INVESTINGATING THE EFFECTS OF GLUCOSE AND SWEET TASTE ON CORTICOSPINAL AND INTRACORTICAL EXCITABILITY

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TITLE: Investigating the effects of glucose and sweet taste on corticospinal and intracortical excitability

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Abstract

Transcranial magnetic stimulation (TMS) is commonly used to measure corticospinal and intracortical excitability in basic and clinical neuroscience. However, the effect of glucose on TMS-based measures is not well defined, despite a potentially impactful influence on precision and reliability. Here, a double-blinded placebo-controlled study was used to test the effects of glucose on two commonly used TMS measures: short-interval intracortical inhibition (SICI), and the area under the motor evoked potential recruitment curves (AURC). SICI and AURC are thought to reflect inhibitory (GABAergic) and excitatory (glutamatergic) neurotransmission respectively. Healthy males (N=18) each participated in four sessions. Session 1 involved TMS familiarization and acquisition of an individualized blood glucose response curve. During sessions 2, 3 and 4, dependent measures were taken before (T0) and twice after (T1 & T2) drinking 300 mL of solution containing glucose (75 g), sucralose-sweetened placebo (control for sweetness) or plain water (control for time). The T1 and T2 measurements were started 5 minutes prior to the blood glucose peak observed during Session 1. Blood glucose and mean arterial pressure (MAP) were also monitored. Sucralose, but not water or glucose increased AURC and none of the treatments altered SICI. There was no association between blood glucose level and TMS measures, but in all three conditions MAP rose after consumption of the drink. There was a positive correlation between the rise in blood pressure and the relative increase in AURC at the higher stimulus intensities. Eleven participants returned for a fifth session to quantify the smallest detectible change in the AURC measurements and it

was confirmed that significant changes were real while non-significant differences in measurement means fell within the range of expected measurement error. This study also suggests a relationship between corticospinal excitability and autonomic tone. Additional investigation is required to understand the mediating factors of this association.

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Declaration of Academic Achievement

This thesis has written entirely by Stephen Toepp and all experiments and TMS analyses were conducted by Stephen Toepp. All treatment solutions and the session randomization schedule were provided the McMaster University Medical Center (MUMC) research pharmacy. Dr. Aimee Nelson aided conception and design of the study and my fellow lab mates and collaborators, Claudia Turco, Dr. Chiara Nicolini, Mitchell Locke, Jenin El-Sayes and Diana Harasym aided the collection of data.

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List of Abbreviations

ACh – Acetylcholine

ACC – Anterior Cingulate Cortex

ANLS – Astrocyte-Neuron Lactate Shuttle

Asp – Aspartate

ATP – Adenosine Triphosphate

AURC – Area Under the Recruitment Curve

CMR_{glc} – Cerebral Metabolic Rate of glucose

CNS – Central Nervous System

CS – Conditioning Stimulus

EMG – Electromyography

GABA – Gamma Aminobutyric Acid

GAP – Glyceraldehyde-3-Posphate

Glc-6-P – Glucose 6 Phosphate

Gln – Glutamine

Glu – Glutamate

GSH – Glutathione

HPA – Hypothalamus Pituitary Adrenal

LDH – Lactate Dehydrogenase

M1 – Primary Motor Cortex

MEP – Motor Evoked Potential

MEP_{TS} – unconditioned Motor Evoked Potential

$MEP_{CS\ TS}$ – conditioned Motor Evoked Potential

MSO – Maximum Stimulator Output

MUMC – McMaster University Medical Centre

OAA – Oxaloacetate

PC – Pyruvate Carboxylase

PDH – Pyruvate Dehydrogenase

PMC – Premotor Cortex

PPP – Pentose Phosphate Pathway

RMT – Resting Motor Threshold

rTMS – repetitive Transcranial Magnetic Stimulation

SAM – Symapathetic Adrenal Medullary System

SMA – Supplementary Motor Area

SM1 – Primary Sensorimotor Cortex

SICI – Short Interval Intracortical Inhibition

tDCS – transcranial Direct Current Stimulation

TCA – Tricarboxylic Acid

TMS – Transcranial Magnetic Stimulation

TS – Test Stimulu

1. GOALS OF THE THESIS

Since its introduction in 1985, the non-invasive brain stimulation technique called transcranial magnetic stimulation (TMS) has become prominent in basic and clinical neuroscience. The specialization and refinement of TMS techniques and equipment has enabled researchers to probe various aspects human neurophysiology that were previously inaccessible *in situ* due to the discomfort or invasiveness associated with earlier brain stimulation procedures such as direct current stimulation.

In addition to technical advancements, increased awareness and understanding of influential subjective factors will be necessary to improve the quality of TMS research. Indeed, most TMS measures exhibit high variability and replication of reported findings is often difficult. One factor that may contribute to this difficulty is diet and feeding. The brain relies heavily on glucose metabolism to meet its high energy demand and glucose is involved in several important aspects of neuronal function to which TMS may be sensitive.

The goal of this thesis is to assess the influence of glucose on human neurophysiology and thereby evaluate the merits of glucose monitoring or experimental controls as strategies for improving TMS research. The thesis begins with an in-depth review of the current literature regarding the involvement of glucose in neural function and dysfunction, and the known effects of glucose on TMS measurements. This thesis will

then describe the rationale, design, and findings of the research which aims to address specific and important gaps in the current TMS-glucose literature.

2. REVIEW OF THE LITERATURE

The brain consumes a disproportionate 20% of all glucose-derived energy despite comprising only 2% of human body mass, driven by high metabolic rate and the reliance on glucose as a source of carbon for biosynthetic processes (Mink et al., 1981; Mergenthaler et al., 2013). Among brain cells, the highest metabolic demand is attributable to neurons, which carry out the information delivery and processing functions typically considered to be the primary purpose of the central nervous system (CNS) (Harris et al., 2012). However, glucose metabolism is related to a wide variety of neuronal and glial cell functions, including neurotransmission, energy storage, control of oxidative stress, and biosynthesis of neuromodulators and neurotransmitters (Mergenthaler et al., 2013; Dienel 2012). This review will start by exploring role of glucose in each of these processes and then describe the state of TMS research with respect to these processes.

2.1 Fueling synaptic activity

The high energetic cost of maintaining ionic gradients, reversing cellular Ca^{2+} influx following the arrival of action potentials and recycling synaptic vesicles (among other processes), makes synaptic transmission the largest CNS consumer of adenosine triphosphate (ATP) (Harris et al., 2012). Neurons obtain the energy substrates required to meet the demand from astrocytic glycogen stores through the astrocyte-neuron lactate shuttle (ANLS), or from extracellular glucose, taken up directly into the neuron after

being imported from the blood (Ashrafi and Ryan 2017; Magistretti and Allaman 2015). There is some controversy concerning whether ANLS or neuronal glycolysis is the predominant source of neuronal fuel provision (Daniel 2012; Pellerin and Magistretti 2012). While it should be noted that both pathways likely contribute to some degree, the major arguments for each are summarized in the next two sections.

2.1.1 The Astrocyte-Neuron Lactate Shuttle

According to the ANLS model, lactate produced and released by astrocytes is the main dynamic fuel source of neurons (Magistretti and Allaman 2015). Cerebro-cortical neurons also produce lactate in hypoxic contexts where reduced oxidative phosphorylation stimulates the acceleration of glycolysis (Malthankar-Phatak et al., 2008). Here, the saturation of pyruvate dehydrogenase (PDH) which controls entry of pyruvate into the tricarboxylic acid (TCA) cycle leads to the production of excess pyruvate which is converted into lactate by lactate dehydrogenase (LDH). Astrocytes exhibit a tightly regulated metabolic profile that can readily produce lactate in the presence of oxygen, a process called aerobic glycolysis (Warburg, 1956). In contrast to hypoxic neurons which must compensate for insufficient oxygen delivery, astrocytic aerobic glycolysis involves the active suppression of PDH via phosphorylation which restricts pyruvate oxidation at the point of entry into the tricarboxylic acid (TCA) cycle (Halim et al., 2010). The glycolysis for ATP production and LDH must be active to rapidly regenerate NAD+ and maintain glycolytic flux. The resulting excess of lactate is released into the extracellular space to be taken up by neurons. Once taken into a neuron, lactate drives the LDH

reaction in the opposite direction, producing pyruvate to fuel oxidative phosphorylation in the mitochondria.

Pellerin and Magistretti (1994) provided evidence that glucose uptake and lactate production are regulated by the neurotransmitter glutamate, suggesting a possible mechanism for coupling between energy delivery and neuronal activity. Astrocytic lamellar processes that sheathe and surround synaptic structures possess strategically located membrane proteins which are sensitive to glutamate reuptake activity (Genoud et al., 2006). Since glutamate uptake and recycling (a main function of astrocytes) is energetically demanding and consumes significant ATP, glucose uptake and catabolism must be facilitated. This is achieved through disinhibition of key glycolytic enzymes like hexokinase and phosphofructokinase. The signalling pathways that upregulate aerobic glycolysis and lactate production are thought to be largely activated by the increase in intracellular Na^{2+} that accompanies glutamate uptake in peri-synaptic projections (Magistretti et al., 2015). Enhancing glycolysis while maintaining suppression of PDH results in the dynamic production of lactate for metabolism in neighboring neurons.

Broadly, the ANLS model predicts that lactate and not glucose provides the larger portion of energy to firing neurons. In contrast to the highly glycolytic astrocytes, neurons rely almost exclusively on oxidative phosphorylation and do not store glycolytic substrate (i.e. glycogen). Neurons also lack the ability to enzymatically upregulate glycolytic flux in response to increased ATP demand due to rapid autosomal degradation of the positive glycolytic modulator 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (Bolanos et

al., 2010). Zhang and colleagues (2014) also found differential splicing in the pyruvate kinase (PK) gene between neurons which express the PMK1 isoform and astrocytes which express PMK2. Only PMK2 has the capacity to accelerate glycolytic flux when ATP demand is elevated (Zhang et al., 2014). This "fixed" glycolic metabolism suggests that direct uptake and catabolism of glucose is less likely to be the pathway by which dynamic neuronal energy requirements are satisfied.

2.1.2 Neuronal Glucose Uptake and Glycolysis

Alternatively, it has been argued that the ANLS mechanism is too slow to accommodate rapid changes in activity-related ATP demand since it relies on the accumulation of lactate in the extracellular space (Chih et al., 2003). Furthermore, when glycolysis is disrupted in isolated rat neurons, the induction of synaptic activity results in a rapid presynaptic ATP decline and subsequent synaptic quiescence (Rangaraju et al., 2014). This suggests that despite its allegedly fixed velocity, glycolytic metabolism is important for synaptic communication and astrocytic lactate is likely insufficient as the primary fuel source.

Several lines of evidence suggest that synaptic transmission is heavily reliant on local glycolysis, fueled by extracellular glucose. First, the greater speed of glycolysis relative to the oxidation of lactate-derived pyruvate would seem to favor the tight temporal coupling of energy delivery to potentially rapid and dynamic activity-related ATP demands (Pfeiffer et al., 2001; Ashrafi and Ryan, 2017). In addition, enzymes associated

with the ATP-generating payoff steps of glycolysis are enriched on the surface of pre-synaptic vesicles and axonal transport organelles, conferring close spatial coupling between glycolytic ATP yield and important energy-consuming processes (Knull 1980; Ikemote et al., 2003; Takamori et al., 2006; Zala et al., 2013). In synaptic terminals, the glycolytic machinery can also adapt during periods of high energy demand by assembling into a metabolic complex called a "metabolon" to rapidly metabolize available glucose and produce ATP and pyruvate (Jang et al., 2016). The importance of potent neuronal glycolysis is further illustrated by the high neuronal expression of the Glut3 glucose transporter (Simpson et al., 2008) and the glutamate-sensitive recruitment of the Glut4 transporter at synaptic terminals in active neurons (Ashrafi et al., 2017).

The heavily oxidative nature of neuronal cells appears to allow them to rapidly capitalize on the delivery of pyruvate from both astrocytic lactate and neuronal glycolysis. However, the strategic enrichment of glycolytic machinery and Glut4 at presynaptic terminals and the inherently more rapid production of pyruvate and ATP suggests that it is mainly neuronal glycolysis and oxidative flux and not astrocytic lactate that accommodates rapid changes in activity-dependant ATP demand.

2.2 Neuromodulators and Neurotransmitters

Only a small portion of glucose metabolized is diverted to provide carbon for the synthesis neurotransmitters and neuromodulators (e.g. acetylcholine synthesis rate is only ~1% of glucose utilization) (Joseph and Gibson, 2007). However, studies showing that

disruption of biosynthetic shunt pathway activity via hypoxia or targeted enzyme silencing causes alterations in neuronal structure and function, interferes with CNS development and blunts cognition, are illustrative of glucose's critical role in the production of these neuroactive compounds (Errico et al., 2012; Punzo et al., 2016, Zhang et al., 2011), Gibson and Duffy 1981; Joseph and Gibson 2007). This section will provide a summary of the biosynthetic role of glucose.

In addition to glucose's requirement as a carbon source for de novo neurotransmitter synthesis, glucose metabolism must provide the ATP necessary to maintain the critical neurotransmitter shuttling processes required for neurotransmission. Indeed, glucose oxidative flux is directly proportional to rates of neurotransmitter cycling between astrocytes and neurons (Dienel, 2018). Although precise mechanisms for the coupling of respiration with glutamate (Glu), glutamine (Gln), and gamma-aminobutyric acid GABA cycling are not yet established, this section will also provide a basic theoretical framework for understanding the importance of glucose in this context.

2.2.1. Biosynthesis

The neurotransmitters acetylcholine (ACh), Glu and GABA as well as the neuromodulators D-serine, glycine and D-Aspartate are synthesized from glycolytic and TCA cycle intermediates which are produced in both astrocytes and neurons (Dienel, 2018). While glucose is metabolized in both astrocytes and neurons to generate these precursors, the synthesis of the amino-acid backbone for these neuroactive compounds

occurs exclusively in astrocytes (Shank et al., 1985; Yu et al., 1983). The astrocyte-specific CO_2-fixing enzyme pyruvate carboxylase (PC) converts pyruvate into oxaloacetate (OAA), which is then combined with acetyl-CoA to produce α-ketoglutarate, and these TCA cycle intermediates are precursors for Asp, Glu, Gln and GABA (Dienel, 2018). Fixation of CO_2 rises with brain activity and oxidative metabolism (Öz et al., 2004), and PC is stimulated by K^+(Kaufman and Driscoll, 1992). It has been suggested that cellular uptake of K+ released into the extracellular space by depolarized neurons is a potential mechanism for the regulated production of Glu by astrocytes (Hertz and Chen, 2016). Although the biosynthetic pathway and regulation of ACh production is less clear, glucose has been also identified as the source of its characteristic acetyl group (Joseph and Gibson, 2007).

2.3. Pentose Phosphate Pathway

In addition to neuromodulators and neurotransmitters, some glucose is diverted from entering glycolysis to enter the pentose phosphate pathway (PPP) which produces NADPH for biosynthetic processes. First, glucose-6-phosphate (Glc-6-P) is converted to ribulose-5-phosphate and NADPH by the sequential actions of Glc-6-P dehydrogenase and 6-phosphogluconate. Ribulose-5-phosphate is then rearranged to form ribose-5-phosphate which is an important ribonucleotide component. In order to prevent the sequestration of phosphate in the shunt pathway, transketolase and transaldolase transform PPP intermediates into fructose-6-phosphate and glyceraldehyde-3-phospate

(GAP) which can then re-enter glycolysis. As such, a rise in PPP flux means an increase in glycolytic rate associated with the metabolism of additional GAP and Fru-6-P.

NADPH produced by the PPP provides substrate for fatty acid and cholesterol biosynthesis, as well as for enzymes like nitric oxide synthase, aldehyde reductase, and aldose reductase (Dienel, 2018). The regeneration of NADPH as a cofactor for the antioxidant enzyme glutathione (GSH) is also an important function of the PPP in adulthood. In combination with catalase, GSH neutralizes destructive hydrogen peroxide molecules made from superoxide ions produced by respiratory chain activity or neurotransmitter metabolism. GSH also detoxifies several additional reactive species including formaldehyde and methylglyoxal (Rae and Williams, 2017).

2.4 Effects of Brain Stimulation on Glucose Metabolism

Brain stimulation has demonstrated a capacity to change glucose metabolism in both targeted and remote areas of the brain. Siebner and colleagues (2000) demonstrated that 5Hz repetitive TMS (rTMS) of the hand area of the left primary motor cortex (M1) increased the cerebral metabolic rate of glucose (CMR_{glc}). The researchers used [^{18}F]fluorodeoxy-D-glucose positron emission tomography ([^{18}F]FDG-PET) to assess brain glucose metabolism in eight participants at rest and after delivering 1,800 pulses of subthreshold stimulation. There was a relative increase in CMR_{glc} in the supplementary motor area (SMA) and M1 after stimulation which approximately 30 minutes after rTMS delivery. This suggests that the metabolic impact was sustained for some time after

cessation of pulses. The same group also demonstrated that 2Hz suprathreshold rTMS increases CMR_{glc} in the hand area of primary sensorimotor cortex (SM1), SMA, lateral premotor cortex (PMC), and the motor portion of anterior cingulate cortex (ACC) (Siebner et al., 2001). In rats, accelerated high-frequency rTMS, which is a higher frequency derivative of a currently accepted intervention for treatment-resistant depression, was shown to reduce striatal levels of the serotonin metabolite 5-hydroxyinlacetic acid also increase motor behavior one day after rTMS (El Arfani et al., 2017).

Brain activity is also modulated by transcranial direct current stimulation (tDCS) which involves passing current through cortical tissue by way of electrodes placed on the scalp. Binkofski and colleagues (2011) used [^{31}P] magnetic resonance spectroscopy to measure brain ATP turnover and a euglycemic clamp to assess glucose uptake from the blood before and after 20 minutes of phasic anodal tDCS. There was a significant increase in ATP turnover starting 65 minutes after the end of tDCS, and a biphasic change in glucose uptake exhibiting a trending decrease followed by a significant increase with the inflection occurring ~75 minutes after tDCS (Binkofski et al., 2011). This change in glucose uptake may be related to a metabolic response to tDCS since in-vitro electrical stimulation of brain slices increases pentose PPP flux (Kimura et al., 1974) which is associated increased glucose sequestration by hexokinase and acceleration of downstream

STUDY	POPULATION	INTERVENTION	MEASURE	RESULT
SPECTERMAN ET AL., 2005	Young adults (N=10, 5 females)	68 g of glucose taken orally	MEPs evoked by TMS at 1.1X RMT	Increase
ANDERSEN ET AL., 2006	Type 1 diabetics, (N=5, 3 females)	3-hour hyperglycemic clamp 16mmol/L	Resting Motor Threshold	No Change
GANT ET AL., 2010 EXP 1.	Young males (N=19)	0.4 g/kg body mass of maltodextrin taken orally 11 min before testing	MEPs evoked during max contraction	Increase
GANT ET AL., 2010 EXP 2	Young adults (N=17, 6 females)	Carbohydrate drink in mouth during TMS measurements	MEPs evoked during max contraction	Increase
BADAWY ET AL., 2013	Healthy young controls (N=10, 5 females)	Meal ingestion 2 hours before testing	Paired pulse intracortical inhibition Resting Motor Threshold	Increase No Change
BAILEY ET AL., 2019	Young adults (N=10, 5 females)	Carbohydrate mouth rinse immediately or 10 min before testing	MEPs evoked by during 50% contraction	Increase

Table 1. A summary of the current literature concerning the effect of carbohydrate consumption on TMS measures

glycolysis (Dienel et al., 2018). The researchers also discovered reductions in blood pressure, cortisol, and ACTH indicating a decrease in hypothalamus pituitary adrenal (HPA) axis activity after tDCS (Binkofski et al, 2011).

2.5. The Effect of Blood Glucose on TMS Measurements

Several studies have been published over the course of the last two decades suggesting that carbohydrate, and more specifically glucose, can influence measures of motor function obtained using TMS. An overview of studies investigated the effects on glucose or other carbohydrates on TMS measures can be found in Table 1. These studies used a variety of paradigms and TMS measurements, which add nuance to the interpretation of the reported effects of glucose on corticospinal and intracortical excitability.

The first study investigating the influence of elevated blood glucose on a measure of brain excitability using TMS was conducted in the context of the energy drink Lucozade. Specterman and colleagues (2005) assessed corticospinal excitability by measuring the area under the rectified electromyography (EMG) trace of motor evoked potentials (MEPs) elicited in the first dorsal interosseous (FDI) muscle of the right hand by TMS delivered at 110% of the resting motor threshold over the left M1 (Specterman et al., 2005). Measurements were taken before and after the ingestion of Lucozade (n=6), or before and after ingestion of 3 different control solutions (n=4). The Lucozade solution given to six of the participants contained 68 g of glucose and 46 mg of caffeine in 380ml of carbonated water. As such, control solutions included (1) 68 g of glucose in carbonated

water, (2) 46 mg of caffeine in 380 ml of carbonated water and (3) 380 ml of carbonated water (Specterman et al., 2005). MEP size was measured at baseline and every 30 minutes after drinking. MEP size was significantly greater starting at 30 minutes after Lucozade and caffeine, and 60 minutes after glucose. Only the glucose solution produced an increase in MEP size that was significant 120 minutes after ingestion and this increase was correlated with a rise in blood glucose levels (Specterman et al., 2005).

Andersen and colleagues (2006) also studied the influence of blood glucose manipulation on TMS measures in five type 1 diabetics. Resting motor threshold (RMT) was measured as an indicator of corticospinal excitability before and after a 3-hour interval with blood glucose maintained at either 5 or 16 mmol/l. No change in RMT was observed, even though participants were subjected to higher blood glucose levels and for longer than in the study by Specterman and colleagues who exhibited peak levels of ~8 mmol/L with a reduction to baseline 2 hours after bolus ingestion (Andersen et al., 2006; Specterman et al., 2005) . This suggests that RMT is insensitive to glucose's modulation of corticospinal excitability which was observed by Specterman and colleagues (2005) who measured suprathreshold MEP area. In support of this notion, a more recent study found that RMT did not to change 2 hours after ingestion of a meal (Badawy et al., 2013). Furthermore, RMT is a measurement that is understood to probe the excitability of smaller motor units with lower thresholds (Rossini et al., 2015) and it may be necessary to measure higher threshold motor units by measuring the size of MEPs evoked by suprathreshold TMS or during active contraction. It may be that sampling data from motor neuron populations

with a range of depolarization thresholds is necessary to detect influences of glucose on corticospinal excitability.

Meal ingestion has also been shown to alter TMS measures of intracortical excitability. These are paired-pulse measures which involve delivering a conditioning TMS pulse (CS) prior to a suprathreshold test stimulus (TS). The CS may inhibit or facilitate the MEP evoked by the TS depending on the interstimulus interval (ISI) between the two pulses. Changes induced by CS are expressed as the ratio of the MEP evoked with the CS to that when the CS is absent (unconditioned) and are thought to be reflective of neurotransmitter receptor subpopulations. Badawy and colleagues (2013) assessed intracortical excitability in 10 healthy young adults (5 males; 5 females) before and 2 hours after consumption of a meal. TMS measurements included paired pulse TMS with ISIs of 2ms and 5ms to assess short-interval intracortical inhibition (SICI), 10ms and 15ms to assess intracortical facilitation (ICF) and 100, 150, 200, 250, 300, 350 and 400ms to assess long-interval intracortical inhibition (LICI) or intracortical facilitation (LICF). The researchers found significant increase in LICI with the 250 and 300ms ISIs (Badawy et al., 2013). Interestingly, a pattern appeared in the data whereby there was a tendency toward more inhibition and less facilitation after food consumption at all ISIs. This probably reflects a broad influence of feeding (and presumably glucose) on intracortical excitability which is not isolated to any particular neurotransmitter receptor subtype.

2.6 Influence of Taste Circuitry on TMS Measurements

Another possibility is that the effects of glucose are related to a more acute influence on sweet taste receptors in the mouth rather than glucose levels in the blood. Activation of oral sweet- and carbohydrate-sensing receptors induces activity in the facial, glossopharyngeal and vagus afferents which project to the nucleus of the solitary tract (NTS) in the medulla and pons (Bailey et al., 2006). Catecholaminergic NTS neurons regulate feeding, cardiovascular reflexes, arousal and stress (Roberts et al., 2017). Here the signal may also access and influence descending corticospinal neurotransmission at the level of spinal alpha motor neurons by way of the reticulospinal pathway (Yates and Stocker 1998). The possibility of an effect of the afferent signal originating in the mouth on corticomotor output is supported by several two notable experiments.

Gant and colleagues (2010) measured MEPs evoked in the biceps brachii during maximal contraction in two experiments. In the first experiment, MEPs were measured 11 minutes after consuming carbohydrate or energy-free placebo solution. Approximately 30% facilitation of the MEP was observed after the intake of carbohydrate solution but not the placebo, even though testing preceded the subsequent rise in blood glucose (Gant et al., 2010). In the second experiment, MEPs were measured while participants held the carbohydrate or placebo solution in their mouths. Once again, even though there was no increase in blood glucose, the size of the evoked MEPs was increased. Indeed, there was no correlation between blood glucose levels and the relative change in MEP size.

In line with these findings, another recent study found that holding a carbohydrate solution in the mouth facilitated quadriceps MEPs both immediately after and 10 minutes post-rinse in 10 young adults (5 females) (Bailey et al., 2019). Blood glucose levels were not reported, but it is reasonable to assume that this occurred in the absence of a change in blood glucose level. Researchers assessed MEP size during submaximal (50% maximal voluntary contraction) quadriceps contraction before and after holding a carbohydrate or placebo solution in the mouth for 20s. The carbohydrate solution was one of maltose, maltodextrin, or glucose and all elicited significant increases in MEP size relative to the placebo wash (Bailey et al., 2019). As with the study by Gant and colleagues (2010) these data support the notion that carbohydrate may influence brain function by some other mechanism outside of its role as an energy substrate taken up from the blood.

3. INTRODUCTION

Glucose is the brain's primary energy substrate and provides the main source of carbon for *de novo* synthesis of large neuroactive compounds which are required for essential processes ranging from neurotransmission to the management of oxidative stress (Ashrafi et al., 2017; Dienel et al., 2012; Deinel et al., 2018; Mergenthaler et al 2013). Unsurprisingly, the relationship between circulating glucose levels and brain function is relevant in a variety of clinical contexts. Ketogenic diets which restrict carbohydrate intake have long been prescribed to manage epilepsy which is more common in hyperglycemic diabetics (Lu et al., 2018), and hyperglycemia is associated with poorer stroke outcomes (Desilles et al., 2013). However, the glucose literature currently lacks a

comprehensive neurophysiological profile of healthy humans during periods of fasting versus post-prandial hyperglycemia.

Some studies have used non-invasive neurophysiological measures to compared hyperglycemic, normoglycemic or fasting conditions, and have reported provocative relationships between circulating glucose and brain excitability (Andersen et al., 2007; Badawy et al., 2013; Specterman et al., 2005). For example, Specterman and colleagues (2005) used single-pulse transcranial magnetic stimulation (TMS) to measure corticospinal excitability and reported a 3-fold increase in the size of motor evoked potentials (MEPs) 60 minutes after ingestion of 68 g of glucose. The authors also found a positive correlation between MEPs and circulating blood glucose levels. In another study, intracortical excitability was measured in fasted (i.e. 12 hours overnight) and fed (i.e. two hours after a meal) epileptic and healthy control participants using paired-pulse TMS (Badawy et al., 2013). They found that intracortical excitability changed with glucose levels in both epileptic and healthy individuals (Badawy et al., 2013). However, not all studies have detected an effect of glucose on neurophysiological measures. Andersen and colleagues (2007) manipulated glucose levels in type 1 diabetics via intravenous glucose pump and did not find any change in cortical motor thresholds, a common measure of cortical excitability. This suggests that some but not all neurophysiology measures are sensitive to the effects of glucose.

There is also little clarity regarding the mechanisms by which glucose influences TMS measures. The repeated measures study conducted by Specterman and colleagues (2005)

tested MEPs in a time-efficient manner which allowed high temporal resolution, but this is at the cost of more comprehensive tests which could probe underlying mechanisms. For example, MEP recruitment curves take longer to assess, but may reflect cortical glutamate levels (Stagg et al., 2011). Investigating a proxy measure of glutamate levels may be productive since glutamatergic neurotransmission has been linked with glucose levels in many in vitro and in situ neurobiological studies (Ikemoto et al., 2003; Pellerin and Magistretti 1994; Roberts et al., 2017). It may also be productive to probe GABA-mediated intracortical inhibition using paired-pulse TMS. Although Badawy (2013) found no significant effect of glucose on short interval intracortical inhibition (SICI), all significant and non-significant intracortical measure differences between fasted and fed participants were in the direction of increased inhibition or less facilitation after feeding (Badawy et al., 2013). This is counterintuitive given the apparent increase in MEP size after glucose ingestion (Specterman et al., 2005), and warrants further investigation. In addition, neither of these studies account for unequal equal rates of glucose metabolism and both lack controls for potential effects of consuming a sweet tasting drink (Specterman et al., 2005; Badawy et al 2013).

Thus, the goal of this study is to test the effect of glucose ingestion on the healthy human brain using a variety of non-invasive neurophysiological measures which have relevant mechanistic underpinnings. This investigation also controls for the influence of a sweet placebo and time-lock measurements individually observed peak glucose latencies. The results of this study have the potential to provide researchers with much-needed clarity

regarding the effects of glucose on TMS measurements which are commonly used in clinical and basic neuroscience and will facilitate the interpretation of past and future studies.

4. METHODS

4.1. Participants

Healthy, young (N=18, 22.6 ± 2.5 years), right-handed, male non-smokers were recruited from the McMaster University student population. The chosen sample size was derived from SICI and AURC data published by the Neuophysiology and Imaging Laboratory at McMaster and from previously published research by other groups. For example, within-subject differences in SICI of ~10% were detected using sample of 13 participants (Lulic et al., 2017), while Badawy and colleagues (2013) found no within-subject differences with a similar sized sample (N=10). Furthermore, within-subject differences in measures of corticospinal excitability were detected using a variety of measurement methods (Specterman et al., 2005; Lulic et al., 2017). Increases after glucose ingestion were detected with a sample of only 4 participants with a dissimilar method to the present study (Specterman et al., 2005). However, the more similar recruitment curve method used by Lulic and colleagues (2017) also detected a change in a sample of 14 participants. The final sample size of 18 participants ensured sufficient sample size and permitted the accommodation of data loss due to collection error, participant withdrawal or corrupted data files.

Participants passed a screening for TMS contraindications (Rossi et al., 2009; Appendix A), and were identified as right-handed using a handedness questionnaire (Appendix B) which required them to indicate which hand they use for several common manual tasks (Oldfield et al., 1971). The International Physical Activity Questionnaire was used to exclude inactive individuals (< 600 MET-minutes/week). Inactive individuals were excluded to reduce the risk of influence from prediabetic impairment of glucose metabolism which is inversely correlated with physical activity level (Hu et al., 2019). This study was approved by the Hamilton Integrated Research Ethics Board (HiREB) and conformed to the declaration of Helsinki.

4.2 Electromyography

Surface electromyography (EMG) of the first dorsal interosseous (FDI) muscle of the right hand was performed using adhesive electrodes (9-mm diameter Ag-AgCl) that were placed over the FDI muscle belly and the metacarpal head of the index finger. EMG signal was amplified 1k times and sampled at 5k Hz with low and high pass signal filters of 2.5 k Hz and 20 Hz respectively. The amplified and filtered signal was recorded and saved for later analysis on an IBM-compatible PC using an analog-to-digital interface (Power 1401; Cambridge Electronics Design, Cambridge, UK) in combination with Signal/CED analysis software (Signal version 6.02; Cambridge Electronics Design).

4.3 Transcranial Magnetic Stimulation

Single and paired-pulse brain stimulation was delivered by holding a 50mm figure-of-eight TMS coil, connected to Magstim Bistim stimulator (Magstim, Whitland, UK), over the left primary motor cortex (M1). The optimal stimulation location or "motor hotspot" was targeted using Brainsight neuro-navigation software (Rogue Research, Canada) with motion-tracking reflective markers fixed the coil and a headband worn by the participant. The motor hotspot for FDI muscle was determined by delivering pulses at 50% of the maximum stimulator output (%MSO) over the approximate location of M1 while adjusting coil placement until the TMS pulses reliably evoked large MEPs. The angle of the coil relative to the midsagittal plane was maintained at 45˚ to induce posterior-to anterior current in cortical tissue. Motor hotspot was defined as the location that elicited consistently large MEPs in the FDI muscle. The optimal location and orientation of the coil was registered in the neuro-navigation software and all TMS stimuli were delivered here for the duration of the testing session.

4.3.1. Resting Motor Threshold

Resting motor threshold (RMT) was defined as the stimulus intensity (%MSO) that evokes and MEP (i.e. peak-to-peak amplitude >50 μV) 50% of the time. This value was determined using TMS_MTAT_2.0 freeware (http://clinicalresearcher.org/software.htm). The starting stimulus intensity was set to 37 %MSO, and twenty TMS pulses were delivered over M1 with stimulus intensity being adjusted after each pulse as prescribed by

the MTAT software based on the MEP occurrence (or lack thereof) on the previous trial (Ah Sen et al, 2017) .

4.3.2. MEP Recruitment Curve

Corticospinal excitability was measured by using single-pulse TMS to assess recruitment curves. Eight TMS pulses were delivered at 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, 200% of RMT in a randomized order. The MEP amplitude is plotted against stimulus intensity and data were fit with a Boltzmann sigmoidal curve. The regression line was segmented 1000 times and the area under the recruitment curve (AURC) was quantified by trapezoidal integration.

4.3.3. Short-Interval Intracortical Inhibition

Short-interval intracortical inhibition (SICI) was measured using a paired-pulse TMS protocol where by 12 unconditioned (MEP_{TS}) and 12 conditioned ($MEP_{CS\text{-}TS}$) pulses were delivered in a randomized order. For conditioned trials, the suprathreshold test stimulus (TS) was preceded by a conditioning stimulus (CS) with a stimulus intensity of 80% of RMT and onset occurring 2 ms prior to the TS. The unconditioned pulses included only the TS which was adjusted on-line to evoke responses of approximately 1 mV in size. The strength of SICI was quantified using the ratio of conditioned to unconditioned MEP size ($MEP_{CS\text{-}TS}$/ MEP_{TS}).

4.4. Blood Glucose and Blood Pressure

Capillary blood glucose measurements were performed via the glucose oxidase method with a hand-held diabetes monitoring device (Abbott MediSense FreeStyle Precision Neo Blood Glucose and Ketone Monitoring System, Abbott).

Because previous research has indicated that blood pressure may be elevated by ingestion of a large glucose bolus (Rebello et al., 1983: Synowski et al., 2013), mean arterial blood pressure (MAP) was measured using an automated blood pressure monitor (OMRON Blood Pressure Monitor, OMRON Healthcare). The mean arterial pressure was calculated from the systolic and diastolic pressures as indicated below:

$$MAP = \frac{2DBP + SBP}{3}$$

Where SBP is the systolic blood pressure and DBP is diastolic blood pressure.

4.5. Experimental Design

This study implemented a double-blinded, three-way crossover design in which fasted participants were assessed before and after ingestion of water, a sucralose-flavored placebo or a 75 g glucose bolus. All solutions were 300mL. Prior to the first experimental testing session, participants completed a preliminary testing session. A schematic of the study schedule for each participant is shown in Figure 1.

4.5.1. Preliminary Testing and Familiarization

Visit 1 was used to familiarize participants with TMS and to assess a time-course for glucose metabolism, allowing the subsequent TMS measures on visits 2, 3, 4 and 5 to be individualized. Participants arrived in the lab having fasted for 10 hours and having fully read the consent from provided at the time of screening. After providing written informed consent, they were given an orientation of the equipment and procedures involved in the testing. TMS measures, including the assessment of the motor hotspot and RMT, were performed to familiarize the participant with the sensations that accompany brain stimulation. Motor hotspot was saved and used for reference during subsequent visits.

Next, participants ingested a 75 g glucose bolus in 300 mL of solution and finger-prick blood samples were collected and analyzed at 10-minute intervals. The latency at which peak blood glucose occurred was used to ensure that TMS tests are conducted during a period of high circulating glucose for each individual.

4.5.2. Experimental Testing Sessions

Visits 2, 3 and 4 were scheduled at least 48 hours apart. On the day of each visit, participants arrived in the lab having fasted for 10 hours. At the beginning of the visit,

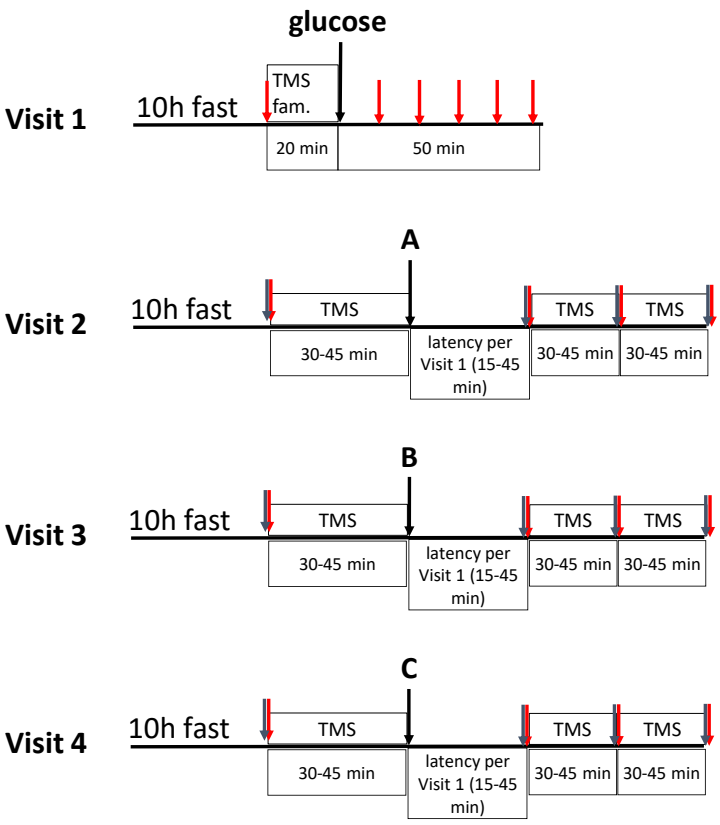

Figure 1. The timeline for each of the 4 visits. A, B and C represent the coded labels that correspond with the consumption of one of the three treatments (i.e. water, sucralose or glucose) as scheduled by MUMC. Red arrows indicate measurement of plasma glucose concentration and grey arrows indicate measurement of blood pressure. Glucose and blood pressure measurements were initiated 10 minutes prior to the start of TMS

participants were administered the TMS screening questionnaire (Appendix A). This was to ensure that participants have refrained from alcohol, nicotine and/or non-prescription medicines in the 12 hours prior to the experimental session, as these substances might affect measurements. Failure to do so resulted in rescheduling the session to a later date. Capillary blood glucose and blood pressure were then assessed before TMS testing was initiated. Next, motor hotspot was re-assessed and RMT was measured. SICI and MEP recruitment curves were measured in a randomized order to avoid an order effect.

Immediately following the baseline neurophysiology tests participants ingested either water, sucralose-sweetened placebo (5g/300ml Splenda® solution), or a 75g oral glucose tolerance test bolus. The visit during which each treatment was delivered was also randomized to avoid an effect of order (section 4.3.4). Participants then rated their degree of liking and the intensity of sweetness of the drink on a 10 cm visual analog scale. For degree of liking, the left anchor was labeled "dislike extremely" and the right anchor was "like extremely". For sweetness, the left anchor was "not at all" and the right anchor was "extremely sweet". After a rest interval which was 5 minutes shorter than the latency of peak blood glucose determined during visit 1, the neurophysiology battery will be repeated twice. TMS measurements acquired twice after the rest interval and are heretofore referred to as the T1 and T2 TMS bouts. The timing of these bouts was chosen with the aim of capturing peak glucose levels in the plasma (2^{nd} bout) and subsequently the peak of glucose levels in the cerebrospinal fluid (3^{rd} bout) which occurs ~30 minutes after plasma glucose (Shestov et al., 2011). Finger plasma glucose and blood pressure were assessed prior to T1 and T2, as well as after T2.

4.5.3 Blinding and randomization

The McMaster University Medical Centre (MUMC) research pharmacy provided a randomized treatment schedule. All treatment solutions were provided in uniform, shrouded bottles with a

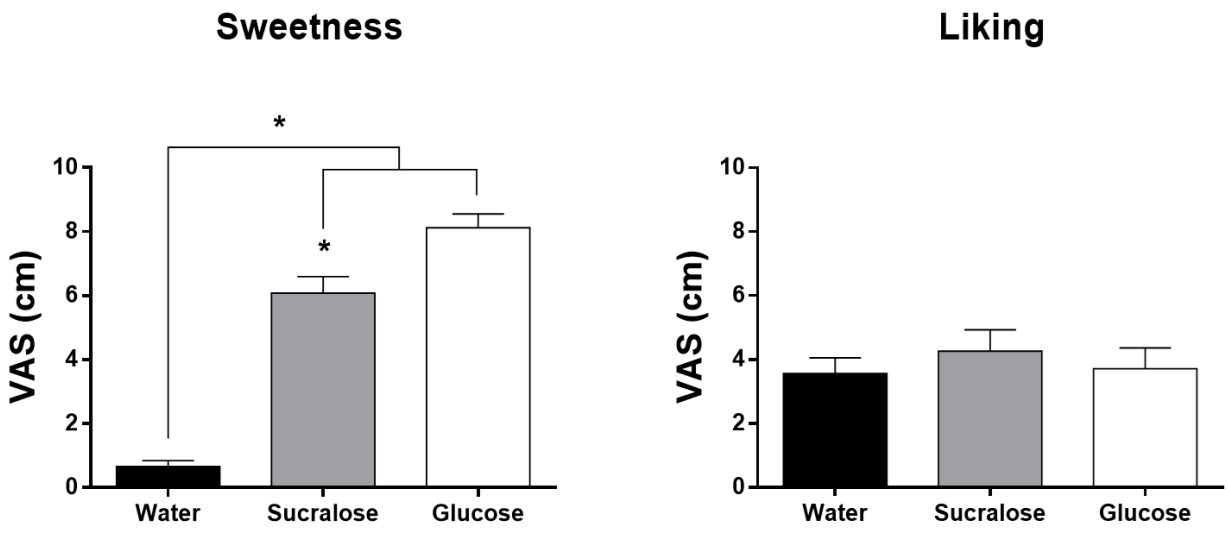

Figure 2. Subjective ratings of sweetness and liking recorded directly after drink ingestion. Sucralose was significantly less sweet that glucose, but both sucralose and glucose were sweet compared to water. *significant difference, two tailed Wilcoxon sign-rank test ($p<0.05$)

letter code corresponding to the order of delivery. MUMC pharmacy held the drink randomization (i.e. drink identity) key until collection was complete to ensure that the experimenters were blind to the identity of the drink. Blood glucose and subjective ratings of sweetness were recorded by an unblinded researcher who did not otherwise take part in data collection or analysis. The sucralose-sweetened placebo was taste-matched with the 75g glucose solution by MUMC pharmacy. Participants were explicitly asked not to say anything about the taste of the drink to the researchers and it was made clear that this was very important to the integrity of the study. The participants were blind to the identity of the drink do the degree that they could not distinguish between the sucralose placebo and the glucose solutions (water was not masked with any taste). While participants reported

that both the sucralose and glucose solutions were much sweeter than water, glucose was rated significantly sweeter than sucralose (Figure 2).

4.5. Smallest detectible change

Eleven participants returned for an additional session to quantify the smallest detectible change (SDC) for the various AURC measurements performed on the MEP recruitment curves (described in section 5.2). SDC is defined by Weir (2005) as, "the difference needed between separate measures on a subject for the difference in measures to be considered real." The session was identical to the previous experimental sessions described in section 4.3.3 except that they were not given any treatment solution after baseline tests. Wait times were set to reflect the same timeline for T0, T1 and T2 measurements as during the previous experimental sessions. The statistical calculation of SDC is detailed in the next section (section 4.6).

4.7 Statistical Analysis

All measures were normalized to the baseline mean (T0). Prior to unblinding, 2-way repeated-measures analysis of variance (ANOVA) was implemented to detect within-participant effects of TIME (3 levels; T0, T1, T2) and TREATMENT (3 levels; glucose, sucralose, water). When data were not normally distributed, values were ranked, and the repeated-measures ANOVA was performed on the ranked data as described by Conover and Iman (1976). This no-parametric analysis has been shown to provide a valid F-

statistic which is powerful and robust when main effects, replication effects and interaction effects are present (Iman and Conover, 1976). The other assumptions of the ANOVA, including that data were ratio or interval, randomly sampled and conformed to sphericity, were satisfied regardless of normality. Wilcoxon sign ranked tests were used assess the hypotheses that glucose would strengthen SICI and increase AURC. Post-hoc analyses were conducted as indicated by significant effects revealed by ANOVAs and the normality (or lack thereof) of the data for each measure. Significance was set to alpha < 0.05.

SDC was assessed using the standard error of means (SEM) estimated as the square root of the mean squared error (MSE) term from a one-way repeated measures ANOVA with three levels of the factor TIME (Eliasziw et al., 1994; Hopkins, 2000; Stratford and Goldsmith, 1997). The SDC for each AURC measurement was calculated as follows:

$$SDC = SEM \times 1.96 \times \sqrt{2}$$

This equation generates a 95% confidence interval which is reflected the corresponding *z* score of 1.96. The $\sqrt{2}$ term reflects correction necessary to account for variability in both scores being compared as explained by Weir (2005). The SDC values calculated from this subset of eleven participants could be applied to the data from the entire sample because the SEM is "a fixed characteristic of any measure, regardless of the sample of subjects under investigation" (Nunnally and Bernstein, 1994).

5. RESULTS

5.1 Blood Glucose

The peak plasma glucose concentration after ingestion of the oral glucose tolerance test dose (75g) during Visit 1 was 9.5 ± 1.0 mmol/L. This represents a 2-fold increase from the fasting glucose (4.8 ± 0.4 mmol/L) and an absolute rise of 4.7 ± 0.9 mmol/L. The frequency distribution of peak glucose latencies is shown in Figure 3A. Sixteen of the participants exhibited peak glucose latencies of 30 or 40 minutes while one participant peaked at 20 minutes and another peaked at 50 minutes. The observed peak glucose latency spread of 30 minutes suggests that the use of an individualized testing time course was justified.

Plasma glucose levels over the course of each experimental visit (i.e. before and after delivery of water, sucralose or glucose) were measured at the timepoints are displayed in Figure 3B. In the glucose delivery condition, plasma levels ~5 minutes before beginning T1 (~10 minutes before the peak glucose latency measured during Visit 1) were 3.4 ± 1.9 mmol/L higher than fasting. The measurement taken after the T2 indicated that plasma glucose remained ~2.0 mmol/L higher than fasting levels. These data confirm that glucose levels were substantially increased during both post-drink TMS testing bouts in the glucose delivery condition.

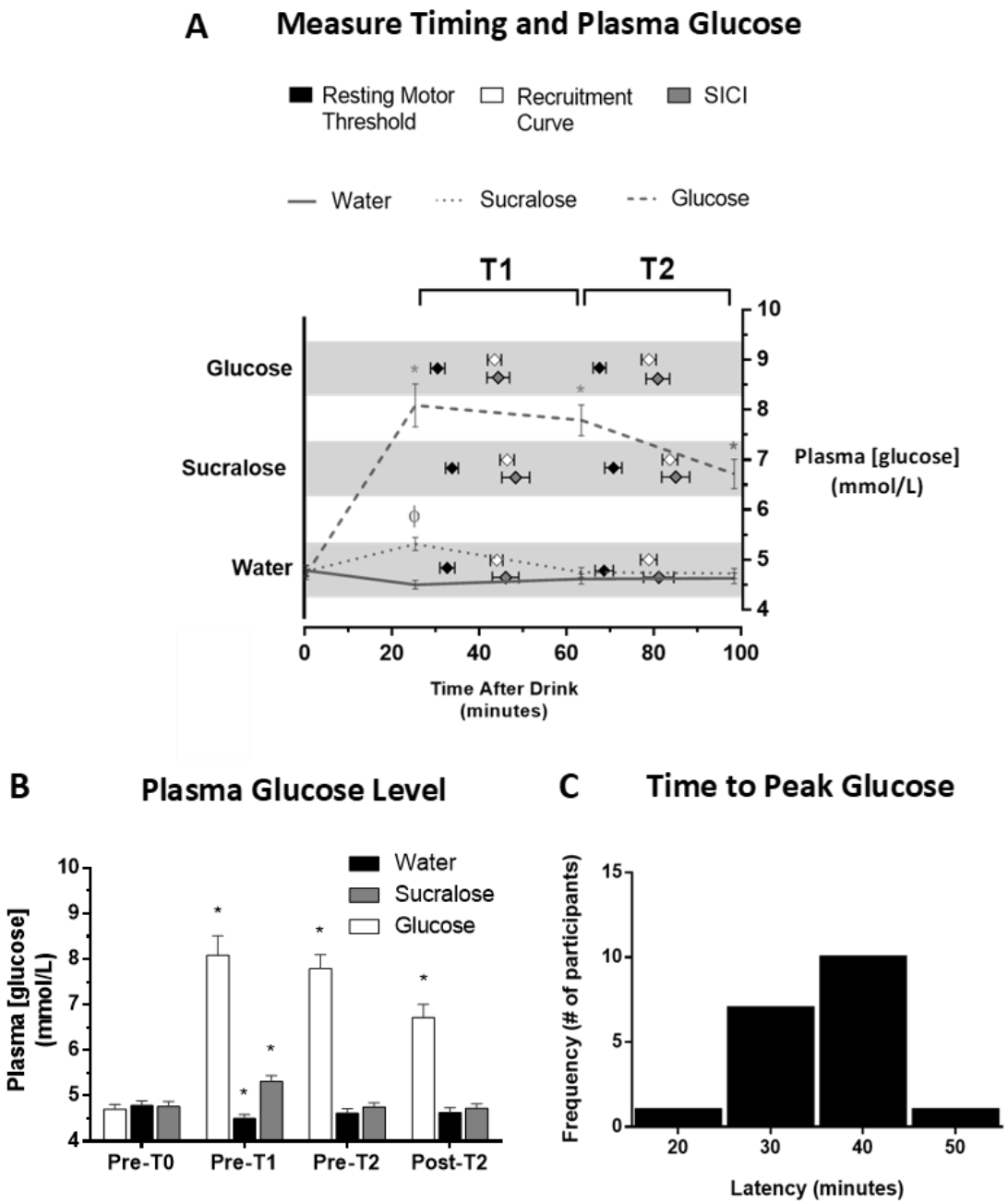

Figure 3. A) the mean and standard error of the of the collection times for RMT, MEP recruitment curves, and SICI with plasma glucose levels for each testing session. The shaded area highlights glucose levels B) Plasma glucose levels at each timepoint before and after ingestion of glucose, sucralose or water C) The frequency distribution of the peak glucose latencies measured on visit one and * significant difference from baseline, ϕ significant difference between sucralose and water ($p<0.05$).

Notably, plasma glucose was also increased prior to T1 after ingestion of the sucralose-sweetened placebo.

The small but statistically significant increase of 0.5 ± 0.3 mmol/L can be attributed to the bulking ingredients that accompanied sucralose in the Splenda sweetener. Glucose levels recorded after T1 and T2 exhibited no difference from fasting, suggesting that the effect of the placebo solution on blood glucose was transient relative to that of the 75g glucose bolus. After the ingestion of water, there was a small decrease blood glucose level relative to baseline (0.3 ± 0.4) which was only observed prior to T1. While this difference reached statistical significance, it is unclear whether this was physiologically meaningful.

5.2 MEP Recruitment Curve Area (AURC)

Two way repeated-measures ANOVA revealed a significant effect of TIME ($F_{(2, 17)}$=11.404; p<0.001), but no main effect of TREATMENT ($F_{(4,17)}$=1.055; p=0.359) or TREATMENT*TIME interaction ($F_{(4,17)}$=2.067; p=0.095). These data are displayed in Figure 4A. Furthermore, the Wilcoxon matched pairs sign-ranked test did not find significant change in AURC after glucose ingestion at T1 (p=0.963) or T2 (p=0.176).

These findings were unexpected because a previous report indicated that corticospinal excitability increased 60 minutes following glucose ingestion (Specterman et al 2005). A series of secondary analyses were performed to better understand this discrepancy. These analyses were guided by the methodological differences between this and previous

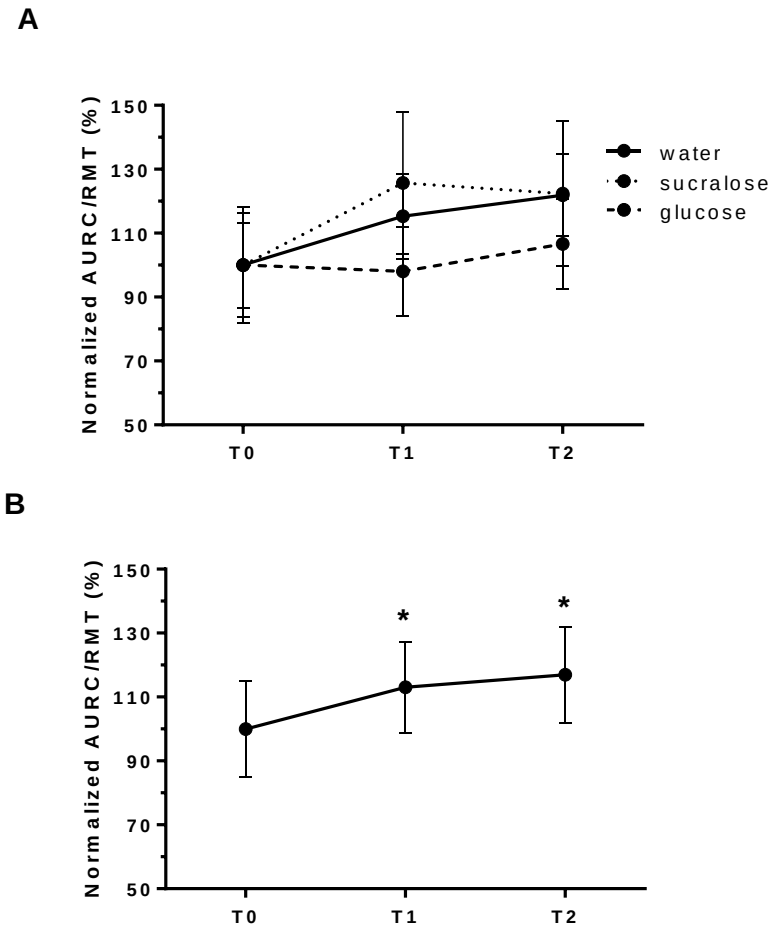

Figure 4. A) AURC data from each treatment condition at each timepoint normalized to the T0 treatment mean (100%). B) Treatment-averaged data showing significant increases in AURC at T1 and T2 as assessed by Wilcoxon matched pairs sign rank tests *significant difference from T0 ($p<0.05$)

research and by patterns which emerged in the supplemental data. These guiding factors include differences in TMS intensity, changes in mean arterial pressure and the unequal sweetness between placebo and glucose drinks.

5.2.1 Effect of Stimulus Intensity

The use of the MEP recruitment curve to assess corticospinal excitability in this research contrasts with the method used by Specterman and colleagues (2005), who measured the size of MEPs evoked at a fixed suprathreshold intensity. Delivering TMS pulses at 12 different intensities to obtain a MEP recruitment curve permits the measurement of motor neurons with a range of depolarization thresholds. While AURC may be sensitive to changes in a larger motor neuron pool, it does not discriminate between changes in neurons with different recruitment thresholds. Indeed, when the recruitment curve data were averaged across treatments as shown in Figure 5A, it appears that MEP amplitudes increase over time preferentially at the higher stimulus intensities (i.e. where higher threshold motor units are being recruited). To probe this apparent interaction of TMS stimulus intensity with time, recruitment curve data were segmented into 10% intensity intervals between 100% and 200% of RMT.

At this point, an anomaly was identified in data from one participant whereby AURC was ~0 for multiple allegedly suprathreshold intensity intervals. Because this likely reflected an error in data collection impacting all other intensities delivered within the recruitment curve, the data for this participant was removed from the analysis.

The ten intervals were then grouped into three intensity bins. The bins were defined using two landmark stimulus intensities derived from the Boltzmann sigmoid fit of the grand-average recruitment curve (Figure 5B). The first landmark intensity is 130% of RMT

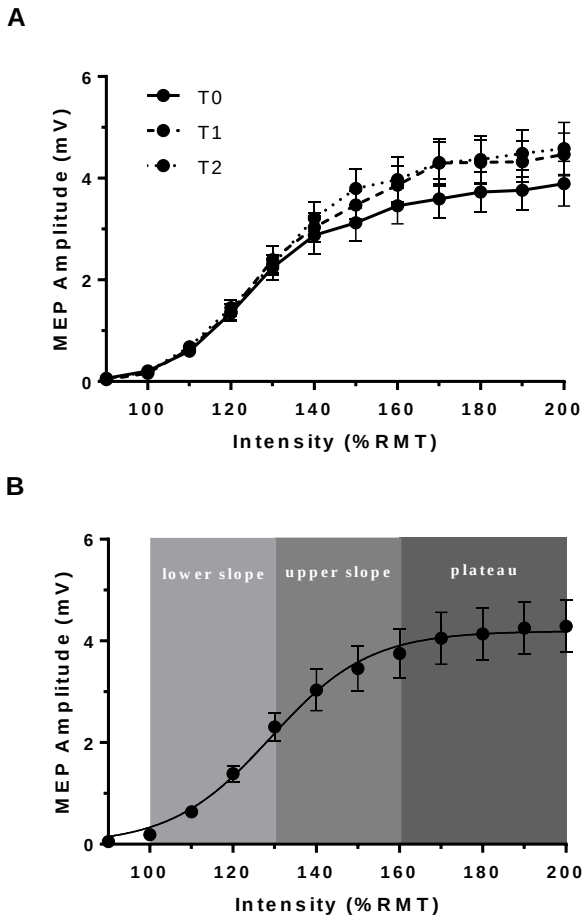

Figure 5. A) Treatment-averaged data showing that the effect of time appears to be more prominent at high intensities. B) The grand-average of all recruitment curves with overlaid bins used to segment the data for the assessment of an intensity effect.

which bisects the lower and upper half of the ascending part of the recruitment curve. This landmark reflects the approximate intensity of the inflection point of the Boltzmann sigmoid which is 129% of RMT. The second landmark intensity is 160% of RMT, which is the first stimulus intensity where the grand average of the MEP amplitudes was significantly lower than the plateau of the Boltzmann sigmoid curve. The first bin is denoted 'lower slope' and covers the interval between 100% and130% of RMT, the

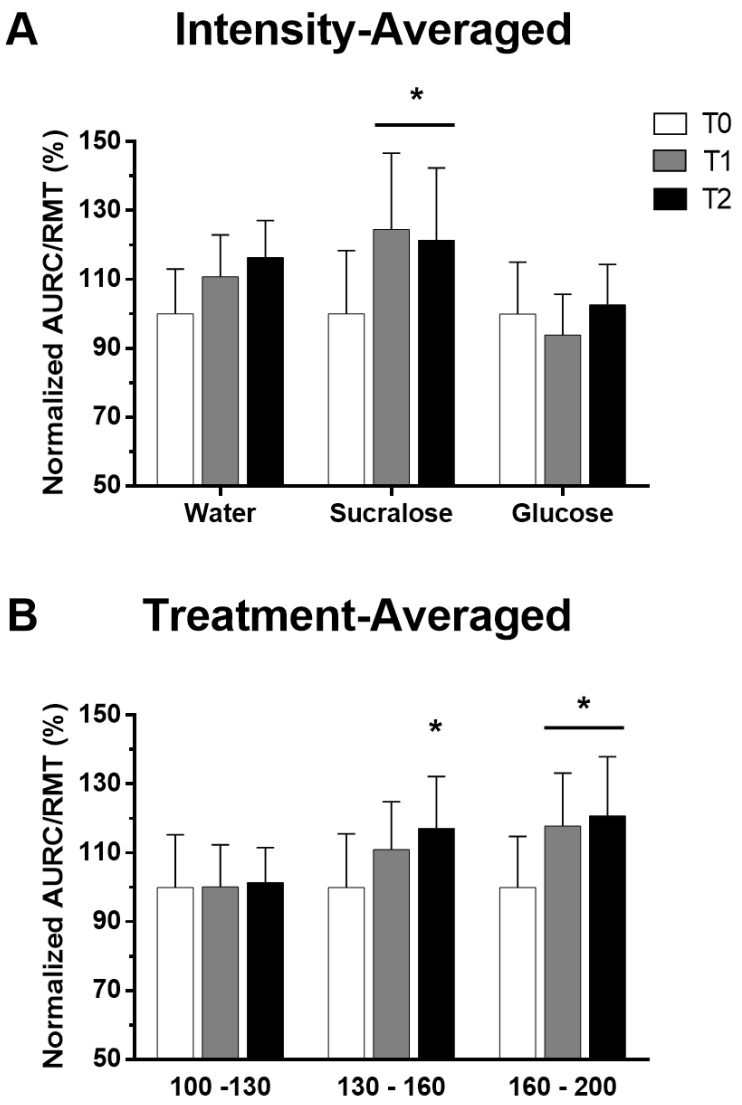

Figure 6. A) Intensity-averaged AURC data from each treatment and B) for each of the three intensity bins when the data is averaged across treatments. Data at T1 and T2 for each treatment or bin is normalized to the corresponding mean at T0 (100%). *significantly different from T0 ($p<0.05$).

middle bin is denoted 'upper slope' and includes area from 130% to 160% of RMT, and the 'plateau' bin includes the remainder of the area between 160% and 200% of RMT.

Three-way repeated measures ANOVA with factors INTENSITY, TREATMENT, and TIME was used to probe time-by-treatment and time-by-intensity interactions. In addition to the previously observed TIME effect ($F_{(2,16)}$=3.83; p=0.05), interactions of TIME*INTENSITY ($F_{(4,16)}$= 3.59; p=0.045) and TIME*TREATMENT ($F_{(4,16)}$=2.76; p=0.04) were both significant. Post hoc analyses were subsequently performed on Treatment-averaged (Figure 6A) and intensity-averaged (Figure 6B) data. AURC data to determine for which treatment(s) and at which intensities AURC changed over time. When the data is averaged across intensity bins, Wilcoxon matched pairs sign rank tests did not reveal a significant change after consumption of the glucose or water solution. Surprisingly, AURC was elevated relative to the fasted baseline after the sucralose placebo by 25% at T1 (p=0.03) and 21% at T2 (p<0.01) respectively. For treatment-averaged data, increases from baseline were present only for upper slope and plateau bins.

AURC MEASUREMENT	F STATISTIC	P VALUE
FULL CURVE	2.12	0.15
LOWER SLOPE	1.02	0.38
UPPER SLOPE	2.31	0.13
PLATEAU	2.37	0.12

Table 2. F Statistics and P-values generated by one-way repeated measures ANOVA performed on data from eleven participants who completed a no-treatment follow-up session. Lower slope AURC was measured between 100 and 130 %RMT, upper slope was between 130 and 160 %RMT, and the plateau was 160 to 200 %RMT.

There was a 17% increase in AURC at T2 for the upper slope bin (p=0.02), and there was an 18% increase at T1 and a 21% increase at T2 in the plateau bin (p=0.02 for T1 & T2).

5.2.2. Smallest Detectible Change

Because the present findings represent a clear contrast to the ~300% increase in MEP size reported by Specterman and colleagues (2005), it was necessary to determine if this method of measuring the MEP recruitment curve was sensitive enough to have detected such a change. To achieve this, SDC was calculated from SEM values produced by a one-way ANOVA of data from eleven participants who completed a no-treatment follow-up session. There was no effect of TIME for measurements of the full curve, or when the data were separated into intensity bins as outlined in section 5.2.1 (Table 2).

AURC MEASUREMENT	SEM (%)	SDC_{IND} (%)	SDC_{GROUP} (%)
FULL CURVE	19.66	54.50	16.43
LOWER SLOPE	38.10	105.60	31.84
UPPER SLOPE	21.03	58.30	17.58
PLATEAU	18.15	50.32	15.17

Table 3. SDC for individuals and group means, as well as the estimated SEM from which SDC was derived. Lower slope AURC was measured between 100 and 130 %RMT, upper slope was between 130 and 160 %RMT, and the plateau was 160 to 200 %RMT.

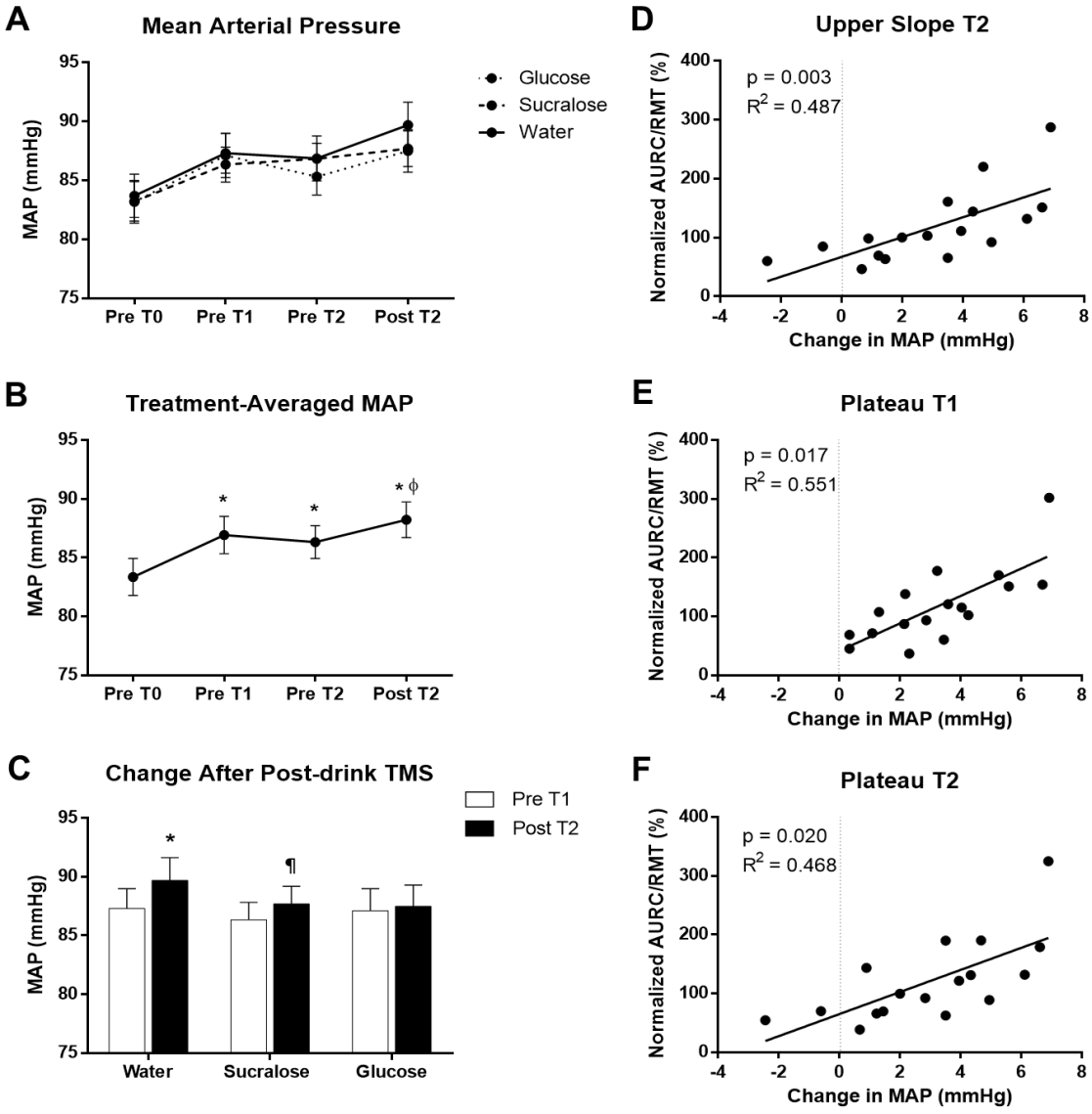

Figure 7. Mean arterial pressure data (left) and significant correlations with AURC data (right). (A) Mean arterial pressure data at all timepoints for all conditions. (B) Treatment averaged MAP data showing significant increases at all timepoints relative to baseline and at Post T2 relative to Pre T1. (C) MAP was significantly increased post-T2 relative to the pre-T1, but only in the water condition, with a trend in the sucralose condition. The three correlations that reached significance were between the change in MAP and (D) the AURC at T2 relative to T0 in the 'upper slope' intensity bin, E) the AURC at T1 relative to T0 in the 'plateau' bin, and F) the AURC at T2 relative to T0 in the plateau bin. *significant relative to Pre-T0 ($p<0.05$), ϕ significant relative to Pre T1 ($p<0.05$), ¶ trend ($p=0.06$)

The SEM and SDC for each measurement method are listed in Table 3. The lower slope SDC is of interest because Specterman and colleagues (2005) evoked MEPs using stimuli within this intensity range. The lower slope SDC was low, indicating that the present method could have detected a change in MEP size greater that 38%. This was also the highest SDC value, suggesting that the upper slope (17.58%), the plateau (15.17%) or the full curve (16.43), may be more sensitive measures of corticospinal excitability. It is reasonable to conclude from these SDC values that the absence of a glucose effect was not due to high measurement error arising from the chosen method of quantifying MEP size.

5.2.3 Changes in Mean Arterial Pressure

Mean arterial pressure was assessed before T0 and before and after T1 and T2 to track the potential confounding factor of blood pressure and related upstream autonomic modulation (Figure 7A). A two-way repeated measures ANOVA with factors TREATMENT and TIME (four levels) found an effect of TME ($F_{(3,16)}$=22.995; $p<0.001$) but no effect of TREATMENT ($F_{(2,16)}$=0.386; $p<0.683$). Post-hoc comparisons found that the treatment-averaged MAP was significantly elevated compared to T0 at each of the three post-drink measurements ($p<0.001$), and T3 was also greater than T1 ($p=0.035$) (Figure7B). To probe which treatment condition was driving the pre-T1 to post-T2 increase paired-sample t-tests (1-tailed) were used to test the hypothesis that MAP increased in each condition. The increase was significant in the water condition ($p=0.01$) and marginal in the sucralose condition ($p=0.06$), but there was no increase in the glucose

condition (p=0.35) (Figure 7C).

5.2.4 Correlations

The increase in the treatment-averaged mean arterial pressure (MAP) from baseline at all post-drink timepoints coincided with a significant effect of TIME on AURC.

SIG. AURC MEASUREMENT	CORRELATE MEASUREMENT	R SQUARED	P-VALUE
$T1_{SUCRALOSE}$	Δ Blood Glucose	0.007	0.998
	Δ Mean Arterial Pressure	0.371	0.366
	Sweetness	0.041	0.493
$T2_{SUCRALOSE}$	Δ Mean Arterial Pressure	0.499	0.313
	Sweetness	0.067	0.753
$T2_{130\text{-}160}$	Δ Mean Arterial Pressure	0.551	0.017
$T1_{160\text{-}200}$	Δ Mean Arterial Pressure	0.487	0.003
$T2_{160\text{-}200}$	Δ Mean Arterial Pressure	0.468	0.020

Table 4. Correlations between AURC data that were significantly different from baseline and glucose, mean arterial pressure and sweetness data that were significantly different from baseline (or water in the case of sweetness ratings). P-values are two tailed and correlations were assessed using Spearman's rho. Goodness of fit is also displayed for the linear regression line since residuals were found to conform to normality which was tested using Shapiro-Wilk's test.

As such, two-tailed nonparametric Spearman's rho correlations were used to probe a potential association between increases in treatment-averaged MAP (i.e. difference from baseline) and the relative changes in the treatment averaged (i.e. intensity binned) AURC. To decrease the number of correlations, and thus the likelihood of type 1 error, pre- and post-TMS values were averaged when a paired-

sample t-test indicated no difference between the two means. This was the case for MAP values measured before and after the T1 testing bout (p=0.268). For T2, correlations were run separately for pre- and post-TMS MAP due to a marginal difference (p=0.06). This approach dictated that a total of three correlations were tested for each dependent variable. Furthermore, correlations were assessed for the intensity-averaged data when both MAP and AURC were significantly different from baseline. This approach resulted in a total of seven correlations performed on the AURC. Bonferroni correction was applied to p values accordingly All tested correlations are shown in Table 4. MAP was significantly correlated with the relative change AURC of the upper slope bin at T2 (Figure 7D), and with the AURC of the plateau bin at both T1 (Figure 7E) and T2 (Figure 7D).

5.3 Short-interval Intracortical Inhibition

Two way repeated-measures ANOVA revealed no effect of TIME ($F_{(2,17)}$=1.693; p=0.204) or TREATMENT ($F_{(2,17)}$=1.455; p=0.252), and no TIME*TREATMENT interaction ($F_{(2,17)}$=1.427; p=0.238) . Wilcoxon matched pairs sign ranked test did not

reject the null hypothesis that SICI was unchanged after glucose ingestion at T1 or T2 relative to T0.

6. DISCUSSION

Contrary to the present hypotheses, this study detected no effect of glucose on corticospinal excitability or short interval intracortical inhibition. However, a 3-way ANOVA did detect interactions of time with treatment and intensity. Surprisingly, sucralose increased AURC while glucose and water did not (Figure 6A). This increase in MEP size after sucralose has not prevously been reported and is difficult to interpret. While there was a significant elevation in MAP in the sucralose condition, this was also the case after water and glucose and the increase was not correlated with the relative increase in AURC.

Interestingly, mean arterial pressure was positively correlated with the relative change in AURC at higher stimulus intensities when the data were averaged across treatments (Figure 7), suggesting a potential association between for SNS or HPA axis activity more generally. The absence of a correlation between changes in glucose level and change in MEP size was also notable since the relationship was observed by Specterman and colleagues (2005). The implications of several aspects of the present testing protocol can be scrutinized to interpret the findings.

6.1 Hydration and Blood Pressure

All participants consumed 300 ml of water on each visit and were also asked to fast for 10 hours prior to all testing sessions. However, the degree to which they voluntarily restricted their water intake prior to the session was not controlled or recorded. The effect on mean arterial pressure could conceivably be impacted by either rehydration from a dehydrated state or hydration relative to a normal state. Since there was a positive correlation between mean arterial pressure and the change in AURC, whether hydration could have impacted blood pressure is of some interest.

While rehydration has been shown to influence mood and cognitive performance (Zhang et al., 2019), there is little evidence to suggest that rehydration or hydration can significantly change blood pressure. For example, a recent study by Zhang and colleagues (2019) found no change in systolic or diastolic blood pressure in a sample of 12 healthy males who fasted for more than 12 hours before rehydration with 1500 mL of water. An earlier study found that hydration with 600 mL of water after being hydrated at baseline caused no change in blood pressure (Rebello et al., 1982). Because our solutions were only 300 ml, and our participants were not directed to dehydrate themselves at baseline, it is unlikely that the blood pressure change was associated with hydration.

6.2 TMS pulse load

The present protocol delivered approximately 180 TMS stimuli at a rate of 0.2 Hz during each of 3 testing bouts lasting 30-45 minutes. In contrast, Specterman and colleagues (2005) delivered only 43 stimuli at baseline and 15 during each post-intervention bout. The effect of prior TMS on MEP size has not been thoroughly investigated. For example, one study by Biabani and colleagues (2018) demonstrated that MEP size has strong intra-session reliability, but only 10-35 pulses were delivered at lower intensities than the were used here. There is insufficient evidence in the literature to exclude the possibility that the TMS protocol in this experiment supressed or masked the expected glucose-induced rise in MEP size. In addition, there is ample evidence that transcranial brain stimulation has a measurable impact on the regulation of blood pressure and glucose tolerance, both of which are central to the interpretation of these results.

6.2.1 Acute effects of TMS on Autonomic Tone

Several studies have reported that TMS can influence sympathetic adrenomedullary (SAM) system and sympathetic nervous system (SNS) drive (Hong et al., 2002; Macefield et al., 1998; Yoshida et al., 2001). In 1997, Macefield and colleagues demonstrated that a single high intensity TMS pulse (85-100% of maximum stimulator output) over the vertex of the cranium supressed sympathetic discharge in the motor fascicles of the peroneal nerve 200 to 600 milliseconds after the stimuli (Macefield et al., 1998). Furthermore, Hong and colleagues (2002) reported that acute 10 Hz rTMS of the

brain reduced mean blood pressure by ~20 mmHg in rats. In this experiment, the researchers used a pharmacological approach in concert with the rTMS intervention to show that this was associated with a decrease in SNS activity (Hong et al., 2002). Heart rate variability (HRV), another index of autonomic nervous system activity, is also altered by rTMS in humans. Yoshida and colleagues (2001) delivered 70 subthreshold TMS stimuli (90% of RMT) at 0.2 Hz or sham TMS and used power spectrum analysis to quantify HRV. The low-frequency component of the HRV measured while participants were standing was elevated after rTMS but not after the sham treatment (Yoshida et al., 2001). Because this component is associated with SNS output, the authors concluded that low frequency rTMS likely increases sympathetic tone (Yoshida et al., 2001). Collectively, these reports suggest that TMS can influence sympathetic tone and blood pressure and that even low frequency TMS (i.e. 0.2 Hz, also used in this protocol) could have an impact.

6.2.2. Long-term effects of TMS on Autonomic Tone

Because there are no reports in the TMS literature describing effects of stimulation at longer latencies than a few minutes, it is necessary to consider the effects of brain stimulation more generally. The modulation of autonomic tone and blood pressure by tDCS, has conveniently been described over a time course that corresponds nicely to that of the present study (Binkofski et al., 2011). Like TMS, tDCS acutely decreases HPA axis activity, but it also has a longer-lasting influence on glucose uptake and brain energy metabolism which overlaps suggestively with the testing timeline of the present study

(Binkofski et al 2011). Binkofski and colleagues (2011) reported an increase in cortical ATP consumption 65 minutes after the end of tDCS, and a biphasic change in glucose uptake exhibiting a trending decrease followed by a significant increase with the inflection occurring ~75 minutes after tDCS (Binkofski et al., 2011). These correspond to the beginning and middle of the T2 testing bout respectively (Figure 3B).

In measuring glucose uptake, Binkofski and colleagues (2011) used a euglycemic clamp technique which maintained blood glucose between 4.5 and 5.5 mmol/L. The context of their findings is therefore more like that of the water condition in the present study than the glucose condition (Figure 3B), although HPA-regulated liver gluconeogenesis and insulin suppression maintained normoglycemia rather than exogenous glucose infusion. Indeed, the significant decrease in blood glucose observed prior to T1 in the water condition may have been enough to elicit sympathetic response that could explain the observed increase in blood pressure in the water condition. Given the timeline described by Binkofski and colleagues (2011), it is conceivable that a TMS-induced increase in ATP turnover and the associated rise in cerebral glucose uptake are responsible for the initial decrease in blood glucose.

6.2.3 Interaction of TMS with Glucose

As outlined in previous sections, it is possible that TMS in this protocol caused short-term and/or long-term effects on autonomic nervous system activity. However, it is difficult to decipher how the predicted effects interact with each other and with the effects of

elevated glucose. Just as low blood glucose elicits an increased HPA response, the ingestion of a large bolus of glucose reduces cortisol levels (Reynolds et al., 2003). If HPA activity was indeed the upstream mediator of the observed changes in MEP size, then one might expect glucose to reduce AURC rather than facilitate MEP growth as demonstrated by Specterman and colleagues (2005).

It is also possible that downstream changes in vascular dynamics are important for the effect of glucose specifically. Glucose ingestion has been shown to increases systolic blood pressure for up to 2 hours (Rebello et al., 1983; Synowski et al., 2013). It was suggested that this could be related to an increase in circulating epinephrine and increased sympathetic drive to the heart muscle as in the overfeeding response previously observed in rats (Rebello et al., 1983). In this case, the acute SNS-suppressive effects of the T1 and T2 TMS bouts (~350 total pulses) may have had a blunting effect on glucose-induced blood pressure rise. Indeed, while MAP was elevated relative to baseline prior to T1 and after T2, there was no increase after T1 and no rise from the pre-T1 to the post-T2 measurement (Figure 7C).

However, the sucralose and water conditions exhibited trending or significant increases from pre-T1 to post-T2, in addition to significant increases from baseline at all timepoints. Because, increases in MAP occurred in the absence of a large carbohydrate bolus, and in the presence of the same TMS stimuli as the glucose condition, the proposed explanation may seem incongruous. Certainly, this interpretation relies on the idea that the increases in MAP in the glucose condition have a different cause than in the other

conditions. For example, while the HPA axis may respond to low glucose after increased cortical ATP consumption after TMS, this seems less likely when circulating glucose is high. Instead, a large glucose bolus was more likely to have increase blood pressure due to the previously described adrenergic response.

6.3. Limitations

The present study is limited by several aspects of its design. Most notably, the number of TMS pulses required to collect the measurements in this protocol exceeded that of previous studies reporting their intra-session reliability. Given that there is some research indicating that cortical brain stimulation can alter SNS output and other aspects of brain metabolism, it is possible that the TMS measurements themselves influenced the results. This study also excluded females to preserve a homogeneous study sample and avoid potential the effect of menstrual cycle. Unfortunately, this also limits the generalizability of the present findings. Lastly, while the water was intended to provide a control for the passage of time and fluid consumption, adding an additional condition where participants receive no fluid treatment would have provided a more effective control for effects of TMS and the passage of time.

7. CONCLUSIONS

The present study found no effect of glucose on corticospinal excitability or inhibitory intracortical excitability. However, there was a surprising ~20% increase in corticospinal

excitability after sucralose. A data-driven analysis of these data indicated that changes in MEP size are related to changed in mean arterial pressure, suggesting some autonomic involvement in the observed effect.

This experiment did not find affirmative evidence supporting the implementation of rigorous glucose monitoring or controls when using TMS measures. However, the present findings do merit further investigation and it is suggested that future studies monitor diet, before and during testing, and consider restricting the number of TMS pulses delivered. Taking these steps will reduce the likelihood of confounding effects of changing brain metabolism or autonomic modulation complicating the interpretation of findings.

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