining cortical excitability in the primary motor cortex (M1) (Mills, Murray, & Hess, 1987; Rossini et al., 2015; Triggs, Calvanio, MacDonnell, Cros, & Chiappa, 1994). Biphasic waveforms complete a full-sinusoidal cycle with waveforms occurring in both directions (Delvendahl et al., 2014; Cadwell,

1990; Kammer et al., 2001b; Sommer et al., 2006). This waveform is more prevalent in present-day research as the capacitor restores the dispensed energy during an oscillation period. This reduces the amount of recharge energy between pulses allowing for a repetitive train (Delvendahl et al., 2014). Biphasic waveforms can be used not only for single-pulse stimulations but also for any other paradigm available by TMS. The majority of stimulators deliver biphasic waveforms, while only single-pulse stimulators generate monophasic waveforms.

Threshold and Intensity

Single-pulse stimulation can be used to disrupt underlying neural tissue briefly. This provides insight into determining the cortical excitability of that region (Kobayashi & Pascual-Leone, 2003; Rothwell, 1997; Vahabzadeh-Hagh, 2014). The motor and visual cortices are two areas that produce an apparent effect from a single-pulse of stimulation (Franca, Koch, Mochizuki, Huang, & Rothwell, 2006). In the M1, a single TMS pulse generates excitability through a series of descending waves of corticospinal activity. The D-waves are the earliest, which occur from direct activation of axons in corticospinal neurons. Following are the I-waves that represent the indirect effect (Di Lazzaro & Rothwell, 2014). It is assumed that a single-pulse would similarly affect the visual cortex; however, less is known about how and exactly where. When applied to an individualized hotspot region in the M1, TMS pulses will cause the active or resting contralateral abductor pollicis brevis to twitch when the intensity is sufficiently strong. A motor threshold (MT) is the lowest level of stimulation capable of causing this twitch in 50% of trials and is measured through motor evoked potential (MEP) amplitudes (Pascual-Leone, Valls-Solé, Wassermann, & Hallett, 1994).

When applied to the visual cortex, TMS pulses can induce the perception of a flash of light, called a phosphene. A phosphene is the perception of a ring or spot of light produced by

direct stimulation of the visual cortex (Cervetto, Demontis, & Gargini, 2007). Generally, it is a spark of light on the contralateral side to the applied stimulation due to the retinotopic nature of the occipital cortex (Elkin-Frankston, Fried, Pascual-Leone, Rushmore, & Cabré, 2010). However, depending upon where the simulation is applied, it can appear ipsilateral to the stimulation and vary in shape, colour, and size (Cervetto et al., 2007; Kammer & Baumann, 2010). A phosphene threshold (PT) is a reliable index of visual cortex excitability, using the measure at which phosphenes are elicited 50% of the time as the threshold (Franca et al., 2006). Although a PT is generally determined using single-pulse stimulation, the details of the practice can vary. There is some debate regarding the technical aspects involved in measuring a PT such as room lighting (Elkin-Frankston et al., 2010; Boroojerdi et al., 2000), the proper identification of a phosphene (Elkin-Frankston, Fried, Rushmore, & Valero-Cabré, 2011), and initial stimulation intensity (Mazzi, Savazzi, Abrahamyan, & Ruzzoli, 2017). If done correctly, a PT positively correlates with an active MT and shows a trend to correlate with resting MT, thereby showing the accuracy of the measure (Deblieck, Thompson, Iacoboni, & Wu, 2008). However, the subjective nature of the participant's verbal report to indicate the presence of a phosphene, unlike the explicitly measurable MEP, can diminish the interrelationship.

MT and PT are often used as guidelines for determining the intensity of stimulation used in a variety of protocols. The stimulation intensity determines the potential magnetic field that is needed to induce an electrical current in underlying neural tissue and can show individual differences (Wassermann, 1998). The heterogeneity of thresholds in individuals varies considerably. Using a stimulation intensity that accounts for the biological efficacy of the stimulus in each individual, rather than using a standardized stimulation output, is considered to be an approach to stimulation that is more systematic and customized (Wassermann, 1988). A

single intensity is often seen as unsuitable in studies in which super or suprathreshold stimulation can produce different neural responses (Kammer, Beck, Erb, & Grodd, 2001a; Abrahamyan, Clifford, Arabzadeh, & Harris, 2015). The majority of protocols are administered at 80-120% of an individual's threshold, rather than a generic stimulator output (Rossi et al., 2009). However, a number of studies have still produced reliable results from standard stimulator output, indicating that there is still debate in the field over its benefits (Mullin & Steeves, 2011; Ganaden, Mullin, & Steeves, 2013). The threshold, and thus, the intensity, will change dependent on the waveform. When monophasic pulses are applied over M1, a lower threshold is observed when the current flows posteriorly-anteriorly in the brain (Sommer et al., 2006). In biphasic pulses, the threshold is the lowest when the current flows in that same direction for the first phase and in the opposite direction for the second phase (Kammer et al., 2001b; Rossi et al., 2009). In the M1, this will indirectly induce the activation of the pyramidal tract via the recruitment of excitatory interneurons. This leads to preferentially elicited late volleys in the corticospinal tract (Klomjai, Katz, & Lackmy-Vallée, 2015). Single-pulse stimulation is one of the most robust methods for finding an individual's threshold (whether motor or visual). It is generally done with biphasic pulses and is used to produce an individualized intensity for TMS protocols providing an outlet for exploratory research or diagnostic purposes.

Frequency, Duration and Pattern of Pulses

Frequency, duration and pattern of pulses also affect TMS mechanisms. The pulses frequency is generally classified as either low or high-frequency, with low being a maximum of 1 Hz and high being above 1 Hz (Rossi et al., 2009). A 1 Hz paradigm delivers one single-pulse each second. Therefore, a 1 Hz protocol administered for 10 min would have a total of 600 pulses. Concerns over safety have limited the use of high frequencies in TMS (Wassermann,

1998). Duration and pattern of stimulation can also vary. The duration of repeated application can range from as short as 40 s or as long as 40 min, while the pattern of pulses can be administered continuously or with various inter-train intervals. The type of coil, waveform, intensity, frequency, duration, and pattern will all differ depending on the TMS protocol applied and the desired effect.

Paired-Pulse

Based on the specifications above, TMS is subdivided into categories. Single-pulse TMS is described above, and the level of associated excitability is generally measured by MEP when applied to the M1 or PT when applied to the V1. Paired-pulse paradigms provide insight into the excitability and integrity of corticocortical connections (Vahabzadeh-Hagh, 2014). Paired-pulse mechanisms utilize two isolated pulses delivered close in time to examine intracortical processes such as cortical excitation and inhibition ratios (Rotenberg, Horvath, & Pascual-Leone, 2014; Udupa & Chen, 2010; Di Lazzaro et al., 1998). The first pulse is the initial conditioning stimulus (CS), and it has a priming effect. The second pulse is the subsequent test stimulus (TS), and it is dependent on the intensity and inter-pulse interval duration (Di Lazzaro et al., 1998). Short-latency intracortical inhibition (SICI) and intracortical facilitation are commonly used physiological measures to assess excitability from a paired-pulse paradigm (Guerra et al., 2019). This occurs when a low-intensity CS is used to suppress the response evoked by high-intensity TS (Ibáñez, Spampinato, Paraneetharan, & Rothwell, 2020). However, repetitive transcranial magnetic stimulation (rTMS) is the most popular TMS subcategory due to the longer-lasting effects. Stimulation effects can be either inhibitory or excitatory based on the parameters mentioned above and may be measured in a number of ways.

Repetitive Transcranial Magnetic Stimulation

RTMS paradigms utilize repeated trains of single biphasic pulses to induce neural noise. RTMS initially was restricted in clinical treatment to neuro-motor disorders until an antidepressant effect was discovered when placed in the prefrontal cortex (PFC) (George, Lisanby, & Sackeim, 1999; Noohi & Amirsalari, 2016). Currently, rTMS is being used in clinical treatment for a variety of disorders, such as, but not limited to, depression, autism, and Parkinson's disease (Schwippel, Schroeder, Fallgatter, & Plewnia, 2019; Benninger et al., 2011). Experimentally, the effect of rTMS has been used to reverse engineer neural networks for a variety of cognitive functions, such as, but once again not limited, to scene processing (Mullin & Steeves, 2011; Ganaden et al., 2013), face recognition (Solomon-Harris, Mullin, & Steeves, 2013) and executive function (Demeter, 2016; Grossheinrich et al., 2009). After-effects arise from an alteration of long-term excitability of neurons and networks following stimulation (Thickbroom, 2007). Excitatory changes in synaptic transmission are labelled long-term potentiation (LTP), and inhibitory changes are called long-term depression (LTD). LTP and LTD are examples of synaptic plasticity and are accepted as the most likely mechanism behind the conditioning effect of rTMS (Thickbroom, 2007).

Parameters and Type

RTMS paradigms are generally differentiated by frequency: low-frequency rTMS (<1 Hz) is applied continuously and typically generates an inhibitory response, while high-frequency rTMS (>1 Hz) is applied in a repetitive patterned fashion and generally induces a reduction in intracortical inhibition (Pascual-Leone et al., 1994; Pascual-Leone et al., 1998). The patterned protocol is designed where short trains of stimulation are separated by periods of no stimulation. The pauses have a significant impact on the power of rTMS in both efficacy and safety (Rossi et

al., 2009). Although higher frequencies, such as 100 Hz with a continuous application, have been used safely in animal studies, the risk of seizures is too high in humans for such high-frequency protocols to be administered (Paulus, 2005). Seizures can be induced by hyper-synchronized discharges of groups of neurons in grey matter tissue (GM) (Oberman et al., 2011; Wassermann, 1998). This is mainly due to an imbalance between inhibitory and excitatory synaptic activity, where the excitation is significantly elevated. This mechanism explains the danger of high-frequency continuous rTMS and the need for pauses during a stimulation train. Low-frequency protocols are less likely to induce seizures due to inhibition of synaptic activity and can be applied continuously. However, seizures have still been reported even with "safe" protocols, although never with stimulation to the visual cortex (Rossi et al., 2009). Medication, sleep patterns, drug use, and general individual differences can contribute to the safety of protocols (Oberman et al., 2011), and this is why participant screening is vital in TMS research.

Many different modifications to the frequency and pattern of stimulation have been adopted in an attempt to augment and refine the effects of TMS. Generally, in a clinical setting, the high-frequency rTMS protocol that is typically used is 10 Hz for 4 s with 26 s inter-stimulation interval (ISI); 3000 pulses; total duration of 37.5 min (Blumberger et al., 2018) and low-frequency rTMS is 1 Hz applied continuously; 600 pulses; total duration of 10 min (Min et al., 2016). Examples of modification in both clinical and experimental settings for high-frequency rTMS is 20 Hz for 2 s with 28 s ISI; 1200 pulses; total duration of 15 min (Rossi et al., 2009) and for low-frequency 1 Hz rTMS applied continuously; 1800 pulses; total duration of 30 min (Rafique, Richards, & Steeves, 2016) or 420 pulses; total duration of 7 min (Mullin & Steeves, 2013). Overall, rTMS is defined by its repeated biphasic trains of stimuli. Protocols are classified as high or low-frequency. The exact frequency, duration and pattern of stimulation can

lead to different results. Changes in neural excitability can be monitored during (online) and after (offline) stimulation. An offline design is when TMS effects are measured following the completion of stimulation. Coil type and stimulation intensity can differ in each experiment.

Physiological Mechanisms

The majority of work exploring TMS neural mechanisms has been done in the motor cortex, while less is known about other regions. The physiological mechanisms studied in the motor cortex are used as a general guideline for other areas of the brain. However, the exact type of neurons involved and specifics of mechanisms may differ. This emphasizes the need for additional TMS studies across the cortex. RTMS effects are known to persist even after stimulation has completed. Primarily, frequency and duration are associated with the direction of synaptic transmission, while numerous factors, such as pattern and intensity, can impact sustainability (Bliss & Lomo, 1973; Tsumoto, 1992; Trippe, Mix, Aydin-Abidin, Funke, & Benali, 2009). Synaptic plasticity is the experience-dependent ability of the synapse between two neurons to modulate in intensity and the timing of converging signals (Bliss & Gardner-Medwin, 1973). The strength of synaptic plasticity can either enhance or weaken LTP or LTD expression, respectively (Pell et al., 2011). This follows the Hebbian theory of learning and memory that in simplified terms: neurons that are constantly firing together will eventually wire together (Hebb, 1949). For this to occur, it requires simultaneous depolarization of the corticospinal tract directly at axon hillock of motor neurons or indirectly via interneurons in the motor cortex (Pell et al., 2011). For LTP and LTD to occur, three defining characteristics are needed: 1) input specificity, 2) cooperativity and associativity of signals, and 3) persistence of signals (Kandel, 2000). A signal needs to be specific, intense, and persistent to cause plastic changes. For a weak signal to

become strong, it needs cooperation from multiple weak signals or an association with a more forceful signal from another pathway.

Changes in LTP and LTD depend upon the *N*-methyl-D-aspartate receptor (NMDAR; a postsynaptic receptor that has a cation channel) and calcium channels (Ridding & Ziemann, 2010). Naturally, glutamate binds to α-amino-3-hydroxy-5-methyl-4-isoxazole (AMPAR; a propionic acid receptor), allowing a small degree of depolarization to occur in the dendritic spine. LTP occurs when high-frequency stimulation activates numerous AMPAR causing a more significant depolarization. This aids in unblocking NMDAR by removing the magnesium ion (Mg^{2+}), causing the opening of the receptor and flooding of sodium (Na^{+}) and calcium (Ca^{2+}) ions (Larson, Wong, & Lynch G, 1986; Huerta & Volpe, 2009; Klomjai et al., 2015). Ca^{2+} activates several mechanisms causing calcium-calmodulin kinase II and phosphorylation. This leads to an increase in AMPARs activity by augmented sensitivity to glutamate (Klomjai et al., 2015). This specific mechanism occurs through nonsynaptic pools of AMPARs that are trafficked into the postsynaptic membrane through protein kinases when needed (Mihic & Harris, 1994). Consistent depolarization allows for an increase in synaptic strength to occur. Generally, cortical excitability is dependent on cortical circuits. Specifically, in the motor cortex, cortical excitability is dependent on corticocortical axons and their excitatory contacts to corticospinal neurons. It is influenced by agents blocking voltage-gated sodium channels, crucial in regulating axon excitability, and AMPAR responsible for fast excitatory synaptic transmission

LTD is an activity-dependent reduction in the potency of the neuronal synapse (Lisman, 1989). LTD is necessary for LTP as it weakens a specific synapse in order to make constructive use of the synaptic strength of LTP. If firing were to occur continuously, LTP would reach a ceiling effect that would prevent the encoding of new information (Massey & Bashir, 2007). In

low-frequency stimulation, the NMDARs are also stimulated; however, contrary to LTP, only mildly. Stimulation produces a small and slow elevation of Ca^{2+} that activates protein phosphatases and leads to phosphorization and downregulation of AMPAR (Lisman, Schulman, & Cline, 2002; Huerta & Volpe, 2009). Most forms of LTP and LTD are fully reversible (Citri & Malenka, 2008).

Intrinsic plasticity is an additional synaptic mechanism (Zhang & Linden, 2003), where changes in synaptic transmission after induction of LTP and LTD are associated with alterations of intrinsic neuronal excitability (Xu, Kang, Jiang, Nedergaard, & Kang, 2005; Delvendahl, Jung, Kuhnke, Ziemann, & Mall, 2012). Intrinsic plasticity is a longer-lasting mechanism that is dependent on use. Synaptic and intrinsic plasticity can occur concomitantly and correlate with each other. Specifically, synaptic plasticity is associated with glutamate receptors (Citri & Malenka, 2008; Douglas & Martin, 1998). Intrinsic plasticity, however, is associated with other postsynaptic voltage-gated receptors, which results in a change in membrane excitability (Hodgkin & Huxley, 1952; Delvendahl et al., 2012). Both of these forms of plasticity cause modifications to the brain; however, synaptic plasticity is classified as reversible, whereas intrinsic plasticity indicates a long-term associated change. Alterations in frequency and duration of stimuli in rTMS modify longevity of effects most likely through LTP and LTD mechanisms.

Theta Burst Stimulation

TBS is a relatively new subcategory of rTMS and is growing in popularity due to its efficiency across individuals. Its acclaim comes from the fact that it can achieve similar results to standard rTMS but in a much shorter time frame (Suppa et al., 2016). The concept of TBS originated in animal studies showing that bursts of three-five pulses at 50-100 Hz, repeated at 5 Hz (theta rhythm), induces plasticity in animals causing LTP or LTD-like changes (Larson et al.,

1986; Davies, Starkey, Pozza, & Collingridge, 1991; Hess, Aizenman, & Donoghue, 1996; Di Lazzaro et al., 2008). Theta rhythms are associated with synaptic plasticity (Huang, Edwards, Rounis, & Rothwell, 2005). TBS is currently being explored as a potential alternative to standard rTMS in both clinical and experimental settings for an array of goals. Analogous to rTMS, TBS has been used in clinical populations to treat individuals with depression (Berlim, Mcgirr, Santos, Tremblay, & Martins, 2017) and obsessive-compulsive disorder (OCD) (Harika-Germaneau et al., 2019). In healthy populations, TBS effects are being studied compared to pharmacological influences on the brain, where certain drugs can affect LTP and LTD-like changes (Huang, Chen, Rothwell, & Wen, 2007). As well, the efficacy of TBS is even being examined in the context of genetics as it shows that stimulation can be inefficient to individuals with a particular genetic make-up. Val/Val homozygote individuals have been shown to respond better to TBS than those with one or two copies of the MET allele (Cheeran et al., 2008). TBS is being explored for use in multiple regions such as the visual cortex to analyze changes in PT (Franca et al., 2006), the M1 to examine changes in neurotransmitter levels (Stagg et al., 2009), and the PFC to analyze changes in cognition and mood (Grossheinrich et al., 2009). Whether as a treatment or in an experimental setting, TBS is actively explored for its multi-purpose effects in the brain.

Parameters and Type

Two standardized TBS protocols were recently designed for humans consisting of high-frequency biphasic bursts. Each burst has three pulses at 50 Hz, repeated at intervals of 200 ms at 5 Hz (Huang et al., 2005). Continuous TBS (cTBS) employs the stimulation continuously for 40 s, and intermittent TBS (iTBS) employs the stimulus in an interval pattern. Specifically, iTBS applies a 2 s train repeated every 10 s for a total of 200 s (Huang et al., 2005). Both protocols are

usually administered at an 80% intensity threshold, whether motor or phosphene, depending on the specified region of stimulation (Stewart, Walsh, & Rothwell, 2001). However, intensity ranges from 80-120% threshold have been used in different experiments (Suppa et al., 2016). Based on the assumption that TBS uses a similar if not the same mechanism as rTMS, the continuous nature of cTBS has led it to be understood as a more inhibitory protocol, while the intermittent fashion of iTBS to be more excitatory. Specifically, the longer train in cTBS has a mix of suppressive and facilitatory effects in synaptic transmission with facilitation building up faster than suppression. Nevertheless, suppression being more powerful long-term, cTBS is associated with LTD-like effects (Huang et al., 2005). This also explains why iTBS may be more excitatory, as 2 s trains are not sufficiently long to allow suppression to overtake the facilitatory effects, and are associated with LTP-like changes (Huang et al., 2005).

These protocols have been accepted as the norm. However, modifications have been explored in the same way as standard rTMS. For example, peripheral visual acuity does not significantly change when the stimulation intensity of iTBS is modified (Brückner & Kammer, 2014), while depressive symptoms are reduced if the protocol is repeated three times consecutively, and the intensity remains constant at 80% active MT (Li et al., 2014). Lastly, a modified cTBS protocol at 30 Hz with 267 bursts repeated at intervals of 100 ms for a total of 801 pulses and the total duration of 44 s over right posterior parietal cortex (PPC), induces a significant increase of left visual extinctions (Cazzoli, Müri, Hess, & Nyffeler, 2009). The majority of studies use the standardized cTBS and iTBS protocol; however, adaptations have been implemented for various reasons. In terms of refinement, it is difficult to assess whether the same outcome would have occurred with the standardized protocol or whether the modified protocol is superior in that domain since they are often not directly compared.

Physiological Mechanisms

In the motor cortex, cTBS and iTBS differ regarding their waves of corticospinal activity. The corticospinal activity provides useful insight into the after-effects of TBS since the synchronous neural volleys are a direct measure of the effectiveness of synaptic input to corticospinal neurons (Di Lazzaro et al., 2008). CTBS suppresses the I1-wave, while later, I-waves and D-waves are less affected (Di Lazzaro & Rothwell, 2014). While iTBS enhances late I-waves with no change in I1-wave (Di Lazzaro et al., 2008). ITBS and cTBS affect different populations of neurons where the input to corticospinal cells suppress or enhance different I-waves. TBS appears to follow the same mechanisms of LTP and LTD that occur after standard rTMS. Prior animal studies have shown that when rodents explore a new environment, pyramidal cells in hippocampus fire in short bursts at a frequency of 5-7 Hz (Hill, 1978). Whether experimentally or naturally, bursts elicited in a theta frequency range initiate synaptic plasticity (Larson et al.,1986; Staubli & Lynch, 1987). TBS activates the trigger factor of Ca^{2+} influx to the postsynaptic neuron using the same receptors identified with rTMS. The amount and rate determine the extent of the build-up of inhibition or excitation that will occur, eventually modifying the synaptic strength and direction (Teo, Swayne, & Rothwell, 2007). The trigger factor indicates the potential for both LTP and LTD like-effects to occur, and the amount and rate determine which will be dominant.

The effects of a single session of TBS can last as long as one hour, with the duration of effects increasing with additional sessions (Suppa et al., 2016). Specifically, this is demonstrated through the differences between early and late LTP. Early LTP involves a change in synaptic strength after releasing Ca^{2+} ions and lasts for less than an hour, utilizing already existent AMPARs (Pfeiffer & Huber, 2006; Chervyakov, Chernyavsky, Sinitsyn, & Piradov, 2015).

While late LTP is associated with altered gene expression and protein synthesis and can last up to several weeks, this occurs from the insertion and upregulation of AMPAR. By increasing the efficiency and number of AMPARs at the synapse, future stimuli will generate a more significant and longer-lasting postsynaptic response (Sutton & Schuman, 2006; Chervyakov et al., 2015). A single session of TBS is likely only to affect early LTP, explaining why effects do not exceed an hour. Properly spaced TBS sessions have been shown to induce long-term effects (Duprat et al., 2016). However, an increase in sessions does not always enhance longevity and sometimes can even reverse effects (Gamboa, Antal, Moliadze, & Paulus, 2010). The previous history of neural activity is a critical factor in response and direction obtained from stimulation. The first round of TBS can be comparable to a CS, while the second mimics a TS (Potter-Nerger et al., 2009). Dependent on spacing in cTBS, the first train can induce inhibition; however, when gating occurs, the level of Ca^{2+} inside the targeted neurons is increased during the conditioning protocol, which provokes a contrasting effect. In prolonged iTBS, effects were converted to inhibitory due to a decrease in the amount of calcium influx (Gamboa et al., 2010). Overall, LTP- and LTD-like effects and receptor function in TBS are similar to standard rTMS. However, it is crucial to understand that most physiological experiments have been conducted in the motor cortex with limited comparability in other regions, specifically the occipital cortex. Very little is known about TMS effects in this area in general and even less regarding TBS. Due to limited research with TBS in the occipital cortex, it is essential to understand the mechanism of response it can have in this region and its ability to induce neuroplasticity.

Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) is a non-invasive, non-ionizing radiation-based investigational tool used to image the body. Specifically, in the brain, imaging of cortical and

subcortical structures provides insight into the specific neuroarchitecture (Schölvinck, Maier, Ye, Duyn, & Leopold, 2010). MRI uses a strong magnetic field and radiofrequency waves in order to generate the images (McRobbie, Moore, Graves, & Prince, 2007). There are several neuroimaging techniques sensitive to a variety of measures that are used to gain a better understanding of the brain's structure, organization, and function. The combination of NIBS and neuroimaging techniques gives insight into the physiological mechanisms of action of NIBS.

Magnetic Resonance Spectroscopy

MRS is a non-invasive analytical MRI technique that measures brain metabolites. Compared to standard MRI, MRS has a lower spatial and temporal resolution (Mullins et al., 2014; Hyder, 2010; Ende, 2015). MRI measures signals from abundant water and fat molecules to produce whole-brain images (McRobbie et al., 2007). In contrast, proton (^{1}H) MRS acquires its signal from hydrogen in less prominent metabolites and therefore requires a single large voxel in order to detect the lower concentration of biomolecules (Hyder, 2010). Hydrogen has two spin orientations with slightly different energy levels. When placed in a strong magnetic field, the unpaired nucleons align their spins either parallel or antiparallel to the axis of the magnetic field applied (Dager, Oskin, Richards, & Posse, 2008). The MRS signal reflects a unique frequency that occurs in response to the magnetic field, i.e., emitting a radiofrequency. The nuclei within different functional groups exhibit different resonance frequencies due to unique magnetic shielding. This occurs from their respective electron clouds configuration, which shields each nucleus differently, dependent on the molecule. The amount of shift in resonance frequency for a given molecule is called its chemical shift (Dager et al., 2008). MRS quantifies metabolite signals from their spins occurring in different chemical environments and that separate along the chemical shift axis. This reveals a spectrum of different peaks (Harris, Saleh, & Edden, 2017).

Many different types of metabolites can be measured. Gamma-aminobutyric acid (GABA) and glutamate and glutamine (Glx) are commonly quantified inhibitory and excitatory neurotransmitters, respectively, and they correspond to LTD and LTP-like changes (Stagg, Bachtiar, & Johansen-Berg, 2011a; Schmidt-Wilcke et al., 2017). MRS metabolite levels are quantified by finding the area under the spectral peak in the analysis (Edden, Puts, Harris, Barker, & Evans, 2014). MRS is a robust tool for measuring metabolites with high concentrations and signals such as N-acetyl aspartate (NAA) and Creatine (Cr). However, many metabolites do not share these characteristics and instead are low in concentration with overlapping signals and multiple sub-peaks (Harris et al., 2017). J-coupling is when a chemical signal splits into several sub-peaks (multiplets). This arises when the field experienced by a spin is affected by adjacent spins within that molecule. The splittings are due to coupling results in signals that have lower peak intensity and are broader along the chemical shift axis, making them harder to detect (Puts & Edden, 2012). The chemical structure of GABA, for example, has three different multiplets, and these signals are overlapped by more intense signals from more abundant metabolites such as NAA at 2 parts per million (ppm), CR at 3 ppm and glutamate and glutamine at 2.3 ppm (Mikkelsen et al., 2017). Many techniques and sequences have been developed in order to solve these problems.

Mescher-Garwood Point Resolved Spectroscopy

Selected options for MRS include stimulated echo acquisition mode (STEAM), which uses magnetization to form a stimulation echo or spin-echo full intensity acquired localized (SPECIAL) acquisition, which uses ultra-short echo time via this technique (Grover et al. 2015; Stagg, 2014). The most popular option is Mescher-Garwood Point Resolved Spectroscopy (MEGA-PRESS). MEGA-PRESS is a J-difference spectral editing technique implemented with

point resolved spectroscopy (Mescher, Merkle, Kirsch, Garwood, & Gruetter, 1998). In most PRESS sequences, echo time (TE) is changed by keeping the first slice selective echo time constant and shifting the second slice selective refocusing pulse. The time between the refocusing pulses is TE/2. For effective editing at a range of TEs, the editing pulse must maintain a separate TE/2 (Saleh et al., 2019). The length of TE is a critical determinant in many sequences, as this permits metabolite signals to be acquired with minimal T2 signal loss (Choi et al., 2013).

MEGA-PRESS exploits the coupling properties of molecules in order to divide their combined signal from other molecules (Mikkelsen et al., 2017). In addition, for molecules with a lower concentration, difference editing techniques uses frequency-selective inversion pulses to help in detection. MEGA-PRESS separates coupling by applying a radiofrequency pulse to one coupled spin, which can modify the time-evolution of its coupled partner and, therefore, the appearance of the corresponding peak of that spectrum (Mullins et al., 2014). MEGA-PRESS quantifies and subtracts peaks leading to separate measurements of metabolites.

In GABA, MEGA-PRESS analyzes each GABA spin system separately. A frequency-selective pulse, "ON resonance," which only directly affects signals close to 1.9 ppm, will have an indirect effect (mediated by the coupling) on GABA signals at 3.0 ppm. However, in order to separate GABA peaks from other metabolites, an inversion pulse needs to be applied elsewhere, "OFF resonance," so the coupling can evolve freely through the echo time, subtracting scans without these pulses. Other signals are removed as the majority of the signals at 3.0 ppm are not coupled to signals at 1.9 ppm. The editing spectrum occurs when two acquisitions are performed with and without the frequency-selective pulses. This allows the subtraction of the "ON" from the "OFF"-spectrum removing everything except the peaks that are affected directly by the pulse

(Mullins et al., 2014). Although the majority of signals are removed, the spectrum still includes not only the GABA signal at 3 ppm, which are coupled to GABA spins at 1.9 ppm but also other signals close to 1.9 ppm and J-coupled macromolecules. Since all these peaks are so close, it is difficult to reliably separate them (Mullins et al., 2014; Edden & Barker, 2007). Thus, it is generally accepted to use GABA+, which includes GABA and the other macromolecules mentioned above (Maddock, Caton, & Ragland, 2018). This technique also provides the concentration of the excitatory neurotransmitter, glutamate. This occurs as the editing pulse bandwidth can be sufficiently broad to include signals close to 2.1 ppm coupled partners and can be retained in the difference spectrum. However, once again, due to the complexity of the overlapping of peaks, it is not solely glutamate with resonances at 3.74 and 2.34 ppm that are included, but also, glutamine with resonance at 3.75 and 2.45 ppm (Maddock et al., 2018). The incorporation of the two is generally reported as Glx. MEGA-PRESS acquisition will change slightly based on field strength, make-and-model scanner, and experimental procedure. Modifications are made to the bandwidth of slice selective pulses and differences in its timing and to the bandwidth of editing pulses (Edden & Barker, 2007). Overall, the mechanism remains relatively the same across scanners.

Analysis of Magnetic Resonance Spectroscopy

Many different versions of GABA+ and Glx can be determined based on different internal references and corrections. An additional separate water scan can be implemented. The unsuppressed water peak can be used as an internal reference standard to control for the signal to noise ratio (SNR) (Dager et al., 2008). This can include comparing GABA+ and Glx against water, NAA, Cr or against water when tissue-corrected. Tissue composition impacts the interpretation of MRS metabolite quantification. Tissue-correction accounts for voxel

segmentation in terms of water relaxation/visibility and accounts for different facets of the voxel tissue fraction (Porges et al., 2017). Large voxels inevitably contain substantial amounts of GM in which the bulk of metabolite activity occurs, white matter (WM), which contains some activity but significantly less, and cerebrospinal fluid (CSF), which contains a negligible amount (Porges et al., 2017). This correction aims to decrease the dependency of metabolite concentrations on the underlying voxel tissue composition. Tissue-correction strategies can alter the interpretation of results, compared to uncorrected (Harris, Puts, & Edden, 2015). The most commonly used tissue-correction is the CSF-correction that aims to account for the voxel's CSF fraction (Mikkelsen et al., 2019). This amends the elevated metabolite concentration that can occur from CSF. Other corrections include the relaxation of water frequency, which improves the visibility and relaxation of water signals in all three tissues, alpha-correction, which differentiates between all the tissue concentrations and the average voxel normalization, which normalizes the average of the voxel.

Neurometabolites

GABA is the primary inhibitory neurotransmitter in the human brain. It is formed by the decarboxylation of glutamate in the GABAergic interneurons (Schmidt-Wilcke et al., 2017). There are two main isoforms: GAD65 and GAD67. Both are distributed in GABAergic neurons. GAD67 is typically found in the cytoplasm, while GAD65 is found in the nerve endings. GAD65 is primarily responsible for GABA vesicular release (Myers, Nutt, & Lingford-Hughes, 2016). GABA either binds to a $GABA_A$ receptor, which allows the influx of chloride, leading to hyperpolarization of the neuron or $GABA_B$ receptors, which interacts with potassium and calcium channels. This interaction creates either a potassium efflux or inhibits calcium channels. Both of these mechanisms lead to an inhibitory response (Ulrich & Bettler, 2007; Schmidt-

Wilcke et al., 2017). Glutamate refers to the anion of glutamic acid and is considered the primary excitatory neurotransmitter in the human brain (Tremblay et al., 2013). The inhibitory and excitatory response from neurometabolites can be associated with LTD- and LTP-like effects. LTP and LTD mechanisms are a principal consequence of the effectiveness of glutamatergic synapses (Thickbroom, 2007; Pell et al., 2011). Other important factors contribute to lasting after-effects. When glutamate is released, it directly affects synaptic plasticity, which initiates LTP (Tremblay et al., 2013). While the inhibition of $GABA_A$ and $GABA_B$ facilitates its potentiation (Hess & Donoghue, 1994; Hess et al., 1996), this occurs from the elimination of Mg^{2+} on NMDARs (Pell et al., 2011). GABA transmission also regulates synaptic integration, probability, and timing of action potentials (Ende, 2015).

The influence of inhibitory processes on LTP is also believed to underlie the improved efficacy of patterned protocols in TBS. LTP occurs as cortical $GABA_B$ inhibition only lasts around 200 ms (Davies et al.,1991), similar to the interval between bursts in TBS. The initial burst activates inhibitory interneurons, eventually leading to GABA-mediated hyperpolarization of the pre-synaptic neuron. When a second train is applied, this induces a lower threshold of $GABA_B$-mediated hyperpolarization on the pre-synaptic neuron, decreasing the release of inhibitory neurotransmitters (Ulrich & Bettler, 2007). This leads to the voltage-dependent NMDAR mediated current associated with LTP induction being enhanced on the post-synaptic neuron (Davies et al.,1991). In LTD, mechanisms occur as a consequence of enhanced cortical inhibition at GABAergic synapses as well as a decrease in the effectiveness of facilitatory glutamatergic activity (Thickbroom, 2007; Pell et al., 2011).

Although MRS assesses GABA+ and Glx levels changes, it is not clear how the total concentration of a metabolite within a large volume of cortical tissue relates to local synaptic

activity. MRS can only quantify the total concentration of a given neurochemical within the voxel and cannot distinguish each specificity's relative contribution (Stagg, 2014). However, for GABA, it is hypothesized that MRS derived GABA levels reflect extra-synaptic GABA tone rather than synaptic $GABA_A$ or $GABA_B$ activity (Myers et al., 2016; Stagg et al., 2011a). Since Glx is measured as a result of coupling with GABA peaks, precise measurements of glutamate as an excitatory neurotransmitter are not possible. Glutamine will not have as direct an effect on synaptic plasticity in the same way as glutamate; however, due to overlap in peaks, it is still measured in combination with glutamate (van Veenendaal et al., 2018). GABA and glutamate represent inhibitory and excitatory measures of neurotransmitters; although the MRS-derivatives of these metabolites are not verbatim, they are hypothesized to represent a reasonably accurate tool assess change in activity and potential LTD and LTP.

Purpose of the Current Work

The present study examines changes in biomarkers following a single session of cTBS or iTBS compared to sham stimulation in the occipital cortex. This project will contribute to our understanding of the commonly used TBS protocol by measuring neurotransmitter concentrations. It aids in establishing a concise understanding of TBS's underlying mechanisms, analyzing real differences in metabolites before and at several time points after stimulation. For TBS to be a useful clinical tool to treat diseases, it is necessary to understand the underlying neural mechanisms in various brain regions. This will help to gain a clear understanding of how it should be implemented in treatment. In non-visual disorders, TBS is used for the treatment of many psychological illnesses. This includes, but is not limited to, depression, schizophrenia, phobias, bipolar disorder, and others. The use of TBS in clinical treatment has been to stimulate the dorsolateral prefrontal cortex (DLPFC), dorsomedial prefrontal cortex, cerebellum and

others. (Rachid, 2017). TBS has also been explored in neurological disorders in the M1 with the rehabilitation after a stroke (Talelli, Greenwood, & Rothwell, 2007), tremors from Parkinson's disease (Benninger et al., 2011) and in neurodevelopmental disorders in the posterior superior temporal sulcus (pSTS) investigating changes in repetitive behaviour with autism (Ni et al., 2017). The potential to treat a wide variety of disorders through many different regions of the brain displays the extensive utility of TBS.

TBS has been used successfully in a variety of brain regions for a variety of disorders. However, there have been limited studies that have investigated TBS in the visual cortex, with even fewer that have analyzed its therapeutic potential. The visual system compromises the most complex circuity of all sensory systems and possesses the ability to undergo synaptic plasticity and neuroplastic changes (Ranieri et al., 2019). Different regions in the visual cortex are associated with different levels of processing. The primary visual cortex, V1, is located in the posterior pole of the occipital cortex. It is highly specialized for processing information about static and moving objects and basic aspects of visual images such as oriented lines (Salminen-Vaparanta, Noreika, Revonsuo, Koivisto, & Vanni, 2012b). V1 has a well-defined map from visual input, such that it is organized in a retinotopic fashion. Surface V1 neurons have robust tuning to ocular dominance, orientation, motion, spatial frequency direction and speed (Hubel & Wiesel, 1959). Deeper layers of neurons in the V1 are sensitive to the more global organization (Lamme & Roelfsema, 2000; Zhaoping, 2014). Regions of the visual cortex beyond the V1 include the ventral stream (V2 and V4) which represents the "what" pathway and the dorsal stream (V3, V5) which represents the "where/how" pathway (Van Essen, William, & Newsome, 1984; Maunsell & Van Essen, 1983; Salminen-Vaparanta, Koivisto, Noreika, Vanni, & Revonsuo, 2012a). The visual cortex is divided into multiple sections; however, there is often

overlap and correlation with the processing of information. Since the V1 is close to the scalp, it is often chosen for stimulation when studying TMS effects on basic visual processing (Allen et al., 2014). TMS has also been used to stimulate other areas such as the inferior occipital cortex, used for face recognition (Solomon-Harris et al., 2016), and the lateral occipital cortex (LO), for object and scene processing (Mullin & Steeves, 2011)

To the best of our knowledge, no study has examined GABA+ and Glx concentrations comparing cTBS, iTBS and sham TBS to the V1 in healthy humans. This project was motivated by a previous study from our laboratory that analyzed the impact on neurotransmitter concentrations following a single or accelerated session of 1 Hz rTMS (Rafique & Steeves, 2018). A single session altered resting-state functional magnetic resonance imaging (fMRI), while the accelerated protocol, which consisted of five rTMS sessions separated by a 15-min break between each session, significantly reduced GABA+ levels at the stimulation site (Rafique & Steeves, 2018). This study demonstrated the potential of neurochemical modulation from rTMS in the occipital cortex, and it was of interest to determine whether TBS would be similar. Due to limited research with TBS in the visual cortex, it is essential to understand the effects it can have in this area and its ability to induce neuroplasticity. Our hypotheses were that if cTBS and iTBS operated in the same way across the brain, neurotransmitter concentrations would fluctuate as expected based on the excitatory and inhibitory mechanism previously discovered in the M1. However, a single session of 1 Hz rTMS shows no effect on metabolites in the V1 (Rafique & Steeves, 2018), but in contrast, a single session of 1 Hz rTMS to the PFC does (Michael et al., 2003). This demonstrates the incongruities that occur with the application of TMS across different brain regions. It is apparent that not all brain areas have the same response to the same TMS protocol. Inter (López-Alonso, Cheeran, Río-Rodríguez, & Fernández-Del-

Olmo, 2014) and intra (Hamada, Murase, Hasan, Balaratnam, & Rothwell, 2012) participant variability, as well as variability across the cortex, makes interpretation and generalisability from previous experiments more difficult.

The LTP and LTD mechanism described above discovered in the hippocampus is only roughly translatable to surrounding regions in the brain. In the cerebral cortex, cortical neurons are placed in multilayered arrangements with numerous additional synaptic connections within each functional module and diverse sets of axons running from each module to its connected counterpart (Huerta & Volpe, 2009). Thus, an exactly translatable mechanism from the hippocampus is not possible. While some research has shown similar mechanisms in the motor cortex, there is limited work done in other regions of the brain. Variability from individuals could be explained by the brain's structural complexities, causing unsuspected responses (Durst et al., 2015). It is difficult to assess if the heterogeneous activity occurs from a change in the efficacy of TBS paradigms for inducing plastic changes across brain regions, or due to inter and intra (Guerra, López-Alonso, Cheeran, & Suppa, 2017) participant variability. GABA+ and Glx are sensitive to many external changes (Suppa et al., 2016), and without proper controls, this can cause unexpected fluctuations in neurotransmitters and variable responses to TBS. Variability in magnitude and direction of effects can occur with both iTBS and cTBS (Hamada et al., 2012; Nettekoven et al., 2015). Thus, it is essential to investigate the effects of TBS on healthy individuals in the visual cortex to set a standard for "normal" biological mechanisms that occur after stimulation, specifically in this area. Prior to the development of a clinical intervention protocol, solid mechanistic knowledge about the casual and specific contribution of brain regions and networks and how they may relate to clinical symptoms needs to be quantified. Initial work in healthy participants can lead to translational therapeutic-validation studies. This creates a

foundation for the treatment of visual disorders such as amblyopia, hallucinations, and other general visual impairments.

Chapter 2:

Visual Cortical Gamma-Aminobutyric Acid and Glutamate Following Theta Burst Stimulation to the V1

Introduction

NIBS techniques such as TBS are an invaluable tool in neuroscience to safely and effectively induce neural plasticity in healthy participants. It is often used to reverse engineer neural networks in the brain. TBS is currently being explored as a potential alternative to rTMS as the protocol can be delivered quicker while still achieving similar results (Blumberger et al., 2018; Suppa et al., 2016; Ridding & Ziemann, 2010). ITBS typically generates an excitatory response, in a similar fashion to LTP in synaptic transmission, while cTBS typically produces an inhibitory response due to its continuous pattern, causing LTD (Larson et al.,1986; Davies et al., 1991; Hess et al., 1996; Di Lazzaro et al., 2008; Huang et al., 2005). The effects of TBS are mediated by changes in the local activity of cortical neuronal pathways. MRS can measure neurotransmitters such as GABA and glutamate, which can be used as markers for inhibition and excitation of these pathways corresponding to LTD- and LTP-like changes (Stagg et al., 2011a; Schmidt-Wilcke et al., 2017).

A number of studies have examined GABA+ and Glx following rTMS or TBS stimulation and most focus on effects in motor or frontal cortices with few examining effects in the visual cortex. Table 2.1 summarizes previously published studies measuring GABA+ and/or Glx following a single session of TBS or rTMS in healthy participants. Response to stimulation differed across experiments with a lack of consistent outcomes.

Table 2.1.

GABAergic and/or glutamatergic changes with MRS that occurred in healthy participants after a single session of TBS or rTMS

Article	Stimulation protocol	Target of stimulation (TS); voxel placement (VP) for MRS	Sample size and participant inclusion criteria	Participant exclusion criteria	Effect on GABA+ and/or Glx

Vidal-Piñeiro et al., 2015	Original iTBS & Original cTBS & Sham (placebo coil used)	TS: Left IPL VP: Left IPL & left PCC	31 right handed participants (age = 23.5 [2.00] years; seven males) in PCC Sample size for each group: 10 iTBS, 10 cTBS, 11 sham One dropped in the sham group for IPL (30 participants) Gender, age and education were matched	No history of head trauma/ neurological or psychiatric diseases No TMS & MRI safety contradictions	Distal GABA+ increased in left PCC after iTBS compared to cTBS & sham No change at stimulation site (left IPL) No change in Glx
Allen et al., 2014	Original cTBS & Sham (coil oriented horizontally with spacer)	TS & VP: V1	18 participants (age =26.3 [5.0] years; 11 males) Repeated measures design	No TMS & MRI safety contradictions	GABA+ levels increased in cTBS condition compared to sham Glx was NP
Stagg et al., 2009	Original cTBS & Sham (stimulation at vertex)	TS & VP: Left M1	15 right-handed male participants (age= 27.5 [NP]) Repeated measures design	No TMS & MRI safety contradictions	GABA+ levels increased compared to sham No change in Glx
Iwabuchi et al., 2017	ITBS (bursts of three pulses at 50 Hz applied at a frequency of 5 Hz for 40 s repeated for a total of three runs with 5 min intervals between runs) & Sham (placebo coil used)	TS: Left DLPFC VP: Left DLPFC & left ACC	27 participants (age =25.11 [7.13]; NP) Repeated measure design	No history of head trauma/ neurological or psychiatric diseases No substance abuse/ dependence or medication use No TMS & MRI safety contradictions	GABA/Glx ratio decreased compared to sham in left DLPFC No change at left ACC No change in GABA+ or Glx individually
Bridges et al., 2018	RTMS (20 min of 1 Hz applied continuously) &	TS & VP: Left DLPFC	11 participants (age = 29.6 [6.2]; 10 males)	No history of head trauma/ neurological or psychiatric diseases	NAA/Cr correlated with Glx/Cr in sham group

	Sham (placebo coil used)		Repeated measure design Minimum of six hrs of sleep before the session	No substance abuse or dependence No TMS & MRI safety contradictions No alcohol 24 hrs before	Glx/Cr correlated with Cho/Cr in active group No change in GABA+ or Glx individually
Michael et al., 2003	RTMS (20 min of 20 Hz, 2 s trains, separated by 58 s ISI for 20 trains) & Sham (coil oriented horizontally with spacer)	TS: Left DLPFC VP: Left and right DLPFC, left ACC	12 right-handed participants (age = 46.1 [13 years]; nine males) Sample size for each group: seven rTMS, five sham	No history of head trauma/ neurological or psychiatric diseases No substance abuse or dependence No TMS & MRI safety contradictions	Glx decreased in left DLPFC-dependent on pre-TMS levels compared to sham No change at right DLPFC or left ACC No change in GABA+
Rafique & Steeves, 2018	Single session rTMS (20 min of 1 Hz applied continuously) & Accelerated session rTMS (20 min of 1 Hz applied continuously repeated for five sessions with 15-min interval breaks)	TS & VP: V1	16 right-handed participants (age = 25.15 [1.21]; 10 males) Sample size for each group: eight single session rTMS group, eight accelerated session rTMS group Normal or corrected-to-normal vision	No history of head trauma/ neurological or psychiatric diseases No substance abuse/ dependence including smoking or medication use including hormonal contraceptives No TMS & MRI safety contradictions No alcohol 48 hrs before No history of frequent or chronic migraines	GABA+ significantly decreased in accelerated session rTMS group compared to single session rTMS group No change in Glx
Gröhn et al., 2019	1 Hz rTMS (20 min of 1 Hz, 10 pulses per	TS & VP: Left and right M1	Seven right-handed male participants (age	No history of head trauma/ neurological	GABA levels increased in left M1 and

	train, separated by 1 s ISI for 120 trains) & 5 Hz rTMS (22 min of 5 Hz, 25 pulses per train, separated by 45 s ISI for 24 trains)	range= 21-40 [NP]) Sample size for each group: seven 1 Hz rTMS (one individual received the additional 5 Hz rTMS)	or psychiatric diseases No TMS & MRI safety contradictions Sleep apnea No medication	decreased in right

Note. This table describes studies that use a single session of TBS or rTMS in healthy participants and measure GABA+ and/or Glx using MRS. TS= target of stimulation, VP= voxel placement, GABA+= gamma-aminobutyric acid and macromolecules composite, Glx = glutamate + glutamine, ISI= inter-stimulation interval, Original iTBS (triplet 50 Hz bursts, repeated at 5 Hz, 2 s train separated by 8 s ISI for 20 trains (3 min, 10 s) designed by Huang et al., 2005), Original cTBS (triplet 50 Hz bursts, repeated at 5 Hz applied continuously (40 s) designed by Huang et al., 2005), IPL= inferior parietal lobe, PCC= posterior cingulate cortex, iTBS =intermittent theta burst stimulation, cTBS = continuous theta burst stimulation, TMS = transcranial magnetic stimulation, MRI = magnetic resonance imaging, V1 = primary visual cortex, M1= primary motor cortex, NP= not provided, DLPFC = dorsolateral prefrontal cortex, ACC= anterior cingulate cortex, rTMS = repetitive transcranial magnetic stimulation, NAA= n-acetyl aspartate, Cr = creatine, Cho= choline

It is evident from this chart that there is variability in the outcome of a single session of rTMS and TBS. This variability may be associated with brain structure distinctions depending on where the stimulation target is, differences in the level of neural activity prior to stimulation, neurochemistry (Greenhouse, Noah, Maddock, & Ivry, 2016; Durst et al., 2015; Mueller et al., 2013; Reithler, Peters, & Sack, 2011) or confounding variables. As a result, given the multifactorial nature of variables contributing to TMS findings, it is essential to measure the effects of TBS in a number of brain regions in a highly controlled manner. This will help to establish an understanding of how TBS affects neurometabolites and how to avoid erroneous generalized assumptions of its impact.

Few studies have evaluated biomarker fluctuations following non-invasive neuromodulation to the occipital cortex. Only one study other than ours, has measured GABA+

in the human occipital cortex after cTBS to the V1. They found a significant increase in GABA+ levels immediately following TBS at the stimulation site (Allen et al., 2014). In addition, TBS has shown the ability to modify other measures in the visual cortex. For example, in humans, cTBS stimulation reduces resting-state connectivity between visual areas (Rahnev et al., 2013), increases PT by 10% (Franca et al., 2006), modifies peripheral acuity (Brückner & Kammer, 2014) and can enhance conscious vision (Allen et al., 2014). ITBS in rodents has been shown to reduce GAD67, increase its counterpart, GAD65 (Trippe et al., 2009) and alleviate dark-rearing effects on cortical development (Castillo-Padilla & Funke, 2016). The reduction or enhancement of physiological and behavioural outcomes in these experiments provides fundamental insight into the potential LTD- and LTP-like effects in the occipital cortex. The diversity of methodological techniques contributes to our understanding of TBS mechanisms through approaches such as fMRI or enzyme quantification.

For TBS to be a useful clinical tool and to understand how it should be implemented in treatment, its effects need to be studied in various regions of the healthy brain. Clinically, TBS can be a useful tool in ophthalmological cases related to the central nervous system function in visual disorders of cortical origin (Mahayana, Gani, & Muggleton, 2017). For example, it has been used to improve "neglect-like" effects with stimulation to the PPC (Cazzoli et al., 2012) and could potentially treat optic neuritis caused by multiple sclerosis (Mahayana et al., 2017). There are limited clinical studies of rTMS applied directly to the occipital cortex. However, our laboratory has successfully reduced visual hallucinations following a stroke (Rafique et al., 2016), and others have used rTMS to treat Charles Bonnet Syndrome (Merabet, Kobayashi, Barton, & Pascual-Leone, 2003). Both TBS and rTMS have been used in experimentation with their treatment of amblyopia (Tuna et al., 2019; Clavagnier, Thompson, & Hess, 2013;

Thompson, Mansouri, Koski, & Hess, 2008; Thompson, Mansouri, Koski, & Hess, 2012); however, the validity of the results has been debated. An examination of the underlying neurobiological mechanisms associated with TBS will further our understanding of potential therapeutic benefits in clinical patients. It will also help constrain its use in the laboratory for safe administration in healthy participants. To summarize, we performed a single session of cTBS, iTBS or sham TBS to the occipital cortex in healthy participants. We measured GABA+ and Glx before stimulation and at two-time points after stimulation, immediately and one hour post-stimulation. To the best of our knowledge, our study is the first to do so. This study was conducted to examine the effects of TBS on the V1 on neurotransmitters and to establish normative data from healthy individuals. In order to control extraneous factors, we employed strict exclusion criteria to exclude potential confounding variables associated with unnatural metabolite fluctuations.

Methods

Participants

A total of 31 healthy right-handed participants were recruited between the ages of 18 and 35 (mean age = 22.5 [3.89] years; 13 males). Participants were assigned to one of three experimental groups (sham, cTBS, iTBS) in a pseudo-random fashion and naive to the stimulation condition. One male participant was discarded from the sham group due to high fit error from motion artifact. The final analysis included 10 participants in each group. All participants had normal or corrected-to-normal vision with no known safety contradictions to MRI or TMS. Exclusion criteria included a history of neurological disorders, mental illnesses, medical conditions, medication including birth control, history of migraines and drug/substance abuse, including smoking or recent usage. Efforts were made to test females in the same self-

reported period of their menstrual cycle when possible. Participants were also asked to avoid drinking 48 hours in advance and asked to have a good night's sleep prior to testing. All participants gave informed consent, and the protocol was approved by the Office of Research Ethics at York University in accordance with the Declaration of Helsinki.

Vision Assessments and Neurological Questionnaire

All participants were required to complete and pass three visual assessments to ensure eligibility— monocular and binocular visual acuity (standardized early treatment diabetic retinopathy study vision chart; Precision Vision; La Salle, Illinois, United States), colour vision (Ishihara test; Kanehara Trading; Tokyo, Japan), and stereo acuity (Titmus circles test; Titmus Stereo Optical Company; Chicago, Illinois, United States).

All participants completed and passed the Montreal Cognitive Assessment (MoCA, v7.1–7.3; Nasreddine et al., 2005), screening for mild cognitive impairment.

Transcranial Magnetic Stimulation

A Magstim Rapid^2 and Plus^1 Stimulator with a 70-mm diameter Double Air Film figure-of-eight coil as well as its sham counterpart (Magstim; Whiteland, Wales) was used to deliver stimulation pulses or mimic stimulation pulses, respectively, to the defined target site.

Phosphene Threshold

A PT was measured for each participant using a single-pulse application. Phosphenes are known to be elicited when stimulation is applied anywhere from 1-5 cm above the inion and 0-3 cm laterally, depending on which hemisphere is being tested (Elkin-Frankston et al., 2010). The left hemisphere was tested for PT, and we marked four locations: one on the inion, another 2 cm above the inion, another 2 cm to the left of the inion and the last 2 cm above the one to the left of the inion to form a small square. In a dimly lit room, wearing a blindfold with eyes closed,

participants were instructed to lean forward with their forehead resting on a table while putting no pressure on their eyes. The coil was held 90 degrees tangential to the midline, which has been shown to be optimal for eliciting phosphenes (Elkin-Frankston et al., 2010). Following each TMS pulse, participants were instructed to respond "yes/no/maybe" corresponding to their perception of a phosphene that could vary in shape, colour, motion and size. A threshold was defined as the intensity at which 50% of pulses (five pulses) resulted in a "yes" response.

The minimum output setting for PT measurement began at 50% intensity, and 10 pulses were administered to the top right corner location of the square with each pulse 6 s apart. This ISI is sufficiently long for the previous pulse's effects to be diminished (Ganaden et al., 2013). After every 10 pulses, the coil was moved to a new position on the square if no phosphenes were reported. The square was used solely as a guideline for where phosphenes are generally expressed. The coil moved beyond the square but keeping within limits above when needed. The coil was placed in a new location at each stimulator output until the individual began to respond "yes/maybe" in a specific area, which was then designated as the hotspot (Mazzi et al., 2017). The experimenter then increased the stimulator output by a value of five until phosphenes were evoked 50% of the time (five pulses; an additional pulse was given if the individual responded "Maybe"). Subsequently, the threshold was reduced by 1% increments to target the precise phosphene level. The maximum output setting used was 90% intensity according to safety regulations (Wassermann, 1998). The blindfold was removed every 10–15 min when necessary, for a minimum of 2 min, to prevent dark adaption (Elkin-Frankston et al., 2010).

Theta Burst Stimulation

We stimulated the occipital cortex, V1, which is the closest scalp coordinate to the mid-hemispheric termination of the left and right calcarine sulci. Participants underwent one of three

TBS conditions: 1) cTBS, 2) iTBS or 3) sham TBS, performed at 80% PT. The TBS protocol consisted of bursts of three pulses at 50 Hz (i.e., 20 ms between pulses), repeated at 5 Hz (i.e., intervals of 200 ms), for a total of 600 pulses. ITBS consisted of a short train of 2 s bursts every 10 s for 20 cycles, while cTBS consisted of 200 bursts applied continuously for 40 s (Huang et al., 2005). Sham TBS mimicked either the cTBS or iTBS protocol using a separate sham coil. The sham coil has a metal plate seated under the physical coil to attenuate the magnetic field but maintains the audible clicks from the coil discharge. Half of the individuals in the sham group underwent sham cTBS, and the other half underwent sham iTBS. Since there were no significant differences between each group, they were subsequently collapsed into one sham group for the remainder of the analyses.

Since the size and anatomical location of visual areas can vary widely across participants, we used a spatial framework where the target stimulation region of interest was individually specified. Participants' anatomical MRI images were reconstructed and co-registered to three-dimensional (3D) cortical surfaces using the image-guided TMS software, Brainsight (Rogue Research; v2.3.10; Montreal, Quebec, Canada). The target stimulation site corresponded to the centre of the MRS volume of interest (VOI), V1. The stimulation site was mapped on each participant's corresponding anatomical images in Brainsight by matching the anatomical landmarks to the MRS VOI images obtained at the baseline MRS acquisition. Common reference points on both MRI images and the participant's head created a co-registration matrix using a Polaris infrared marked system. The image-guided software coil positioning was monitored in real-time during stimulation. The coil was held tangent to the head to minimize coil to cortex distance. The participant sat upright with the coil at the back of the head and their chin resting on a chin rest.

Magnetic Resonance Imaging

The anatomical MRI and MRS were acquired with 3 Tesla (3T) Siemens Magnetom Prisma Magnetic Resonance Scanner with a 32-channel high-resolution brain array coil (Siemens; Munich, Germany). The sequence began with an anatomical high-resolution T1-weighted image followed by MRS. Participants were instructed to remain still and refrain from falling asleep. The room was dark, and participants were instructed to keep their eyes closed.

Anatomical T1-Weighted

The T1-weighted magnetization-prepared rapid gradient echo (MPRAGE) imaging sequence was: number of slices = 192; in-plane resolution = 1 mm^3; slice thickness = 1 mm; imaging matrix = 256 x 256; repetition time (TR) = 2300 ms; TE = 2.26 ms; inversion time (TI) = 900 ms; flip angle = 8 degrees; field of view (FoV) = 256 mm; acquisition time = 5 min. This produced an anatomical image which was subsequently used to define the VOI for TBS and MRS.

Magnetic Resonance Spectroscopy

A single 25 mm^3 square voxel was placed on the occipital cortex containing the V1 in the occipital pole's posterior region and centred on the calcarine sulcus. The lower edge of the voxel followed the cortical surface and aligned alongside the cerebellar tentorium. The VOI position was verified in three planes and, landmarks were used for accurate placement and re-placement on subsequent visits.

^1H spectra were obtained using MEGA-PRESS. A C2P collaboration between Siemens and the University of Minnesota (CMRR) MEGA-PRESS sequence was specifically designed for the Siemens Magnetom Prisma. The parameters were: TR = 3000 ms; TE = 68 ms; averages = 32; The scan was repeated four times for a total of 128 on averages and 128 off averages;

spectral bandwidth = 1500 Hz; acquisition time =18 min. A separate water suppression scan was performed to allow for a tissue concentration reference and had an acquisition time =1 min. Two automated shimmings were done using Siemens B1 Shim mode and TrueForm. Participants were in the scanner and began the T1-sequence within approximately 5 min following TBS; Figure 2.1A shows an example of the standard voxel placement in the occipital cortex. Figure 2.1B shows an example of the difference edited spectrum peak of GABA+ with Glx, as well as Cr and water reference signal peaks by Gannet.

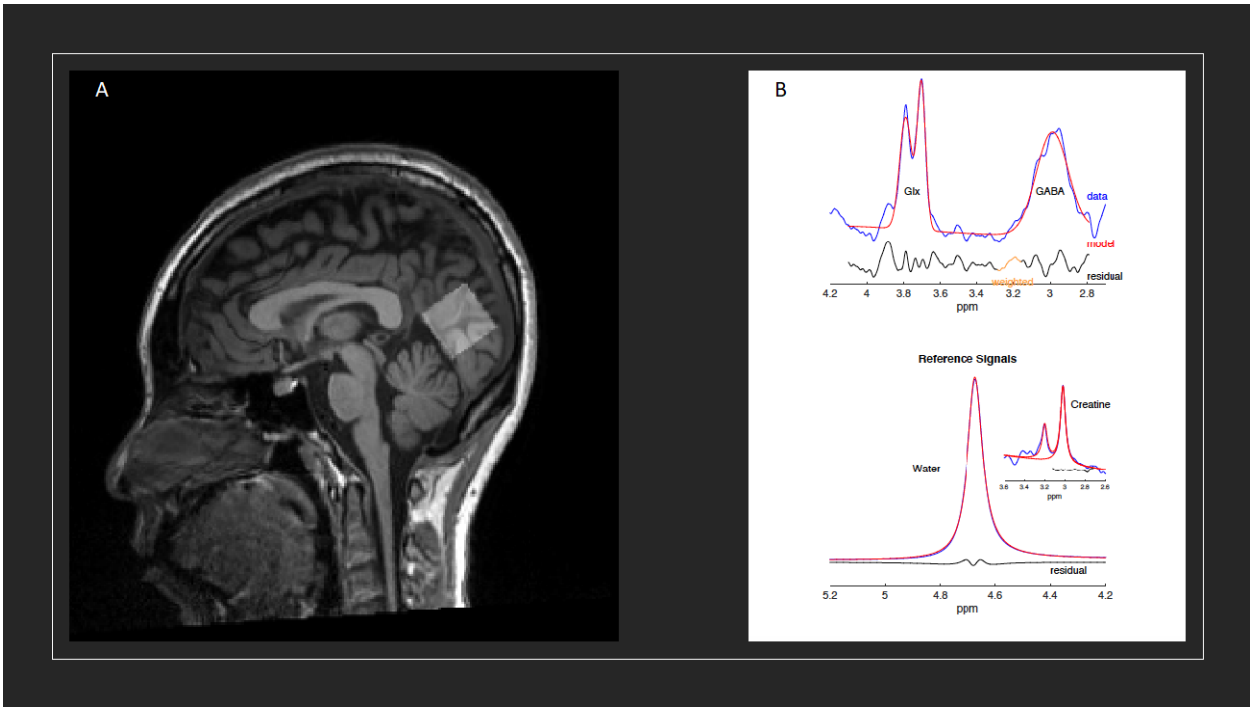

Figure 2.1. ^{1}H MR Spectra Acquired from the Visual Cortex. (A). Example of standard voxel placement within the occipital cortex on a T1-weighted image for a single participant shown in the sagittal plane. (B). An example of sample processing of MEGA-PRESS, the difference edited spectrum (blue line) corresponds to voxel using Gaussian fitting (red line) with Gannet. This example shows typical signal peaks for GABA, Glx, Cr, and water for a single participant at a single time point.

Experimental Design Overview

The experiment was divided into two sessions. Participants initially underwent pre-TBS eligibility questionnaires, vision assessments and the MoCA as screening devices. To minimize the potential diurnal variation of neuromodulators, participants underwent testing midday at approximately 1 pm. The second session was approximately a week later to limit any residual effects from the PT measurement and to avoid any long-term fluctuations in metabolites. MRS baseline measures were acquired upon eligibility, followed by a PT on the first day. In a separate follow-up visit, the participant received either cTBS, iTBS, sham iTBS (control) or sham cTBS (control) to the V1. MRS was repeated immediately following TBS and one hour later. Vision assessments and the MoCA were repeated after the first scan on the second day since it was not possible to test immediately after TBS. The PT was measured after the final scan. Start and end time points were recorded for MRI, TBS and PT. In addition, participants self-reported time at which they woke the day of testing and went to sleep the evening before and their menstrual cycle stage if female. Figure 2.2 shows a diagram of the experimental procedure.

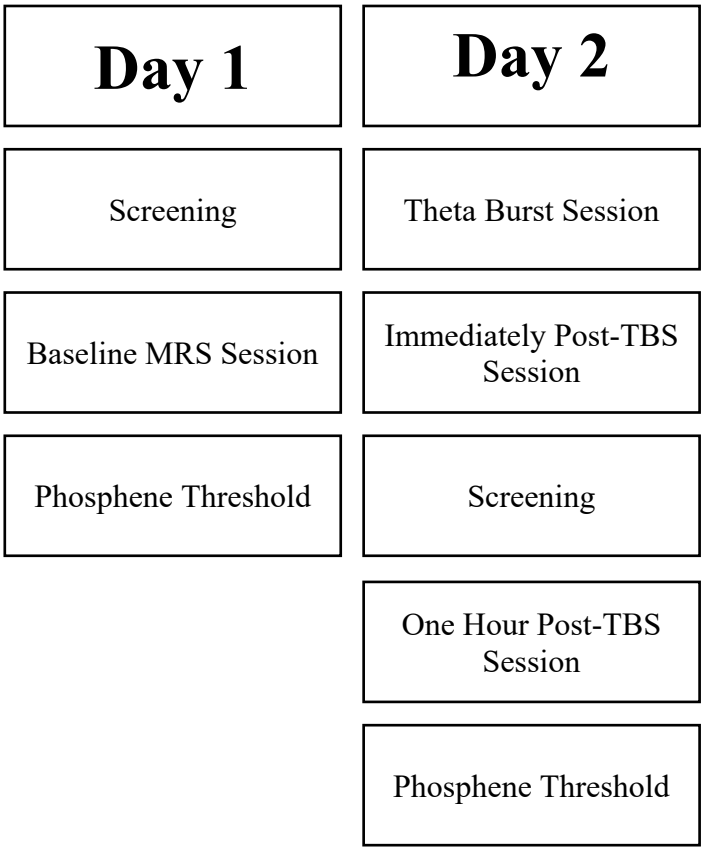

Figure 2.2. Diagram of the Experimental Procedure. Day 1: all participants were screened and completed a baseline T1 and MRS scan followed by measurement of PT. Day 2: all participants received either cTBS, iTBS or sham TBS, and follow-up MR imaging was done immediately post-TBS. After completion of the first scan, participants were re-screened for any changes in visual and cognitive assessments. Participants returned to the MRI scanner at the one hour mark and completed the PT measurement at the end of the experiment.

Data Analyses

MRS data were processed using MATLAB (MathWorks; v2015; Natick, Massachusetts, United States; https://www.mathworks.com/products/matlab.html) add-on Gannet (v3.0; Baltimore, Maryland, United States; http://www.GABA+MRS.com; Edden et al., 2014) and SPM12 (Statistical Parametric Mapping; Wellcome Centre for Human Neuroimaging; London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm). Gannet is a free software package designed for the batch analysis of edited difference spectrum double Gaussian fit MRS data. The Cr signal is measured using the equivalent Lorentzian model and the unsuppressed water spectrum using the Gaussian-Lorentzian model. Gannet is used for preprocessing individual data acquisition, as well as for filtering the spectrum of interest and improving SNR. This software method performs as adequately as other popular technology such as LCModel but is open-source (O'Gorman, Edden, Michaels, Murdoch, & Martin, 2011). The area under the edited GABA signal describes the total number of molecules and thus represents the concentration of that metabolite (Edden & Baker, 2007; Edden et al., 2014). Individual acquisitions underwent standard processing including frequency and phase correction, exponential line broadening, fast Fourier transform of time-domain acquired data to frequency-domain spectra, outlier rejection, time averaging and phased-array channel combination. Difference editing is inherently sensitive to experimental instabilities such as scanner drift and participant movement, and as a result, the best practice for

filtering the spectrum of interest and improving SNR includes frequency and phase correction before time averaging (Edden et al., 2014).

The concentrations of GABA+ and Glx were considered relative to the separately acquired water signal using nonlinear least-squares fitting. This integrates the edited GABA+ peak at 3 ppm and subsequently produces GABA+ concentration estimates (Edden et al., 2014). Gannet provides GABA+ and Glx concentrations uncorrected using Cr as an internal reference and both the corrected and uncorrected versions against water as an internal reference in institutional units (i.u.). In addition, it provides information on tissue fractions (GM, WM, CSF), fit error and overall stability of the experiment (field drift, participant motion). There are three different versions of the type tissue-correction for metabolite levels given. The first is solely relaxation corrected, the second is relaxation and alpha corrected, and the third incorporates previous corrections but also normalizes voxel average. Lastly, Gannet provides a registered image of the MRS VOI by creating a binary mask of the VOI with the same geometric parameters as the anatomical image. Our principal analysis compared the relaxation-and alpha-corrected GABA+ and Glx value across all three-time points. The uncorrected-tissue GABA+ and Glx compared to Cr as an internal reference is also reported (see Appendix A). There is currently a debate in the field of the necessity of tissue-correction and the superior internal reference. Since a tissue-corrected value that uses Cr as an internal reference is not provided, we based our discussion on the tissue-corrected water referenced value. In our opinion, this is the preferred measure as it corrects tissue differences in voxel fractions that may lead to unnecessary variability. Lastly, any participant with a fit error over 10 or high field drift and motion was removed (Mikkelsen et al., 2019).

Statistical Analyses

For statistical analyses, the alpha level was set at 0.05, and 0.1 was considered a trend. All statistical analyses were conducted in R (v1.1.456; Vienna, Austria; www.R-project.org).

We assessed the TBS effect on metabolite concentrations (GABA+, Glx, and GABA+ and Glx composite ratio) across three-time points. We analyzed both tissue-corrected compared to water and uncorrected-tissue compared to Cr. Tissue-corrected compared to water is our principal analysis. The GABA+ and Glx difference score as a composite ratio equation is (Glx-GABA+)/ (Glx + GABA+). This measure was used to determine whether changes in one metabolite may be related to those in the other metabolite and to calculate their normalized differences. Multilevel modelling was used for statistical analysis since the data was non-parametric. Akaike information criterion (AIC) was used to measure the goodness of fit.

Multilevel modelling is highly robust for dealing with repeated-measures data and overcoming issues where the assumptions of Analysis of Variance (ANOVA) are violated (Peugh, 2009). It has less stringent demands, permits hierarchically structured data and can handle missing values (Hox, 1998; Peugh, 2009). Restricted maximum likelihood (REML) equalled to false, while model comparison occurred and was set to true for the final output to reduce bias (McNeish, 2017). REML provides a correction to each model, but each correction may not be identical, thus creating inaccuracies (McNeish, 2017). In GABA+, we compared models with random slopes and random intercepts and a fixed slope with random intercepts. Since all baseline values are different, the model would undoubtedly include random intercepts. However, descriptively looking at the data, we could see for each condition, the slopes varied, but we could not be sure the magnitude by which they varied was sufficient to use the random slopes model. Thus, comparing the two models, we found the random slope and random

intercept model was almost perfectly correlated, indicating it was overfit. This shows that the fixed slope and random intercepts model is superior in describing the data. To double-check, we used the AIC values. The fixed slope and random intercept model showed the lowest AIC value, indicating the best fit. As well, the structure of the data showed that a quadratic model would not have been appropriate. There is a lot of debate in the field over the perfect model selection method. It was decided that a minimal model would be the best choice given the structure of the data. We can also see this model gives the lowest AIC for GABA+. Results from GABA+, Glx and their composite ratio are presented separately below. This type of model was chosen for all subsequent analyses, including post hoc analyses, to maintain consistency.

Multilevel modelling was also used to compare tissue proportions within VOI to ensure proper voxel placement and the effect TBS had on PT. Lastly, we determined the correlation coefficient between GABA+, Glx and PT using Kendall tau. Kendall tau is used for small samples and non-parametric data.

Results

GABA+ Concentration

There was a significant main effect of Time $F(2,58) = 2.98$, $p = .00415$ and a significant interaction between Time and Condition $F(2,58) = -3.03$, $p = .00368$. There was no significant effect of Condition $F(2,58) = 1.40$, $p = .166$. A post hoc analysis showed that across all time points only sham was significant: $t(19) = 2.56$, $p = .0190$, while cTBS showed a trend towards significance: $t(19) = -1.82$, $p = .0848$ and iTBS was not significant: $t(19) = 0.582$, $p = .568$. We then explored the differences across time points in each condition using the same model. We compared time point changes from baseline to immediately post-stimulation (B-I), baseline to one hour post-stimulation (B-O) and immediately post-stimulation to one hour post-stimulation

(I-O). All three time points showed no significant effect from B-I: *ps*> 0.1. Only the sham group was significant from B-O, $t(9) = 2.72$, $p = .0237$, while cTBS and iTBS were not: *ps*> 0.1. Only the cTBS group showed a trend toward significance I-O, $t(9) = -2.24$, $p = .0518$, while sham and iTBS did not: *ps*> 0.1. Unstandardized effect sizes were; sham: 0.227, iTBS: 0.0595, and cTBS: -0.203. Figure 2.3 shows GABA+ tissue-corrected concentration across all time points.

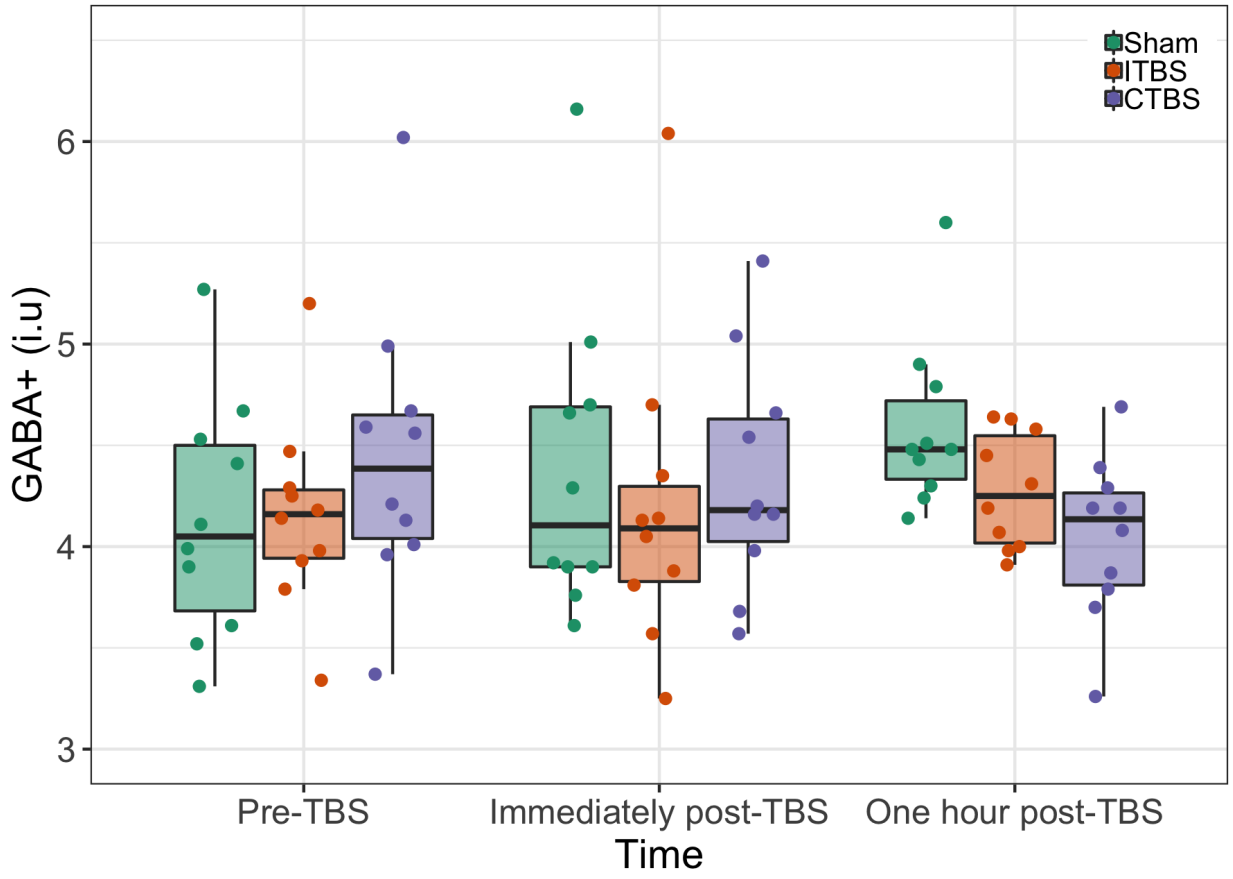

Figure 2.3. GABA+ Tissue-Corrected Concentration Across all Time Points. There are three times points: pre-TBS, immediately post-TBS and one hour post-TBS for the three stimulation conditions: sham, iTBS and cTBS. Error bars represent ± SEM.

Glx Concentration

There was no significant main effect of Time $F(2,58) = 0.826$, $p = .412$, Condition $F(2,58) = -0.526$, $p = .601$ nor an interaction between Time and Condition, $F(2,58) = -0.574$, $p =$

.568. Across all time points there was no significant effect for any of the groups; sham: $t(19) = 0.343$, $p = .735$, iTBS: $t(19) = 1.405$, $p = .176$, nor cTBS: $t(19) = -0.51$, $p = .616$. All three groups showed no significant effect from B-I nor B-O: *ps*> 0.1. Only the iTBS group showed a trend toward significance I-O, $t(9) = 1.95$, $p = .0666$, while sham and cTBS did not: *ps*> 0.1. Unstandardized effect sizes were; sham: 0.0595, iTBS: 0.276, and cTBS: -0.0890. Figure 2.4 shows Glx tissue-corrected concentration across all time points.

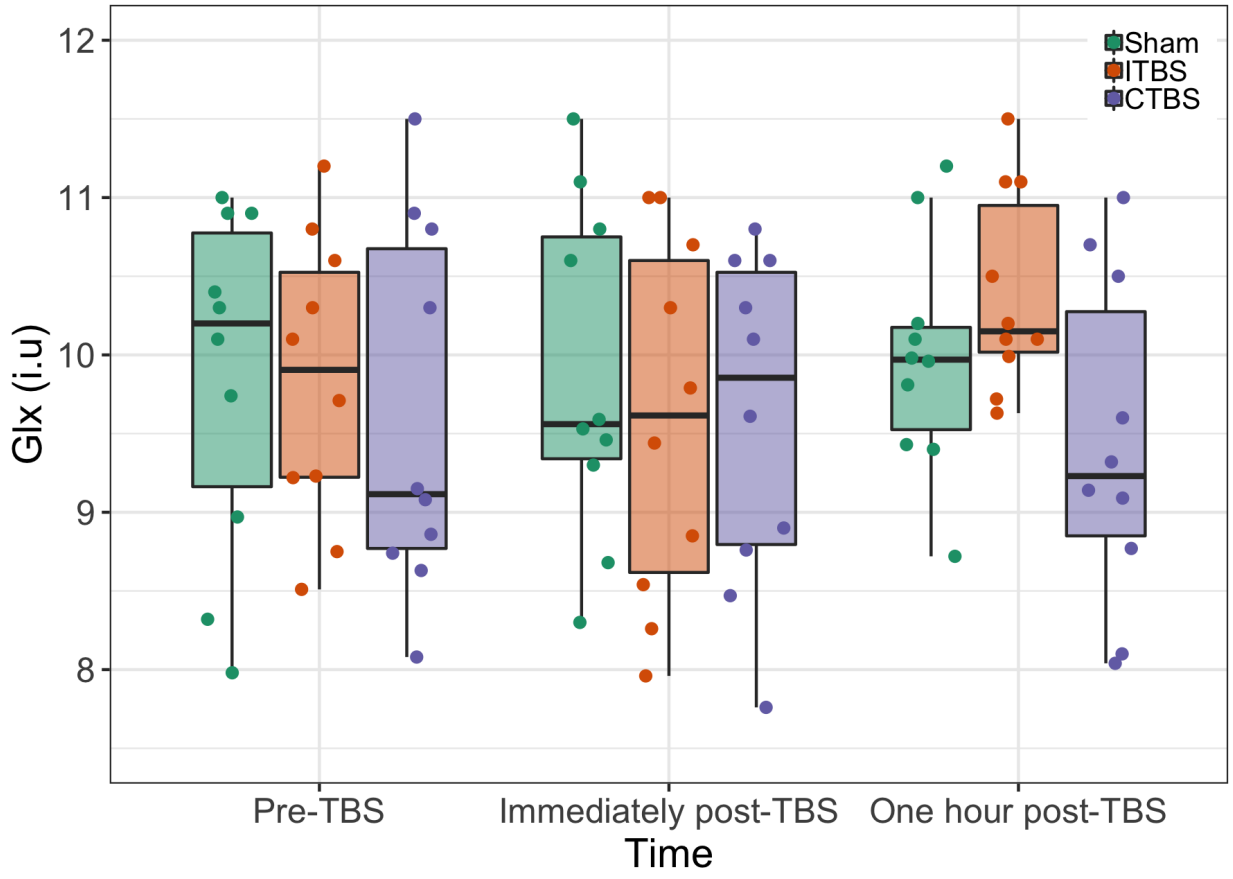

Figure 2.4. Glx Tissue-Corrected Concentration Across all Time Points. There are three times points: pre-TBS, immediately post-TBS and one hour post-TBS for the three stimulation conditions: sham, iTBS and cTBS. Error bars represent ± SEM.

GABA+ and Glx Composite Ratio Concentration

There was a trend towards significance for Condition $F(2,58) = -1.85$, $p = .0685$ and a significant effect of Time $F(2,58) = -2.28$, $p = .0263$ and a significant interaction between Time and Condition $F(2,58) = 2.50$, $p = .0152$. Across all time points only sham showed a trend towards significance: $t(19) = -1.89$, $p = .0737$; while iTBS: $t(19) = 0.609$, $p = .550$ and cTBS: $t(19) = 1.41$, $p = .173$ did not. All three groups showed no significant effect of Time from B-I, B-O nor I-O: *ps*> 0.1. Unstandardized effects sizes were; sham: -0.0197, iTBS: 0.00521, and cTBS: 0.0162. Figure 2.5 shows GABA+ and Glx composite ratio concentration tissue-corrected across all time points.

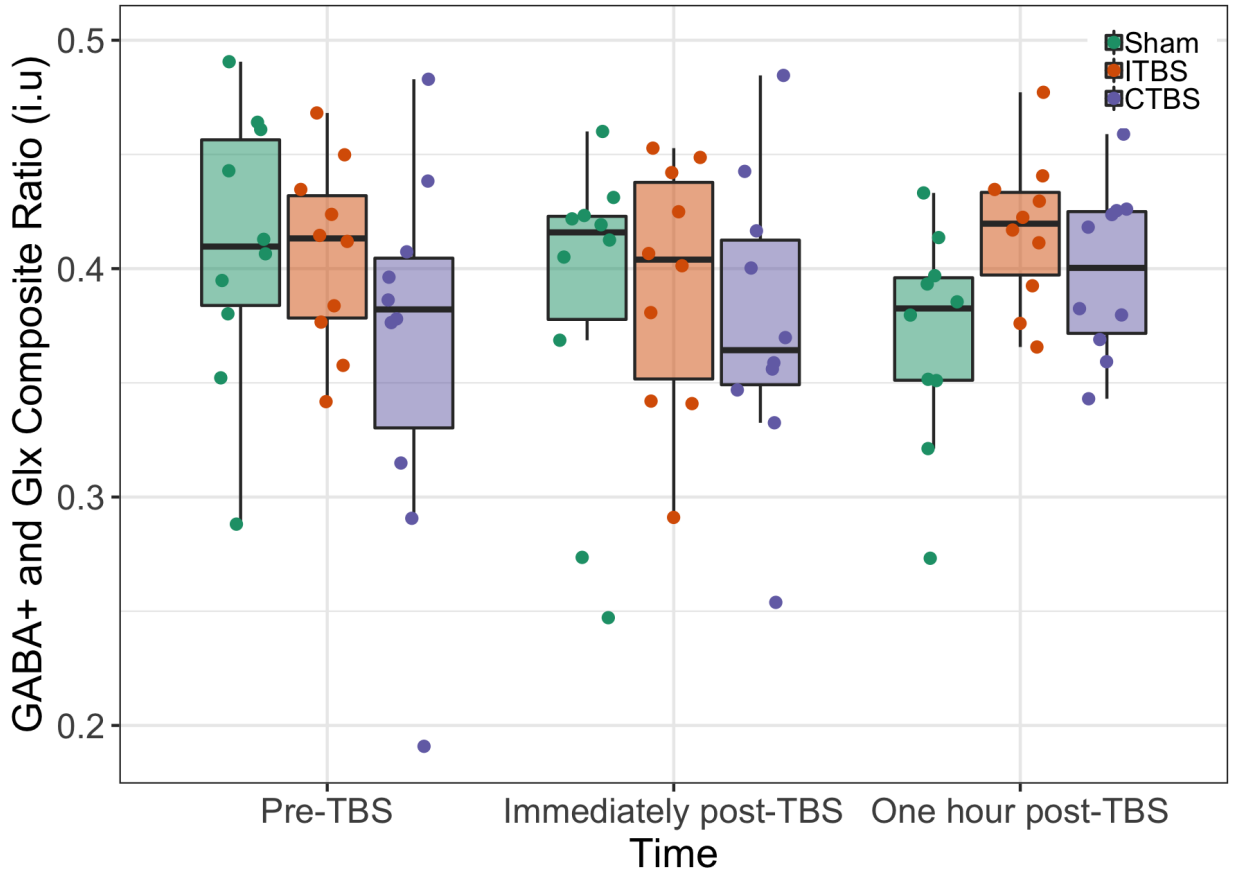

Figure 2.5. GABA+ and Glx Composite Ratio Tissue-Corrected Concentration Across all Time Points. There are three time: pre-TBS, immediately post-TBS and one hour post-TBS for the three stimulation conditions: sham, iTBS and cTBS. Error bars represent ± SEM.

Phosphene Threshold

There was no significant effect of Time $F(2,29) = 1.01$, $p = .323$, Condition $F(2,29) = 0.9$, $p = .375$ nor interaction between Time and Condition $F(2,29) = -0.961$, $p = .345$ for PT. Across all time points only iTBS showed a trend towards significance with its effect on PT: $t(9) = 2.043$, $p = .0714$; while sham: $t(9) = 0.294$, $p = .776$ and cTBS $t(9) = -0.981$, $p = .352$ did not.

Correlation Between GABA+, Glx Concentration and Phosphene Threshold

There was a was a trend towards a positive correlation between pre-TBS GABA+ and Glx concentrations, $\tau = 0.246$, $p = .0582$. PT was not significantly correlated with pre-TBS measures of GABA+ concentration, $\tau = 0.109$, $p = .401$ nor Glx concentration, $\tau = - 0.0375$, $p = .775$

Voxel Fraction

There was no significant effect of Time $F(2,58) = 0.714$, $p = .478$, Condition $F(2,58) = 0.768$, $p = .449$ nor interaction between Time and Condition $F(2,58) = -0.681$, $p = .499$ for GM. There was no significant effect of Time $F(2,58) = -0.371$, $p = .712$, Condition $F(2,58) = -0.242$, $p = .811$ nor interaction between Time and Condition $F(2,58) = -0.808$, $p = .422$ for CSF. There was no significant effect of Time $F(2,58) = -0.349$, $p = .728$, Condition $F(2,58) = -1.03$, $p = .312$ nor interaction between Time and Condition $F(2,58) = 1.33$, $p = .189$ for WM.

Discussion

The present study provides the first set of data from a single session of cTBS, iTBS or sham to the occipital cortex in healthy humans on GABA+ and Glx. The main findings were that a single session of TBS does not alter GABA+ or Glx concentrations at the stimulation site.

Statistical significant or a trend toward effects shown at diverse time-points in the sham and cTBS group, respectively, with GABA+, a trend across all time points in the sham group with the composite ratio and in the iTBS group with Glx, I-O is likely due to drift and not from legitimate changes to biomarkers. The size of the effects was small and did not follow any systematic pattern. Others have observed drift in baseline measures of cortical excitability in the occipital cortex resulting in a statistically significant result in their control group as well (Herpich, Contò, van Koningsbruggen, & Batteli, 2018). My experiment shows that a single session of cTBS or iTBS to the visual cortex has little ramification, if any, on brain biomarkers and our hypotheses were not supported, despite numerous studies showing its effects on visual cognitive behaviour. As a result, we suggest that a single session of TBS can be used safely in a laboratory setting to examine visual cognition without altering brain biomarkers.

Theta Burst Stimulation on GABA+ and Glx Concentrations

Theta Burst Stimulation and Behaviour

TBS applied to the occipital cortex has successfully shown the ability to modulate visual-cognitive behaviour. When TBS is applied to the LO cortex, cTBS influences the ability to judge object size (Chiou & Ralph, 2016). When applied to the right occipital place area in the transverse occipital sulcus, TBS impairs the spatial memory for boundary-tethered but not landmark-tethered objects (Julian, Ryan, Hamilton, & Epstein, 2016). The behavioural effects of TBS are further shown to modulate the BOLD signal as measured by fMRI. For example, a modified cTBS protocol to the right occipital face area in the inferior occipital gyrus reduces the neural response to both static and dynamic faces in the fusiform gyrus (Pitcher, Duchaine, & Walsh, 2014).

TBS also modulates visual-cognitive abilities when applied to visual regions beyond the occipital cortex. CTBS to the right pSTS impairs performance accuracy of facial expression recognition (Sliwinska, Elson, & Pitcher, 2019) and reduces the neural response to faces but not bodies or objects in right hemisphere pSTS, anterior STS and amygdala (Pitcher, Japee, Rauth & Ungerleider, 2017). As well, a modified cTBS protocol applied in the prefrontal cortex improves visual memory accuracy (Carbajal, O'Neil, Palumbo, Voss, & Ryals, 2019).

Although TBS is effective in altering behaviour in the visual domain, my study demonstrates that the effect does not appear to be powerful enough to cause modifications to neural biomarkers. Behavioural experiments often only generate transient effects which do not always correlate with changes in physiological measures. 1 Hz rTMS to the occipital cortex can alter behaviour by impairing working memory in visual coding as measured by the digit span backwards test (Hilbert et al., 2019), but it has not shown the capacity to modulate neurotransmitter levels in this same area using the same stimulation protocol (Rafique & Steeves, 2018). In addition, rTMS to the DLPFC can modify recognition memory performance (Turriziani et al., 2012) but once again has not been shown to significantly alter its metabolites in this region compared to a sham group (Bridges et al., 2018). In general, significant behavioural effects may not be related to significant changes in biomarker concentrations with a single TMS application. Since neurometabolites are often used as an indicator for large, long-lasting effects, it is possible that a single session of TBS only results in immediate but transient weak alterations to neurometabolites that are not perceptible on our timeline but are apparent in immediate behavioural measures.

Furthermore, the link between behavioural and physiological measures is not always straightforward. For example, an improvement in motor learning performance surprisingly has

been shown to reduce activity in motor networks (Pascual-Leone et al., 1994). One might expect a behavioural improvement to correlate with increased brain activation; however, an unexpected reduction in activity shows the discrepancy with the generalizability of behavioural effects with physiological outcomes (Moisa, Polania, Grueschow, & Ruff, 2016). It could be that instead, a reduction in brain activation occurs as efficiency in processing is gained. Overall, behavioural and physiological results may not necessarily always correspond. There is increasing discussion in the field about the mechanisms of action of TBS and the ability to cause long-lasting physiological changes (Polania, Nitsche, & Ruff, 2018). My experiment provides insight into neural processes associated with TBS.

Theta Burst Stimulation and Brain Biomarkers

By conducting a carefully designed study, this experiment provides substantial evidence that a single session of TBS does not cause appreciable changes to brain biomarker concentrations. TBS can thus be safely used in experimental settings as a tool to assess short-term behavioural changes. This experiment applied strict controls in order to provide a reliable account of GABA+ and Glx concentrations after TBS. Only one other study has looked at GABA+ after cTBS in the V1, and they found that levels significantly rose (Allen et al., 2014). However, this study had a number of potential limitations, including liberal exclusion criteria, the use of a round coil rather than a figure-of-eight coil, and the administration of a visual-attentional task during MRS. Our experiment is the first experiment to compare GABA+ and Glx concentrations following cTBS, iTBS and sham to the occipital cortex in healthy humans, not only immediately-post TBS but also one hour later.

There are a number of controllable factors that can influence metabolite levels. The present study employed extensive participant screening in an attempt to mitigate such factors.

GABA and glutamate levels are impacted in psychological (Levinson et al., 2010; Rowland et al., 2013; Schür et al., 2016), neurological (Bai et al., 2015), neurodevelopmental disorders (Drenthen et al., 2016; Bollmann et al., 2015), and after head trauma (Kim et al., 2019). This has been shown with or without medication in patients (Goddard et al., 2004; Bhagwagar et al., 2007), and with medication in healthy individuals (Stell, Brickley, Tang, Farrant, & Mody, 2003). Metabolite levels or TMS effects can be impacted by female contraceptives (Kaore et al., 2012; Smith, Adams, Schmidt, Rubinow, & Wassermann, 2002), frequent migraines (Bohotin et al., 2002; Russo et al., 2005), history of drug/substance abuse or recent drug/substance usage, including smoking and drinking (Ke et al., 2004; Epperson et al., 2005; Lobo & Harris, 2008), age (Gao et al., 2013), and a female's menstrual cycle (Epperson et al., 2002; Harada, Kubo, Nose, Nishitani, & Matsuda, 2011). Diurnal effects such as time of day (Ridding & Ziemann, 2010; Sale, Ridding, & Nordstrom, 2007) and quantity of sleep also modulate NIBS effects (Gorgoni et al., 2015). The magnitude of the initial burst of cortisol observed in an awakening response is associated with the magnitude of the neuroplastic feedback to cTBS (Clow et al., 2014). This paper controlled for all of the above potential confounding variables in an attempt to minimize controllable metabolite fluctuations. To control for external factors, we tested on the same scanner with no visual or attentional cues during MRS and assessed participants only approximately a week later. With the possibility of so many confounding variables, it is essential to have strict exclusion criteria to ensure that results can be attributed solely to NIBS.

We also ensured proper target stimulation by the use of neuronavigation and a figure-of-eight coil, which has more precise focal stimulation than the majority of other coils. Coil placement and direction of current can impact which intracortical networks are activated (Hamada et al., 2012), potentially resulting in a variety of responses. When late I-wave circuits

are activated, responses are more likely to occur in the expected direction (Hamada et al., 2012). In order for this to occur, in the motor cortex, the current needs to be anterior-posterior, and the coil held tangent to the head (Di Lazzaro et al., 2001). As well, the amount of induced electrical field in TBS is different in each retinotopically defined region of the visual cortex. Phosphenes are generated more easily when the coil is placed in the posterior-anterior direction (Kammer et al. 2001b; Salminen-Vaparanta et al. 2012b; Thielscher, Reichenbach, Ugurbil, & Uludag, 2010). We thus, applied our stimulation in the posterior-anterior direction. With Brainsight, we were able to monitor, in real-time, the location and orientation of the TMS coil on the targeted stimulation site. In addition, by using a figure-of-eight coil that produces focal stimulation, it is more likely that only the selected target received stimulation with limited diffuse effects.

Our meticulously designed experiment demonstrates reliably that brain neurometabolites do not fluctuate after one session of TBS to the V1. Although metabolites may be altered in other areas of the brain such as the motor cortex following TBS (Stagg et al., 2009), the lack of physiological evidence of the effect of TBS in the occipital cortex specifically, raises questions about its mechanisms of action in other regions of the brain. It cannot be assumed that protocols known to enhance or reduce excitability in a well-studied region will have the same physiological effect when applied to other brain areas (Polania et al., 2018). In fact, low-frequency rTMS in the frontal cortex decreases inhibition and disrupts feedforward and feedback connections, while the opposite effect occurs in the occipital cortex (Castrillon et al., 2020). This demonstrates that a difference in the cerebral organization, cell type and connectivity may alter the type of response produced from TBS. The functional connectivity of retinotopic visual areas displays dense local functional coupling that is organized respecting topography (Yeo et al., 2011). Both visual and somatomotor systems are organized into separate hierarchies

(Ungerleider & Desimone, 1986; Van Essen, Anderson, & Felleman, 1992). Since there are apparent differences between the motor and visual cortex, the mechanisms from TBS may differ throughout the brain, impacting their effects on metabolites. Our experiment was rigorously conducted, and despite previous evidence that TBS to the occipital cortex can alter visual behaviour, a single session does not seem to create lasting changes in brain biomarker concentrations.

Relationship between GABA+, Glx and Phosphene Threshold

There was no significant relationship between PT and baseline-GABA+ or Glx values and only a trend towards a relationship between iTBS on PT. It is likely that a single session of TBS is not sufficient to cause substantial changes to PT. The lack of a significant relationship between PT and baseline-GABA+ or Glx values could be due to the variability in PT and its ability to detect cortical excitability accurately. TMS to the V1 potentially activates back-projecting fibres arising from extrastriate areas and target neurons within the V1 (Pascual-Leone & Walsh, 2001). Phosphenes represent a direct and intensity-dependent physiological response to stimulation and can, therefore, be utilized as an independent and robust measure of excitability (Elkin-Frankston et al., 2011; Cervetto et al., 2007). However, although single-pulse stimulation is generally the most common procedure used to measure PT, mixed results have been found with the induced perception of a phosphene. This could be due to the efficiency of single-pulse stimulation or subjective understanding of the protocol.

PT positively correlates with active-MT when properly applied (Deblieck et al., 2008). The hurdle with PT measurement, however, is quantifying a response. PT involves some aspects of subjective perceptual awareness of a subtle visual effect. MT can be more easily objectively measured by observing the finger's physical movement, thereby removing potential human error

with personalized reporting that may occur in a PT measurement. Phosphenes can differ across individuals and often require extensive training for participants to fully appreciate what to expect when perceiving a phosphene.

Furthermore, the subjectivity of a PT measurement could have affected the precision needed to determine the stimulation intensity for TBS in visual cortices in order to generate a sufficient neural response. PT varied drastically per participant in our study, causing a wide range of intensities to be used. Forty percent had a PT below 60% intensity, 30% had a PT below 70% intensity, 20% had a PT below 80% intensity, 10% had a PT above 80% in the baseline session. In the M1, sub-MT paired-pulse TMS suppressed the M1 excitability by activating intracortical inhibitory circuits, while supra-MT intensity, facilitated a response (Nakamura, Kitagawa, Kawaguchi, & Tsuji, 1997). As well, supra-MT inhibitory rTMS to the M1 does not suppress MEPs as would be expected (Chen et al.,1997). Thus, an accurate intensity is needed to ensure a proper response to stimulation. The lack of correlation between GABA+, Glx, and PT may due to the subjective reporting of PT. This could have affected the efficacy of TBS, which in turn would affect GABA+ and Glx measures. However, on average, the PT for both sessions was 65% intensity, indicating that although there was variability in response, it appeared to be stable across time. This can also be seen with a lack of significant change in the cTBS and sham group and only a trend in the iTBS group.

Alternatively, perhaps, change in cortical excitability may not directly relate to changes in inhibitory and excitatory neurotransmitters measured by MRS. Since the mechanisms of phosphene induction from stimulation are still unclear, if they occur via GABA receptor activity, rather than extracellularly, neither MRS measured GABA+ or glutamate levels would be expected to be associated directly with PT (visual cortical excitability). Previous reports have

shown the use of a $GABA_A$ agonist can increase PT, i.e., it can reduce cortical hyper-excitability in the visual cortex in migraine patients with aura (Mulleners, Chronicle, Vredeveld, & Koehler, 2002). This displays the possibility that MRS-measured metabolites and cortical excitability may never, in fact, correlate due to the outcomes occurring in different cellular locations. The relationship between GABA+, Glx and PT is still under debate, as well as the use of a PT compared to a standard stimulator intensity for all participants. While we conducted the PT measurement as carefully as possible, it is feasible that variation in subjective reporting could adversely affect its measurement. However, it is more likely that a single session of TBS is not sufficiently strong to cause long-term changes and thus does not affect brain biomarkers or PT. This is especially true since PT was performed nearly two hours post-TBS for the second session.

Alternative Explanation of Significant Effects

An Argument for the Validity of Significant Effects

One could argue that our significant or trend toward effects may, in fact, be representative of valid changes. The use of a sham coil provides a placebo-controlled condition. It is likely that the change in GABA+ concentration over time in the sham group is due to drift effects (Herpich et al., 2018). However, we cannot eliminate the possibility that a small change may have occurred since the sham coil does, in fact, administer a minimal amount of stimulation (Duecker & Sack, 2015). The coil isolates the impact of ancillary effects, including scalp stimulation and auditory activation (Duecker & Sack, 2015). An ideal sham coil would mimic the stimulation feeling with no concomitant electric field (E-field) effects. The Magstim sham coil is similar to the active coil, but with a metal shield that attenuates the current, however, not all stimulation can be blocked. In the active coil, the center of the two coils has the most potent

stimulation. Whereas in the sham coil, there is a nearly zero E-field under the center of the coils, however, the peak E-field forms a diffuse oval 3-7 cm from the center, displaying stronger stimulation in the periphery (Smith & Peterchev, 2018). Subthreshold E-fields of less than 10% of the threshold can still evoke action potentials in various regions of the brain such as the motor and frontal cortex (Opitz et al., 2015; Volkow et al., 2010; Rohan et al., 2014; Rohan et al., 2017). The Magstim sham coil induces peak E-fields corresponding to 25.3% of its respective active values (Smith & Peterchev, 2018). Considering that the voxel is significantly larger than the target of stimulation, and the sham coil produces E-fields of above 10%, perhaps diffuse effects generated changes in GABA+ and Glx in the outer limits of the voxel.

The use of a sham coil has shown changes in electroencephalogram dynamics (Opitz et al., 2015), clinically, by reducing symptoms of auditory hallucinations (Koops et al., 2016), functionally through BOLD signals (Baeken, Wu, & van Heeringen, 2019) and even metabolically (Bridges et al., 2018). It is unclear whether this is due to stimulation from the sham coil itself or other confounding factors such as carry-over or placebo effects. Sham-accelerated iTBS performed in the prefrontal cortex significantly influenced brain perfusion using arterial spin labelling and suicidal thoughts. No change was detected with the active coil, and this was irrespective of depression symptoms (Baeken et al., 2019). Even with sham iTBS, where the coil was flipped from the scalp to a 90-degree angle, there was a significant increase in functional near-infrared spectroscopy during a phonological task in the inferior frontal gyrus with no effect in the active condition once again (Deppermann et al., 2014). Although placebo effects may still be the cause, it is of interest that no change occurred in the active groups, similar to our study. If a placebo effect can change the brain's physiological nature, this may also explain why we see changes in metabolites in our control group. A single session of 1 Hz rTMS to the DLPFC has, in

fact, shown metabolic correlations with total NAA/Cr and Glx/Cr for the sham group only and Choline/Cr and Glx/Cr for both sham and active condition using a sham coil (Bridges et al., 2018). Additionally, increased frontal-parietal phase coupling in the alpha-beta bands concomitant with an increase in the amplitude of the parietal P50-N70 complex has been shown in both the active and sham group (Opitz et al., 2015). However, it is essential to note that all of these studies were done in the frontal region of the brain, where cognitive thoughts and future mental expectations may have affected the legitimacy of the physiological response. With significant changes possible even with minimal E-field, we cannot discount the fact that it is possible that the control group was subject to weak TMS effects.

Secondly, it may also be conceivable that iTBS increased PT over time in our experiment, since cTBS has shown the possibility to significantly raise PT by 10% immediately after stimulation (Franca et al., 2006). Although iTBS did not significantly change PT in that same experiment, we can see the potential for TBS mechanisms to alter cortical excitability.

Thirdly, pre-TBS GABA+ and Glx most likely do, in fact, correlate with each other. However, it would be sensible for GABA+ and Glx to negatively correlate rather than positively, since they have an inverse relationship, one being inhibitory and the other excitatory. In addition, it is possible that Glx only increases immediately post- to one hour post-iTBS, and cTBS decreases GABA+ across all time points with I-O driving the effect if our "baseline" is not an accurate baseline. This could be due to potential weekly metabolite fluctuations. Some may argue that the one hour post-stimulation may, in fact, represent a more appropriate baseline demonstrating the immediately post-stimulation time-point to be indicative of a genuine change from TBS. Neurometabolites can fluctuate over time; thus, a baseline measure taken approximately a week prior may be less accurate than one taken on the same day, especially if

effects were expected to terminate prior to the one hour mark. This could explain why a trend toward effects is only seen on the same day, rather than from baseline to immediately after stimulation.

An Argument Against the Validity of Significant Effects

Despite the fact that some may contend that our significant or trend toward effects are valid it is more than likely that they are due to variability in GABA+ measures from external and internal factors or drift, rather than from a relevant effect. Firstly, in the sham group, many other studies have used the sham coil and have found no biological differences (Vidal-Piñeiro et al., 2015; Iwabuchi et al., 2017). Although significant changes can be seen with the sham coil, they are often justified as carry-over and placebo effects, or noise due to inter-participant variability. To the best of our knowledge, no study has ever suggested that an effect in their control group is due to the stimulation admitted from the sham coil. In our study, it is more than likely that significant effects are associated with drift rather than minimal stimulation from the sham coil. Secondly, if iTBS were to modulate PT, it would be logical that PT would decrease over time since iTBS is an excitatory measure. The threshold to modulate PT should become reduced as iTBS would increase the effectiveness of synaptic connections (Franca et al., 2006). Thirdly, with this logic, it would be sensible that an effect with neurometabolites would occur immediately after iTBS and that specifically, Glx levels would rise (Maddock et al., 2018). Although we do, in fact, see Glx rise, this is not shown relative to the baseline. For cTBS, we would expect that GABA+ would rise, and there would be a change from B-I, considering GABA+ is an inhibitory metabolite and is thought to produce LTD-like effects (Stagg, 2014). However, neither of these phenomena occurred. There was no significant effect B-I and only a trend towards a significant change, which was a decrease I-O.

If we revisit the argument that perhaps there is too much variability over several days and the one hour mark is more representative of an accurate baseline, it would then be sensible that GABA+ levels would decrease after cTBS I-O, as seen in our experiment. This would suggest that cTBS caused GABA+ levels to rise initially and then return to the regular "baseline." However, this hypothesis would not be supported for iTBS and Glx as it would be expected for Glx levels to drop under this notion. Thus, it is apparent that the lack of systematic trends with our results leads to the conclusion that a single session does not cause neurometabolic changes. It is sensible to assume that metabolic effects should have occurred B-I, which is not apparent in any group. Although some may argue that perhaps our baseline is not a true "baseline" and the one hour post-TBS is more representative since it occurs on the same day, this still does not show a clear trend in our results with GABA+, Glx, PT appearing to decrease/increase in a random fashion. As well, although the sham coil does produce minimal stimulation, it is highly unlikely it was sufficient to cause a relevant change in biomarkers. It is probable that our statistical or trend toward effects is due to drift, and a single session of TBS does not affect brain biomarkers.

Overall, a single session of TBS does not appear to lead to long-term changes in neurometabolite concentrations in the occipital cortex. Although prior experiments have found changes in neurotransmitters after a single session (Stagg et al., 2009; Iwabuchi et al., 2017), it is clear that TBS mechanisms are not homogenous across the cortex. Statistically significant or a trend in differences shown at diverse time-points in the sham and cTBS group, respectively, with GABA+, a trend across all time points in the sham group with the composite ratio and in the iTBS group with Glx, I-O is likely due to drift and not from legitimate changes to biomarkers. The rigour of this experiment provides reliable and valid results in the fact that brain biomarkers

and cortical excitability do not change after a single session, demonstrating its ability to be safely used in an experimental setting for short-term behavioural studies.

Chapter 3:

General Discussion

Summary

In this thesis, we assessed how a single session of TBS would affect healthy human GABA+ and Glx concentrations in the visual cortex. We tested three different stimulation groups, cTBS, a purportedly "inhibitory" stimulation paradigm, iTBS, a purportedly "excitatory" stimulation paradigm and a control group. We took measures at three different time points: 1) baseline, 2) immediately post-stimulation, and 3) one hour post-stimulation. We assessed how each stimulation condition affected GABA+, Glx, the relationship between GABA+ and Glx and PT. Although some statistically significant differences and trends were found, the effect sizes were small and did not follow any systematic pattern. All appear to resemble drift rather than legitimate stimulation effects. This is similar to what we found previously in our laboratory, where a single session of 1 Hz rTMS did not significantly alter GABA+ or Glx. Considering that both TBS and rTMS operate on similar neural mechanisms, it is not surprising to have comparable findings. A single session of TBS has no lasting effect on GABA+ or Glx concentrations at the stimulation site.

Limitations

Sample Size

There are a few potential limitations to this study. Firstly, the sample size used in our experiment may limit explanatory power. While our sample size is comparable to that used in other studies with a similar experimental design (Vidal-Piñeiro et al., 2015; Michael et al., 2003; Stagg et al., 2009), a larger sample would help to mitigate potential noise from participant variability. Some studies have used a smaller total sample size than ours, but since they had a within-participant design, their within-group sample size was larger (Allen et al., 2014; Iwabuchi et al., 2017). Ideally, a larger sample size would always be better as low statistical power

undermines the purpose of scientific research and reduces the chance of detecting an actual effect (Button et al., 2013). Often, with small sample sizes, lack of power can not only lead to significant effects to go undetected but also the reverse (Button et al., 2013). A significant p-value that occurs with small effect sizes indicates the possibility of a type one error (Lyu, Xu, Zhao, Zuo, & Hu, 2020). This could offer a potential explanation for some of the small, but significant effects we found with our study, likely due to drift. Since effect sizes are often not always reported, it can be difficult to compare findings with other experiments. It is also essential to note the editorial preference for "positive studies" (Mlinaric, Horvat, & Smolcic, 2017). It cannot be ignored that many TBS experiments show variability in individual participant results but emphasize the positive effect that is found. Variability, small sample sizes, small effects sizes, but significant p-values can point to misleading conclusions that cannot be discounted in other studies. Our study highlights the importance of meaningful effects, reporting effect sizes even if unstandardized and the need for randomized controlled studies.

Variability and Reproducibility in Theta Burst Stimulation

Secondly, mechanisms of action from a single session of TBS show mixed results in terms of variability and reproducibility. In the motor cortex, when MEPs are re-tested after several months, cTBS modulation shows limited reproducibility and inter- and intra-participant variability is considerable (Vernet et al., 2014). Even across different studies, the timing of MEP reliability is different. Some indicate that MEPs are the most reliable 5 min after stimulation across sessions (Vernet et al., 2014), while others say that it is the most reliable after 50 min (Jannati et al., 2019). As well, some individuals do not always respond to stimulation (Brownjohn, Reynolds, Matheson, Fox, & Shemmell, 2014; Vidal-Piñeiro et al., 2015), and the direction of the effect is not always the same (Vernet et al., 2014). CTBS has been shown to lead

to facilitatory effects when resting instead of active-MT is used for intensity (Gentner, Wankerl, Reinsberger, Zeller, & Classen, 2008). Similar conflicting findings have been shown with iTBS as well (López-Alonso et al., 2014; Franca et al., 2006).

It is important to note that many studies do find the "expected" significant effect in the motor, frontal, or visual cortex with cTBS (Stagg et al., 2009; Allen et al., 2014) or iTBS (Hinder et al., 2014; Brownjohn et al., 2014), indicating adverse reports on effectiveness. However, the significant intra- (Vallence et al., 2015; Vernet et al., 2014; Jannati et al., 2019) and inter-participant (Hamada et al., 2012; Goldsworthy, Müller-Dahlhaus, Ridding, & Ziemann, 2014; López-Alonso et al., 2014; Vallence et al., 2015; Guerra et al., 2017; Heidegger et al., 2017; Jannati, Block, Oberman, Rotenberg, & Pascual-Leone, 2017) variability in a single session of TBS is precarious and needs to be acknowledged. To mitigate some of these potential issues, our study included extensive constraints to minimize variability between and within individuals as much as possible in order to yield more reliable results.

Experimental Design

Thirdly, another potential limitation was our between-participant model. There are pros and cons to every study design. It would have been ideal to conduct a within-participant experiment where the same individuals would have undergone each TBS condition. This would limit inter-participant variability. However, due to strict exclusion criteria and the number of sessions needed, this would have been difficult to achieve. If a within-participant protocol was used, participants would have had to undergo a minimum of four sessions, which would have been arduous to schedule as each session must be spaced by at least five days to eliminate potential residual TMS effects. As a result, complications with scheduling can result in participant attrition. Furthermore, it is possible that additional spaced out sessions can increase

the likeliness of a change in response to questionnaires or create added noise due to potential natural neurotransmitter fluctuations. Even if different participants were placed in each active condition and served as their control, it would still be challenging to ensure consistency with questionnaire responses and commitment for more than two sessions.

Participants were not tested in the active condition on the same day as the baseline due to the potential residual effects of PT measurement. Even if the PT was performed on a separate day, TBS could not take place directly after baseline sessions due to the lengthy set-up time for neuronavigation. This could increase the potential for motion artifact due to fatigue and boredom (Power et al., 2014). Overall, it would be ideal to have participants serve as their own control and reduce the number of sessions. However, there is a trade-off between these limitations; if sessions are reduced, added variability occurs when participants do not serve as their own control. However, individuals serving as their own control will increase the required number of sessions, still leading to additional variation.

Mescher-Garwood Point Resolved Spectroscopy

Fourthly, the limited sensitivity of MEGA-PRESS may explain the lack of significant TBS effects on Glx concentration. Although MEGA-PRESS is the most used sequence for the analysis of GABA+ and Glx in MRS (Maddock et al., 2018; Mullins et al., 2014), a newer OFF-PRESS sequence has shown to provide more reliable estimates of Glx while still providing consistent results with GABA+ (Maddock et al., 2018). Additionally, recent positive developments have shown the potential of acquiring GABA without macromolecule contamination (Mikkelsen et al., 2019). However, we chose to use the MEGA-PRESS sequence when we started acquiring data as both other methods were too preliminary and had yet to be shown to be reliable.

As in any MRI sequence, MEGA-PRESS is susceptible to instability/motion artifact. However, to mitigate this concern, the difference editing approach is acquired in an interleaved fashion and post-processing phase- and frequency-corrections are applied to fix potential instabilities (Mullins et al., 2014). The size of the voxel can also limit results. If a voxel is too large, it can be challenging to constrain to a specific localized region of the brain. Effects may also be bleached out by unaffected metabolite concentrations and CSF (Porges et al., 2017). It would be ideal to have a smaller voxel for better temporal resolution; however, this can limit the ability to measure diffuse effects and can limit signal (Mullins et al., 2014). Reduction in voxel size also increases acquisition time, which raises scanner fees. We chose a voxel size that attempted to mitigate the trade-off between spatial and temporal resolution.

Variability does exist in MRS measures, and this could be due to external and internal variability among and within-participants. On average, when GABA+ measures are repeated over separate sessions for up to eight days in healthy males, the coefficient of variation (CV) ranges from 3.5-21% (Evans, McGonigle, & Edden, 2010; Stephenson et al., 2011; Wijtenburg, Rowland, Edden, & Barker, 2013) and similarly, within-participant CV measurements range from 7-13% (Bogner et al., 2011; Near et al., 2013). This indicates that small changes in metabolite levels could be due to inherent natural variability rather than stimulation per se. Water referenced GABA+ measurements corrected for tissue contamination, similar to what we measured in the present study, found the cohort-wide CV to be 17%, and the mean within-site CV to be 10% (Mikkelsen et al., 2019). Specifically, in the occipital cortex, CV with GABA+ during a single day is 9% across-participants and 6.5% within-participants (Evans et al., 2010); CV separated by a few weeks is 13-17% across and 10-12% within-participants (Bogner et al., 2011) and CV across several months is 4.3% within-participant (Near, Ho, Sandberg,

Kumaragamage, & Blicher, 2014). Inherent variability exists across different areas of the brain. For example, over several days, within-participants' CV in the M1 is 5.6% and 21% and in the DLPFC is 7.2% and 27.9% for Glx and GABA+, respectively (Yasen, Smith, & Christie, 2017). Variance in neurotransmitter measurement can create noise within the data, impacting the interpretability of results. Different GABA+ and Glx values are shown with MRS even when scans are done consecutively. Variance from external hardware can occur even when using the same scanner and same sequence due to transmit grain calibrations, leading to potential imperfect inversions from editing pulses (Edden & Barker, 2007). Although fluctuations may still occur within the scanner, this is uncontrollable and would affect every experiment more or less the same way. Our experiment mitigated any erroneous internal and external variability by using the same scanner for each session, carefully controlling for voxel placement so that it was almost precisely the same in each session, preprocessing data and removing participants with high motion/fit error. We also chose a sample size large enough that if a real effect with our experiment were to exist, it would be shown.

Suitability of GABA+ and Glx as a Measure

Lastly, GABA+ and Glx may not be ideal to measure the effect of TBS. While TBS is thought to potentially induce intracortical excitability and inhibition, the underlying neurochemical substrate for this effect remains unclear. MRS-measured GABA+ and TMS-affected GABA may be different. MRS-derived GABA+ is associated with extra-synaptic GABA rather than synaptic $GABA_A$ or $GABA_B$ activity (Stagg et al., 2011a). In the M1, TMS-derived changes occurred in GABA receptors rather than extracellularly ($GABA_A$ mediated by SICI and $GABA_B$ mediated by LICI) and did not relate to changes in MRS-measured GABA+

(Stagg et al., 2011b). However, GABA+ has shown the potential to be modulated by other TMS protocols (Stagg et al., 2009).

As well, little is known about MRS-derived Glx and TMS-affected glutamate. The majority of glutamate in the brain is involved in non-synaptic metabolic roles, and therefore the potential link between local glutamate levels and local excitability is less clear (Stagg et al., 2011b; Stagg, 2014). The majority of glutamate synaptic strength occurs via receptor sensitivity and density, which does not appear to be possible to detect with MRS (Taylor, Tiangga, Mhuircheartaigh, & Cowen, 2012; Stagg, 2014). However, promising results have shown changes in Glx in the motor cortex following TBS (Stagg et al., 2011b; Tremblay et al., 2013; Maddock et al., 2018). Glx levels correlate positively with the TMS-induced silent period duration (Tremblay et al., 2013), and MRS-measured glutamatergic metabolites are also associated with visual plasticity (Wijtenburg et al., 2017). As a result, the reliability of GABA+ and Glx as a direct measure of LTD- and LTP- like TMS effects is still under debate (Mason et al., 2001; Stagg, 2014). It is clear that more research is needed to understand TBS mechanisms and the relationship to LTD- and LTP-like effects and MRS-measured GABA+ and Glx. Our experiment explores this ambiguity by studying GABA+ and Glx concentrations in the occipital cortex, a region that has received little attention.

Future Directions

Multiple Sessions

The optimization of stimulation time and sessions are needed to produce longer-lasting effects. For clinical application, multi-day (Blumberger et al., 2018, Berlim et al., 2017) or accelerated sessions (Goldsworthy, Pitcher, & Ridding, 2015) with TMS are more common. There is also evidence showing cumulative effects with increased plasticity over multiple

sessions (Pell et al., 2011). Repeated sessions of rTMS applied on different days may be required to modulate brain function to a clinically significant extent (Pell et al., 2011). Many individuals are inaccurately classified as non-responders, but in fact, are merely slow responders and require more sessions to initiate a response (Blumberg et al., 2018; López-Alonso et al., 2014). Our laboratory, found that a single session of low-frequency 1 Hz rTMS did not show significant changes in GABA+ or Glx. However, an accelerated protocol of five 20 min sessions performed in one day significantly decreased GABA+ levels (Rafique & Steeves, 2018). As well, a case study done in our laboratory showed that multi-day sessions reduced disruptive visual hallucinations following stroke (Rafique et al., 2016). Numerous sessions need to be done to change gene expression and protein synthesis in order for late LTP to occur. The advantages of multiple TBS sessions are beginning to be explored (Thimm & Funke, 2015; Trippe et al., 2009). However, more research is needed to understand its effects. Considering our study showed that a single session of TBS was not sufficient to change absolute metabolite levels in the V1, future studies should explore the effects of multi-session TBS on metabolite levels in this region.

Alternative Parameters

Many variables can impact TBS from being properly transmitted. Although the original Huang et al. 2005 protocol is the standard, it is possible that the parameters can be further refined for different regions of the cortex. In this experiment, we used Brainsight to ensure precise and continuous stimulation of the target area. However, no alterations were made to the original Huang et al. 2005 protocol for stimulation. Varying modifications have been made to TBS for use in treatment. This includes tripling pulses (Li et al., 2014), reducing burst frequency (Stubberman, Zarrabi, Bastea, Raglan, & Khairkhah, 2018), and decreasing inter-train burst time (Tendler et al., 2018). Such modifications have shown significant effects in treatment. However,

it is difficult to compare across studies, since, they are often used in extended protocols that occur over numerous sessions. Also, they are generally not sham-controlled or compared to the original Huang et al. 2005 protocol (Schwippel et al., 2019). Although these studies give preliminary results for TBS modifications, more work is needed to establish how differences in protocols vary across the cortex compared to the standard treatment. This could help to understand the mechanisms of synaptic plasticity.

Refinement of Phosphene Threshold

Modifications need to be made to PT measurement to produce more reliable and precise thresholding. Accurate and Rapid Estimation of Phosphene Threshold (REPT) method is an improved and automatic psychophysical procedure for estimating PT based on the Bayesian adaptive staircase approach (Abrahamyan et al., 2011). The Bayesian adaptive staircase employs a new approach to choose which stimulation intensities efficiently converge on a threshold. After the participant responds, the Bayesian procedure updates the occipital cortex distribution across a set of psychometric curves, covering a broad sampled space of stimulus thresholds and slopes. The upcoming stimulus is computed to select a stimulation intensity that minimizes the uncertainty as to which one is the actual psychometric function corresponding to the participant's performance (Abrahamyan et al., 2011). This eliminates the human error component of intensity selection and reduces the time required to measure PT significantly. Our study's method required the experimenter to test a threshold a minimum of 10 times to get an accurate measure of the 50% response rate. In the REPT method, the participant only responds once to the stimulation pulse, and then the next intensity to test can be derived. Although this allows for quicker administration, this does not account for participant response variability. The participant may not fully comprehend what a phosphene is, leading them to answer incorrectly, thereby altering the

staircase course. When the stimulation is applied numerous times at the same intensity, this helps to mitigate potential false positives or false negatives in responses. Future studies would be of interest that compare the effectiveness of REPT and single-pulse PT in determining intensity for TBS.

Other methods aid in the categorization of the phosphene location. This is done through elaborate hand-drawing captured techniques that can be translated into coordinates. However, this also produces confounds as phosphenes can change with eye movements, causing recordings to be inconsistent due to inevitable shifts in eyes, head and body (Elkin-Frankston et al., 2011). As well, the application of double pulses may be more reliable than a single-pulse (e.g. Boroojerdi et al., 2000; Kammer & Baumann, 2010). In sum, the refinement of TBS and its future implications needs to be tested and verified in additional experiments.

Clinical Use of Theta Burst Stimulation in the Treatment of Visual Disorders

TBS aids in the treatment of non-visual disorders such as depression and OCD. Since TBS mechanisms are known to be associated with related LTP- and LTD-like effects, this can allow for modulation of cortical excitability in regions with aberrant neuronal firing (Pascual-Leone et al., 1994). Multiple sessions allow for plastic changes to occur, aiding in the individual's recovery. Each disorder is defined by different neuroanatomical, functional, and metabolic abnormalities. Depression and OCD are often associated with a hypoactive left DLPFC and a hyperactive right DLPFC (Guo, Li, & Wang, 2017). TBS alleviates numerous psychological symptoms and can activate or inhibit designated brain areas. However, limited TMS research has been done in the treatment of visual disorders.

Previously in our laboratory, multi-day 1 Hz rTMS to the visual cortex mitigated chronic and continuous phosphene hallucinations in a patient who had suffered an occipital stroke

(Rafique et al., 2016). Low-frequency 1 Hz rTMS was applied to the lesion site for 30 min daily over five consecutive days. Before rTMS, there were focal excitatory discharges at the lesion's border as measured by fMRI, highlighting an imbalance of functional activity across hemispheres. Increased application of rTMS resulted in a cumulative reduction in the intensity of the perception of phosphene hallucinations. This was related to a redistribution of cortical activity at the lesion site and remote regions, creating more balance across hemispheres (Rafique et al., 2016). Eighty-seven percent of all strokes are ischaemic strokes, and 17-40% of these strokes are subdivided into posterior circulation strokes, involving the posterior and middle cerebral arteries. This affects cortical regions involving the visual pathway (Benjamin et al., 2017), leading to a variety of visual and perceptual deficits, including visual agnosia, visual field loss and achromatopsia (Marinković, Milisavljević, Lolić-Draganić, & Kovacević, 1987). These deficits can affect daily life and therefore indicates a need for treatment. RTMS and TBS can create virtual lesions, triggering visual cortical plasticity necessary for reorganization and restitution of visual cortical function. The reorganization is presumably dependent on GABAergic modulation and associated LTP that enables nearby cells to adopt the topographical representation of damaged cells and recovery of the affected region (Eysel et al., 1999; Rafique et al., 2016). Since TBS mechanisms are comparable to standard rTMS, it is likely able to create plastic changes aiding in the rehabilitation of visual function from a stroke.

TMS to the V1 improves contrast sensitivity in individuals diagnosed with amblyopia. Amblyopia is a cortical disorder with a difference in visual acuity between eyes. This is typically due to a chronically blurred image in one eye from a lack of optical correction, a misalignment of the visual axes or a partial occlusion during childhood (Holmes & Clark, 2006). It was initially thought that amblyopia could not be treated in adulthood due to limited plasticity in the mature

brain. However, evidence shows that this may not be the case (El Mallah, Chakravarty, & Hart, 2000; Levi & Polat, 1996; Hess, Mansouri, & Thompson, 2010). One study showed that rTMS could treat amblyopia with a single 10-15 min application of either 1 Hz or 10 Hz, which aided in contrast sensitivity (Thompson et al., 2008). CTBS has shown similar success by improving high spatial frequency sensitivity in the amblyopic eye with no stimulation effect in the fellow eye in five patients (Clavagnier et al., 2013). However, contrast sensitivity was still weaker in the amblyopic eye. Although these studies use relatively small sample sizes, increasing the risk for type I errors, they show preliminary data on the effectiveness of rTMS and TBS in visual disorders.

TBS has also been used as a treatment in other brain areas related to visual-demand. Left-sided spatial neglect is a common neurological syndrome following a right-hemispheric stroke. This occurs when interhemispheric inhibition is impaired and leads to hyperactivity of the contralesional hemisphere. Over two days, eight trains of cTBS applied to the contralateral PPC cause a significant improvement in neglect rehabilitation that persists for three weeks (Cazzoli et al., 2012). When stimulation was applied for two weeks, visuospatial neglect improvement was enhanced even further (Fu et al., 2015). This demonstrates the ability of TBS to modulate hyperactivity and potentially hypoactivity in non-psychiatric visual disorders. Although a single session does not seem sufficient to cause long-lasting neurobiological changes, further experiments can refine protocols needed in the development of TBS for visual therapeutic uses.

Conclusion

To conclude, upon completing an extensive search, to the best of our knowledge, this is the only study to compare GABA+, Glx, and their composite ratio in the occipital cortex after cTBS, iTBS or sham in healthy participants immediately post- and one hour post-stimulation.

This study shows that although significant and a trend toward significant effects occurred in metabolites, they did not follow any systematic trend resulting in no relevant overall change. The rigour of this experiment provides reliable and valid results to suggest that brain biomarkers and cortical excitability do not change after a single session of TBS in the occipital cortex, demonstrating its ability to be safely used in an experimental setting. A single session may be acceptable to cause small short-term behavioural changes but is not sufficient to alter brain biomarkers. This is comparable to previous work done in our laboratory, where a single session of 1 Hz rTMS also did not cause any changes to neurotransmitters. This provides a foundation for healthy participants to be used as a reference for future therapeutic intervention.

We conducted this experiment as it is essential to understand the exact mechanisms of TBS in various brain areas and assessing its different outcomes while still controlling for as many factors as possible. Additional research is necessary to determine underlying neural structure and differences in brain chemistry response to non-invasive neuromodulation in other regions. Our laboratory has provided a strong base of TMS effects in the occipital cortex with both behavioural and physiological measures. This experiment concludes that a single session of TBS does not cause consistent results across brain areas. Specifically, a single session of TBS does not cause changes to GABA+ and Glx in the V1, making it safe to use in a laboratory setting.

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Appendix A:

Analyses of Metabolites done with Uncorrected-Tissue Compared to Cr as an Internal Reference

Results

GABA+ Concentration

There was a trend towards significance for Time $F(2,58) = 2.00$, $p = .0505$ and a significant effect of Condition $F(2,58) = 2.081$, $p = .0411$ and a significant interaction between Time and Condition $F(2,58) = -2.24$, $p = .0288$. Across all time points only cTBS showed a trend toward significance: $t(19) = -2.01$, $p = .0545$; while sham: $t(19) = 1.24$, $p = .231$ and iTBS: $t(19) = 0.486$, $p = .632$ did not. All three groups showed no significant effect from B-I: ps> 0.1 Only the cTBS group showed a trend toward significance B-O, $t(9) = -1.89$, $p = .0796$, while sham and iTBS did not: ps> 0.1. Only the cTBS group was significant from I-O, $t(9) = -3.45$, $p = .00726$, while sham and iTBS were not: ps> 0.1 Unstandardized effect sizes were; sham: 0.00290, iTBS: 0.00125, and cTBS: -0.00505. Figure 2.6 shows GABA+ compared to creatine concentration across all time points.

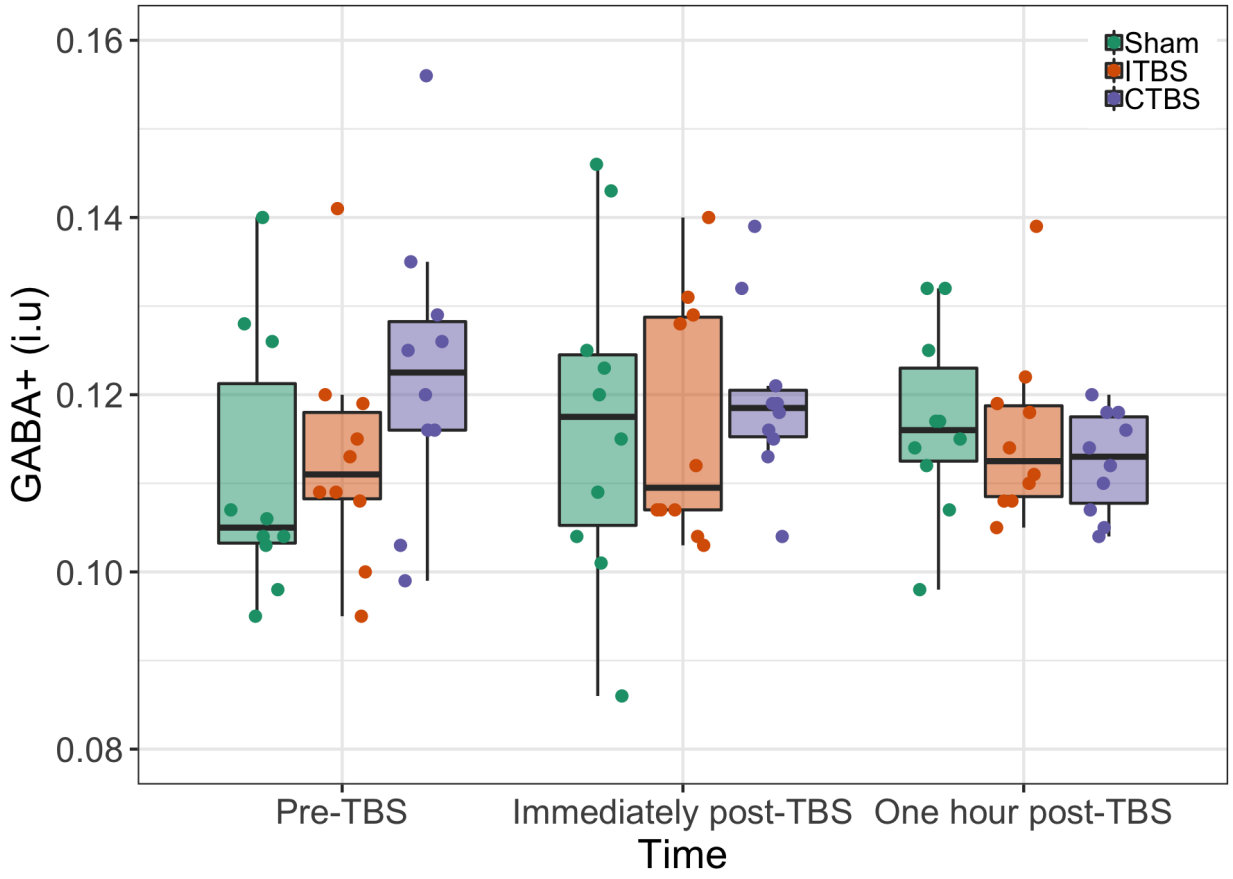

Figure 2.6. GABA+ Compared to Creatine Concentration Across all Time Points. There are three time points: pre-TBS, immediately post-TBS and one hour post-TBS for the three stimulation conditions: sham, iTBS and cTBS. Error bars represent ± SEM.

Glx Concentration

There was no significant effect of Time $F(2,58) = -0.788$, $p = .434$, Condition $F(2,58) = -0.139$, $p = .890$ nor an interaction between Time and Condition $F(2,58) = 0.886$, $p = .379$. Across all time points only iTBS showed a trend towards significance: $t(19) = 1.96$, $p = .0651$; while sham: $t(19) = -1.42$, $p = .172$ and cTBS: $t(19) = -0.21$, $p = .836$ did not. All three groups showed no significant effect from B-I nor I-O: $ps > 0.1$. Only the iTBS group showed a significant effect from B-O, $t(9) = 2.54$, $p = .0316$, while sham and cTBS did not: $ps > 0.1$. Unstandardized effect

sizes were; sham: -0.00145, iTBS: 0.00180, and cTBS: -0.000200. Figure 2.7 shows Glx creatine concentration across all time points.

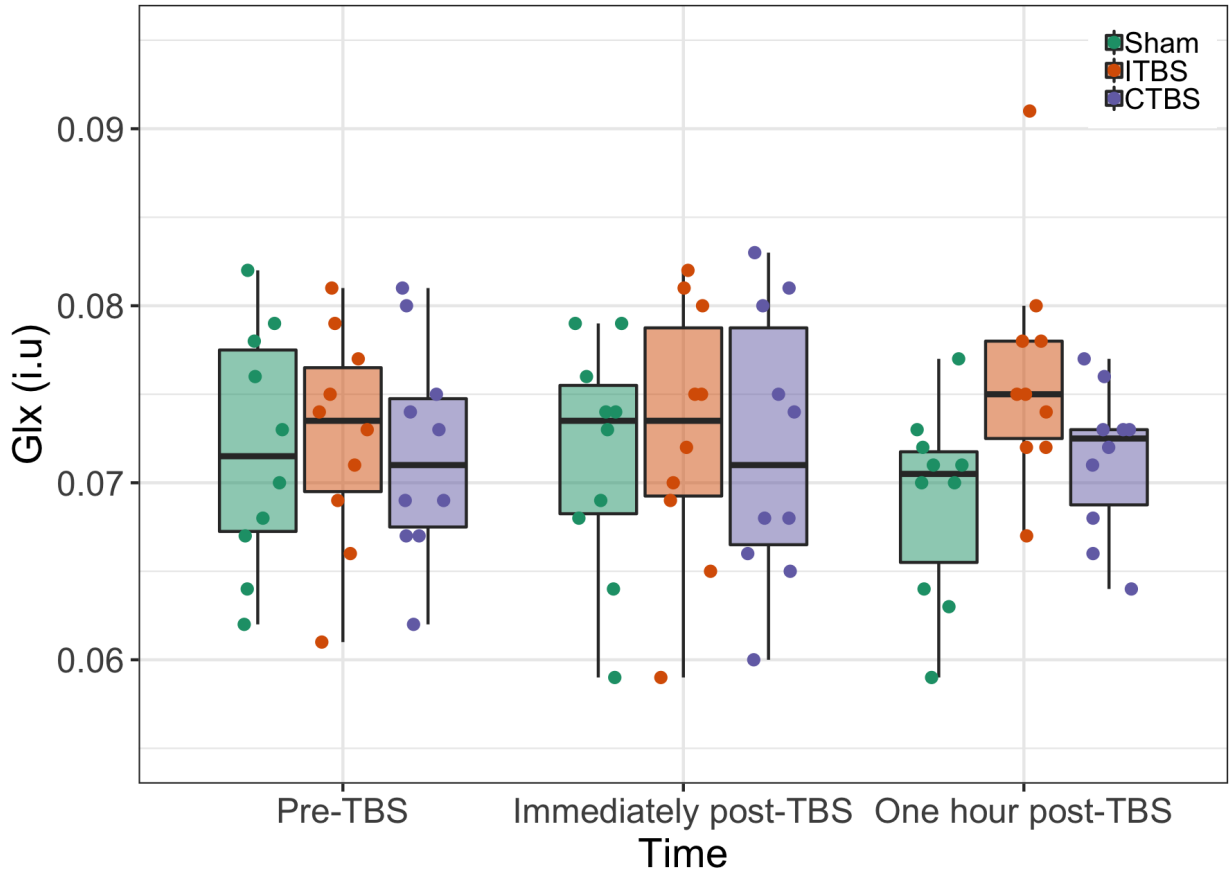

Figure 2.7. Glx Compared to Creatine Concentration Across all Time Points. There are three time points: pre-TBS, immediately post-TBS and one hour post-TBS for the three stimulation conditions: sham, iTBS and cTBS. Error bars represent ± SEM.

GABA+ and Glx Composite Ratio Concentration

There was a trend towards significance for Condition $F(2,58) = -1.85$, $p = .0687$ and a significant effect of Time = $F(2,58) = -2.35$, $p = .0222$ and a significant interaction between Time and Condition $F(2,58) = 2.54$, $p = .0137$. Across all time points only sham showed a trend toward significance: $t(19) = -1.99$, $p = .0608$; while iTBS: $t(19) = 0.607$, $p = .551$, and cTBS: $t(19) = 1.40$, $p = .179$ did not. All three groups showed no significant effect of time from B-I, B-

O nor I-O: *ps*> 0.1. Unstandardized effects sizes were; sham: -0.0225, iTBS: 0.00577, and cTBS: 0.0170. Figure 2.8 shows GABA+ and Glx composite ratio concentration creatine across all time points.

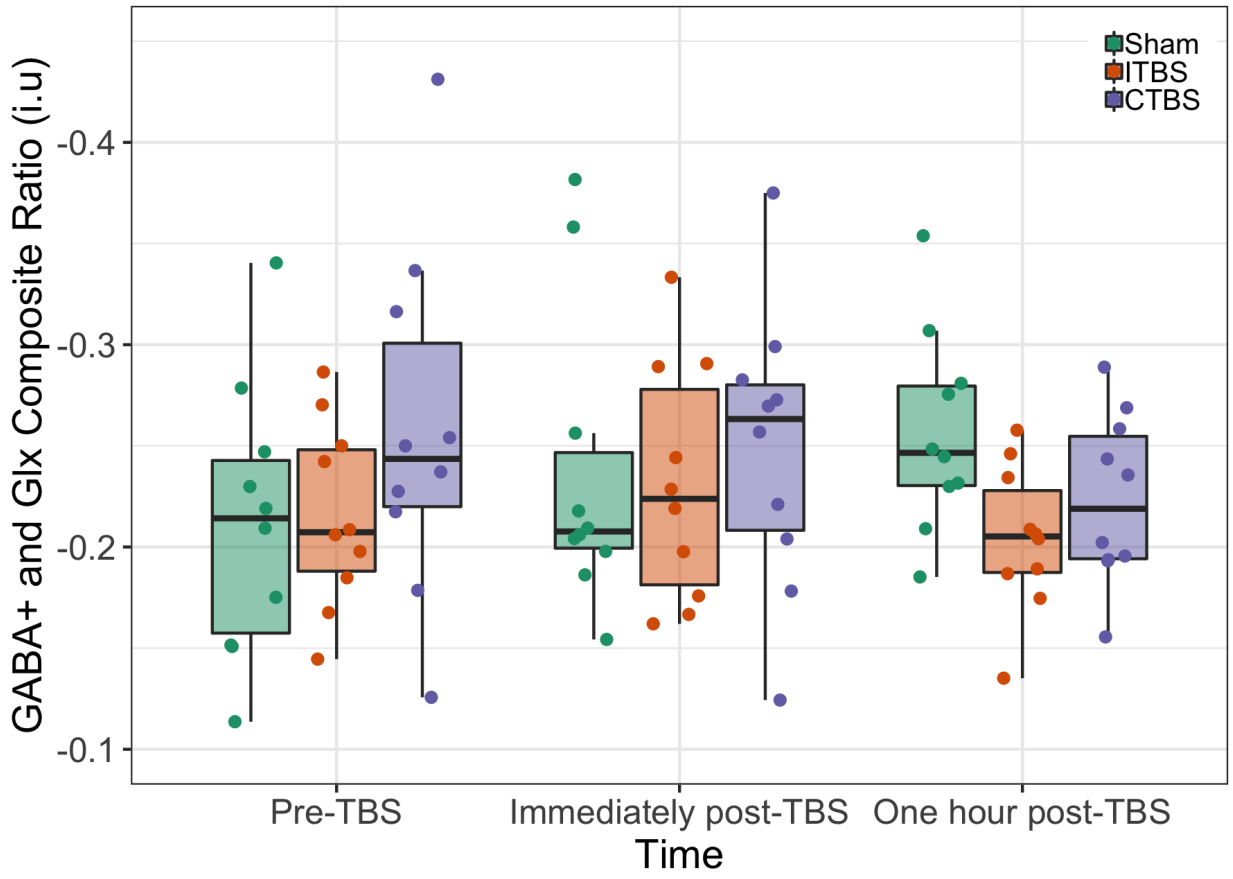

Figure 2.8. GABA+ and Glx Composite Ratio Compared to Creatine Concentration Across all Time Points. There are three time points: pre-TBS, immediately post-TBS and one hour post-TBS for the three stimulation conditions: sham, iTBS and cTBS. Error bars represent ± SEM.

Correlation Between GABA+, Glx Concentration and Phosphene Threshold

There was no significant correlation between pre-TBS GABA+ and Glx concentrations, $\tau = 0.0660$, $p = .616$. PT was not significantly correlated with pre-TBS measures of GABA+ concentration, $\tau = -0.0609$, $p = .642$; but was significantly correlated with Glx concentration, $\tau = -0.312$, $p = .0181$

Analysis of Magnetic Resonance Spectroscopy

Gannet software provides consistent and robust measurements of metabolite concentrations. There is ongoing debate concerning correction strategy and internal reference selection. MRS findings driven by bulk tissue differences are generally held to be of less interest. Therefore, correction in measurements for differences in voxel composition has received an increasing amount of attention. The limitation of tissue-correction is that it does not change the variability of tissue inter-participant wise. In any group of participants, there will be some degree of variation in the tissue composition in the voxel. Given the differences in GABA+ concentration in different tissues, this will lead to variance in GABA+ and Glx measurements (Harris et al., 2015). Furthermore, changes in acquisition parameters for a T1 anatomical can alter image contrast, which may impact segmentation routines (Porges et al., 2017). In our study, issues with metabolite measurement would be equivalent across groups and, therefore, would not impact one group over the other. Since tissue-correction is still relatively new, it is not entirely refined, and differing segmentation routines can lead to potential problems with the validity of the correction. Using Cr as an internal reference may be favourable with the comparability of effects since it is more commonly used in the literature (Mikkelsen et al., 2017). However, advances in tissue-correction are beginning to show outdatedness of this method, with the majority of field advocating for tissue-correction even if not wholly refined.

My study shows that tissue-corrected compared to water and uncorrected metabolite values compared to Cr produce relatively consistent results. However, there are some key differences. In the group using uncorrected-tissue with Cr as an internal reference, GABA+ in the sham group is not significant. The cTBS group is still showing a trend towards significance over all time points, but also showing a trend towards significance B-O and is significantly

different I-O rather than only trending. In the Glx condition, iTBS is no longer showing a trend towards significance I-O but over all time points and B-O instead. Lastly, there is no longer a trend towards a significant relationship with GABA+ and Glx, but there is a significant relationship with pre-TBS Glx and PT. Although tissue-corrected and uncorrected-tissue compared to Cr measure shows similar outcomes, key differences may affect the interpretation of results. We have based our discussion on the relaxation-alpha-tissue-corrected values because it is more precise in our opinion with a correction method for differing tissues and visibility and relaxation of water signals. However, we still wanted to compare this analysis to uncorrected-tissue compared to Cr for additional information. Although Cr reduces the risk of error in the propagation of signal scaling, it also may be at the expense of lower signal quality (Mikkelsen et al., 2017). Opinion in the field suggests that there is no reference signal that is optimal in all applications and discussions (Alger, 2010). As well, both methods have shown to be comparable in reliability (Mikkelsen et al., 2019). This can generally be seen with our results. However, we believe tissue-correction generates progress in the refinement of the measure of metabolite concentration and have thus decided to use this as the principal analysis.