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RESEARCH ARTICLE

Higher Neural Functions and Behavior

Causal role of frontal-midline theta in cognitive effort: a pilot study

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Abstract

Frontal-midline theta (FMT) oscillations are increased in amplitude during cognitive control tasks. Since these tasks often conflate cognitive control and cognitive effort, it remains unknown if FMT amplitude maps onto cognitive control or effort. To address this gap, we utilized the glucose facilitation effect to manipulate cognitive effort without changing cognitive control demands. We performed a single-blind, crossover human study in which we provided participants with a glucose drink (control session: volume-matched water) to reduce cognitive effort and improve performance on a visuospatial working memory task. Following glucose consumption, participants performed the working memory task at multiple time points of a 3-h window to sample across the rise and fall of blood glucose. Using high-density electroencephalography (EEG), we calculated FMT amplitude during the delay period of the working memory task. Source localization analysis revealed that FMT oscillations originated from bilateral prefrontal cortex. We found that glucose increased working memory accuracy during the high working memory load condition but decreased FMT amplitude. The decrease in FMT amplitude coincided with both peak blood glucose elevation and peak performance enhancement for glucose relative to water. Therefore, the positive association between glucose consumption and task performance provided causal evidence that the amplitude of FMT oscillations may correspond to cognitive effort, rather than cognitive control. Due to the COVID-19 pandemic, data collection was terminated prematurely; the preliminary nature of these findings due to small sample size should be contextualized by rigorous experimental design and use of a novel causal perturbation to dissociate cognitive effort and cognitive control.

NEW & NOTEWORTHY We investigated whether frontal-midline theta (FMT) oscillations tracked with cognitive control or cognitive effort by simultaneous manipulation of cognitive control demands in a working memory task and causal perturbation of cognitive effort using glucose consumption. Facilitation of performance from glucose consumption corresponded with decreased FMT amplitude, which provided preliminary causal evidence for a relationship between FMT amplitude with cognitive effort.

cognitive control; cognitive effort; EEG; frontal-midline theta; glucose facilitation effect

INTRODUCTION

Frontal-midline theta (FMT) oscillations (4–8 Hz) are commonly observed in cognitive control tasks (1–3). In observational studies, FMT oscillations have been linked to both cognitive control (2) and cognitive effort (4). Cognitive control is the ability to guide perception and behavior toward internal goals (5–7) and is known to be limited in its capacity, e.g., the number of items that can be held in working memory (8) or decreased performance with divided attention (9). Cognitive effort is the strain of recruiting cognitive control over a prolonged period of time and is also known to be limited based on evidence of reduced accuracy and increased reaction time in cognitive control tasks across an experimental

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session (10). However, cognitive control and cognitive effort are often conflated in experimental designs that utilize a single task contrast (see Ref. 10 for review). For example, experimental paradigms with two conditions will often increase the task difficulty (i.e., cognitive effort processing) and engage cognitive control (i.e., as opposed to purely bottom-up processing. Furthermore, the time course of behavior, let alone neural activity, across an experimental session if often ignored. Thus experiments that dissociate cognitive control and cognitive effort with a causal intervention are required to better characterize the role of FMT oscillations.

One approach to differentiate cognitive control and cognitive effort is to manipulate task difficulty and cognitive control demand in two different experimental variables. For example, a functional magnetic resonance imaging (fMRI) study used working memory load to drive cognitive control demands and visual degradation to increase perceptual difficulty, which lowered accuracy (11). This experiment found different regions that tracked with cognitive control demands (lateral prefrontal cortex) and task difficulty (anterior cingulate cortex). Numerous studies controlled for "task difficulty" to investigate cognitive control processes particularly in lateral prefrontal cortex (see Refs. 12, 13 for example). However, tasks that increase difficulty in a manner that cannot be overcome with greater cognitive effort, such as visual degradation, have been challenged because participants might not increase cognitive effort as a function of task difficulty (10).

Another approach to study cognitive effort is to drive cognitive control over an extended task period and analyze time-dependent changes in behavior and neural activity. In cognitive control tasks that are administered over the course of an hour or more, the amplitude of task-driven FMT oscillations progressively increases, presumably tracking with cognitive effort (4). In addition to increased amplitude of FMT oscillations, sustained cognitive effort depletes blood glucose levels proportional to the amount of cognitive control required to perform the task (14, 15). Therefore, circulating glucose might provide a physiological signal for expended cognitive effort and a potential avenue for causally manipulating cognitive effort. Indeed, by providing glucose, there is a temporary improvement in performance of cognitive control tasks (see Ref. 16 for meta-analysis) including attention (17), visual discrimination (18), and working memory (19). Furthermore, changes in blood glucose levels after glucose consumption are predictive of improved behavioral performance especially during tasks that require cognitive control (15). Across several meta-analyses (16, 20, 21), glucose facilitation is a robust effect, particularly for cognitive control tasks that recruit short-term (i.e. working) and long-term memory, and this evidence suggests that glucose manipulation can be used as a reliable driver for decreasing cognitive effort.

However, there is no study to our knowledge that increased blood glucose levels by administration of a glucose drink and measured its impact on the amplitude of FMT oscillations in a cognitive control task. The present study utilized an experimenter-blinded crossover design where the administration of a glucose drink was used to replenish the blood glucose resources typically depleted during cognitive control tasks (14, 15), thus presumably decreasing cognitive effort while increasing cognitive control. If experimentally boosting cognitive control by glucose consumption resulted in an increase in FMT amplitude, then this would provide evidence that FMT amplitude tracks with the recruitment of cognitive control. Alternatively, if glucose consumption resulted in a decrease in FMT amplitude, then this would provide evidence that the amplitude of FMT oscillations tracks with cognitive effort. Participants performed a visuospatial working memory task with a high and low load condition to manipulate cognitive control demands and to isolate FMT oscillations during the delay period of the task. The task was repeated six times over a 3-h period following administration of the glucose drink. We hypothesized that the amplitude of FMT oscillations would increase over the course of this 3-h time period consistent with a gradual increase in cognitive effort. Finally, the primary analysis was whether glucose consumption would increase or decrease the amplitude of FMT oscillations during a working memory task when blood glucose levels were elevated.

MATERIALS AND METHODS

The present study utilized the glucose facilitation effect to causally increase performance presumably by reducing cognitive effort without changing task parameters related to cognitive control to investigate whether frontal-midline theta (FMT) amplitude tracked more closely with cognitive effort (decreased FMT amplitude) or cognitive control (increased FMT amplitude). A 75-g glucose drink (Lemon Lime flavor, Azer Scientific, Morgantown, PA) was administered in an experimenter-blinded crossover design to manipulate blood glucose levels with volume-matched water as a control. Participants performed a visuospatial working memory task at multiple time points over the 3-h period following consumption. Before the experimental sessions, participants attended a screening session in which the working memory task was titrated in difficulty based on performance. Titration was performed such that the amplitude of FMT oscillations during the delay period reflected the exertion of cognitive effort when cognitive control was engaged. Importantly, the working memory task consisted of two conditions that required high and low cognitive control to isolate the FMT oscillations hypothesized to be driven by cognitive control demands. The screening visit was also used to exclude participants with an abnormal resting glucose level after fasting for 12 h. In addition, a structural MRI was acquired for source localization. Eyes-open resting-state EEG was acquired at each time point to assess whether the modulation in FMT amplitude was specific to the cognitive control task.

The study was approved by the institutional review board at the University of North Carolina at Chapel Hill, and data were collected at the Carolina Center for Neurostimulation. This experiment was a substudy within a parent experiment that was preregistered on ClinicalTrials.gov (NCT04031404). The parent study investigated the impact of glucose on cortical excitability as assessed by single-pulse transcranial magnetic stimulation (TMS). The single-pulse TMS protocol involved 10 pulses, separated by 1,000 ms each, with intensity at or below 120% of resting motor threshold. These pulses were delivered before the drink administration and at each of the five follow-up time points (i.e. 0, 30, 60, 120, and 180 min postdrink). Several minutes elapsed between single-pulse TMS and the working memory task. Note that TMS was used

for its ability to probe cortical excitability (22) and single-pulse TMS is known to have no lasting effects on the brain (23).

Participants

Twenty-three participants were enrolled in the study and provided written informed consent. Participant eligibility was determined during the first study visit, in which participants fasted 12 h before the session to calculate fasting blood glucose levels and to titrate the difficulty of the working memory task. Participant inclusion criteria were as follows: *1*) between 18 and 65 yr old; *2*) right-handed; *3*) body mass index <30; *4*) fasting blood glucose levels <95 mg/dL; and *5*) no history of major medical, neurological, or psychiatric illness. Individuals with a fasting blood glucose level >95 mg/dL were excluded because this blood glucose level approaches the level of prediabetes, and previous studies have established that those with diabetes experience altered glucose metabolism (24). Additionally, female participants were required to test negatively for pregnancy. Seven of the 23 participants failed the blood glucose level screening and two were lost to follow-up. Participants were asked to maintain a regular sleep schedule and fast for 12 h before experimental visits. All participants received monetary compensation for their time. Data collection was terminated prematurely due to the COVID-19 pandemic. At the time of study termination, 10 of 15 participants had completed the study, and 1 of these participants was excluded from analysis due to technical reasons. Four participants that had not completed all four experimental sessions when the study was terminated were excluded from analysis. The nine participants included in the analysis (7 female) were ages 19–62 yr with a means ± SD of 25.7 ± 12.5 yr.

Blood Glucose

The oral glucose tolerance test involves the consumption of a 75-g glucose drink to causally upregulate blood glucose levels with periodic monitoring of blood glucose levels after consumption (25). In accordance with standard procedure for the oral glucose tolerance test, participants were instructed to fast for 12 h before their experimental sessions. The fasting requirement minimizes glucose fluctuations caused by recent, uncontrolled sugar consumption outside of the study protocol. Participants were assigned to receive either the glucose or water drink in a crossover design that was randomized and counterbalanced in two separate visits (5 participants received glucose first and 4 received water first) that were separated by a minimum of 3 days. The glucose consumption triggered the rise and fall blood glucose levels, following either a monophasic or biphasic pattern (26). Glucose levels were expected to return baseline levels in healthy individuals 2 to 3 h after consumption.

Capillary blood draws were taken for each participant before drink consumption and at five follow-up time points (i.e. 0, 30, 60, 120, and 180 min postdrink), and the blood glucose content of each sample was measured with a glucose level analyzer (HemoCue Glucose 201 System; HemoCue America, Brea, CA). Before the first blood sample was analyzed each day, a two-point calibration was conducted with control solutions containing known glucose concentrations to ensure proper device functioning. Glucose level (mg/dL) and the exact time of blood draw at each time point were recorded on an encrypted server using data management software, REDCap (27), provided by University of North Carolina Chapel Hill School of Medicine.

Glucose levels at each time point were baseline corrected using the glucose level before drink consumption: $\frac{post-pre}{pre}$. Participants performed a working memory task ~15 min after the capillary blood draw at each time point. To address the difference in time from blood draw to working memory task, we fit a three-degree polynomial to the blood glucose levels across time with one additional point that represented a return to baseline after 4 h. Missing data points were omitted from the analysis. Given the predictability of blood glucose level changes over time, the polynomial fit was used to interpolate the blood glucose level at the time points that the participant completed the working memory task.

Student's pairwise t tests were performed at each time point after drink consumption to localize the time points at which blood glucose was significantly elevated for glucose drink relative to water. A significance threshold of $P < 0.05$ was used with Bonferroni correction for the five tests (adjusted alpha of 0.01). To reduce multiple comparisons in subsequent analyses, only time points with elevated blood glucose were used to evaluate the glucose facilitation effect on working memory accuracy and the amplitude of FMT oscillations.

Visuospatial Working Memory Task

For all EEG recordings, participants were comfortably seated and a chin rest was used to maintain a fixed distance from a 120 Hz LCD monitor. Participants performed a visuospatial working memory task at every time point to estimate changes in accuracy and FMT amplitude from glucose consumption. The working memory task involved the maintenance of an array of colored squares, similar to other tasks designed to drive cognitive control in pervious literature (28). The task consisted of three epochs (Fig. 1*A*). In the encoding epoch, participants were presented with an array of colored squares for 500 ms and were instructed to remember the location and color of every square. The array consisted of a variable number of colored squares (width of 1.25° visual angle, 9 possible colors) that were presented randomly in 12 equidistant locations around a circle (eccentricity of 6° visual angle) centered on a white fixation point (width of 0.2° visual angle). Half of the trials were high working memory load and half were low working memory load, and working memory load was intermixed and randomized. High load consisted of two more squares than low load, and the load for each participant was titrated based on task performance in a baseline session. After the encoding period was a delay of 2,000 ms. In the delay epoch, a small white fixation point was presented at the center of the screen and participants were instructed to maintain fixation, not to blink, and to hold the location and color of each square from the encoding array in mind. After the delay epoch, participants were probed on their memory for an item within the encoding array. In the probe epoch, an array of empty white outlines of squares was presented in the same locations as the encoding array. A single square in the probe was filled with a color and participants were instructed to indicate whether the

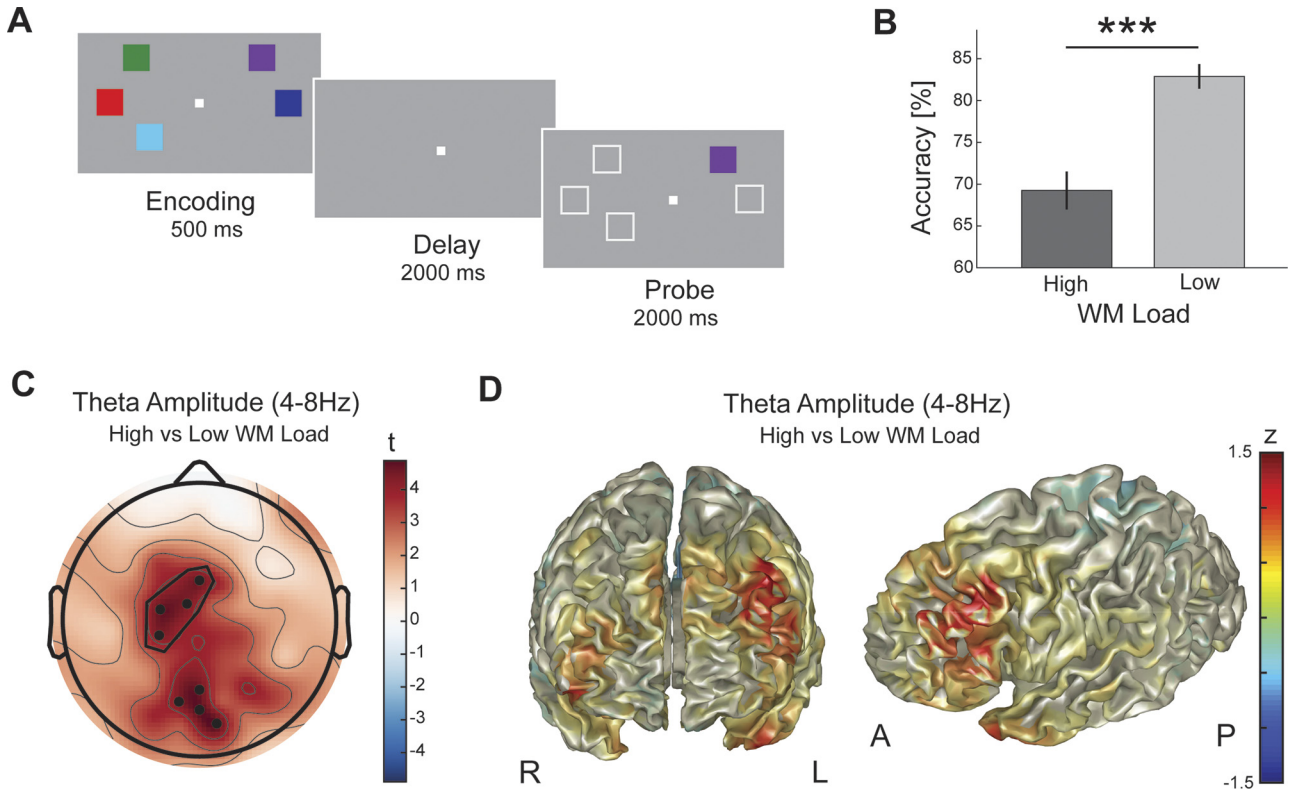

Figure 1. Working memory task and frontal-midline theta oscillations. *A*: the visuospatial working memory (WM) task comprised 3 epochs: encoding (500 ms), delay (2000 ms), and probe (2000 ms). After encoding a variable number of colored squares (high load or low load), participants indicated whether the probe square was matched in color and location to the corresponding square in the encoding array with a button press. *B*: after titration, accuracy was decreased for high load relative to low load. Error bars are SE. ***$P < 0.001$ *C*: a single tail *t* test was performed at each of the scalp electrodes to compare theta amplitude for high versus low load. Black dots are significant electrodes (multiple comparisons adjusted $P < 0.05$, raw $P < 0.002$). Region of interest over frontal-midline used for future analyses is outlined. *D*: spectral source localization using structural MRI data revealed that the increased amplitude of frontal-midline theta oscillations identified by topographic analysis originated in bilateral dorsolateral prefrontal cortex. *Left*: a coronal view of the frontal cortex. R, right; L, left. *Right*: a sagittal view of the lateral left hemisphere. A, anterior; P, posterior.

color of that square was a "match" or "nonmatch" with the corresponding square in the encoding array. Half of the trials were match and half were nonmatch randomized. Of the nonmatch trials, half of the trials included a novel color not seen in the encoding array and the other half of trials included a color from a different location in the encoding array. Participants were given 2,000 ms to make a response and the probe remained on the screen for the full 2,000 ms. Participants used their index fingers to indicate whether the square was the same ("match") or different ("nonmatch") as the square in the encoding array via a button press. The hand used for each response was consistent for all experimental sessions and was randomized and counterbalanced across participants. After the response, participants briefly rested during an intertrial interval. At the start of the experiment, participants were instructed to blink between trials if possible and to avoid blinking during the delay period. The length of the intertrial interval was jittered between 2 and 3 s to reduce prediction of when the next trial would start.

At each of six time points (pre, 0 min, 30 min, 60 min, 120 min, and 180 min), participants completed 2 blocks of 40 trials each which amounted to ~4.7 min of task per block. Participants were allowed a self-paced rest period between successive blocks that was typically <30 s. Thus, each participant completed 40 high load trials and 40 low load trials at each time point. With 12 total time points per participant and 80 trials per time point, each participant completed a total of 960 trials. Despite the relatively low number of participants due to the COVID-19 pandemic, the within-participant design and sufficient power at each time point (40 trials per condition and 960 trials total) is a strength of the experimental design. Trials in which the participant failed to make a response during the 2-s probe epoch were removed from behavioral and EEG analysis. Working memory accuracy was calculated as the average performance for high and low load of each time point.

The optimal working memory load for each participant was titrated during an initial baseline visit. The titration process categorized participants as either low, average, or high performers and this categorization determined their working memory loads during the experiment: 3 and 5, 4 and 6, or 5 and 7. Task titration was implemented to optimize engagement by avoiding floor (accuracy near chance <60%) and ceiling effects (>90% accuracy); therefore, titration ensured that all participants were cognitively engaged and that glucose consumption would be able to influence accuracy. For both experimental sessions with a drink, participants performed the task with the same high and low load.

Eyes-Open and Eyes-Closed Resting-State

To track changes in background FMT amplitude, 3 min of eyes-open and 2 min of eyes-closed resting-state EEG was recorded at every time point. During the eyes open condition, participants were instructed to maintain fixation on a central fixation cross, to relax their body, and to let their mind wander naturally. Each resting-state period preceded the visuospatial working memory task. The data were preprocessed separately from the working memory task, and only the eyes-open periods were analyzed to assess FMT amplitude, given that posterior alpha oscillations dominate the EEG during the eyes-closed state.

Frontal-Midline Theta Amplitude in Working Memory

During the resting-state recording and task, EEG was recorded with a high-density 128-channel electrode net (HydroCel-128; Electrical Geodesics, Inc., Eugene, OR) at 1,000 Hz and an EGI amplifier (NetAmps 410, Electrical Geodesics, Inc.). The impedance of each electrode was required to be <50 kΩ before the start of data collection. Electrode impedance was not assessed after this point, but our group has previously used gel electrodes for temporally extended recordings, such as overnight (29). EEG preprocessing was conducted using custom scripts written in MatLab and the EEGLAB toolbox (30). EEG data were downsampled to 250 Hz with antialiasing filtering and band-pass filtered from 1 to 50 Hz. The data underwent artifact subspace reconstruction to remove high-variance signal and reconstruct missing data (31). Channels with noise above threshold were spherically interpolated. All data were common average referenced. Manual trial rejection was conducted that removed trials with excessive noise during the delay epoch (an average of 5.3% of trials removed per participant with standard deviation of 2.0%). Independent component analysis was performed to identify signal components that corresponded to channel noise, eye blinks, eye movements, muscle activity, and heartbeats (32). These components were visually inspected and manually removed from potential neural activity.

FMT amplitude was hypothesized to increase as a function of cognitive control demands. Thus, we investigated the 2-s delay epoch and applied the Fast Fourier transform to the electrical signal of all channels. Theta amplitude was calculated as the average of frequencies within the canonical theta band (4 to 8 Hz). To determine the electrodes with increased theta amplitude in the frontal-midline as a function of cognitive control, we ran a region of interest localizer using the time point before consumption of both drinks. The region of interest was defined using the baseline sessions to reduce multiple comparisons in the statistical analysis for the glucose facilitation effect. Pairwise Student's t tests for theta amplitude of high versus low working memory load was run for each of the 90 channels on the scalp. A significance threshold was defined as a $P < 0.05$ after Bonferroni multiple-comparisons correction for 90 channels and cluster correction of four: $(\alpha = \left(\frac{0.05}{90}\right) * 4)$. Thus the region of interest was defined as a contiguous cluster of four electrodes or more in the frontal-midline (near FCz) with greater theta amplitude for high versus low load (one-tail, adj-$P < 0.002$, $k = 4$). Two clusters were identified with this method of selection: one in prefrontal cortex and one in the parietal-occipital region. Since we were interested in the top-down processing associated with prefrontal cortex (2, 33, 34), we restricted our analysis to the cluster of electrodes within the prefrontal cortex. As a confirmation that our contrast for region of interest localization (high vs. low working memory load) was qualitatively similar to each of the conditions independently, we calculated the topographic distribution of theta amplitude for high and low working memory load versus one second of the intertrial interval after the end of each trial. This topographic plot revealed a spatially overlapping increase in theta amplitude over the same frontal-midline electrodes. The average theta amplitude from these frontal-midline electrodes was defined as FMT amplitude for all time points during both the working memory task and eyes-open resting-state recordings. FMT amplitude was baseline corrected using the FMT amplitude before drink consumption: $\frac{post-pre}{pre}$, and these values were submitted to statistical analysis for the effects of glucose on FMT amplitude.

The high-density EEG nets paired with individual anatomical MRIs allowed us to perform source localization analysis to determine the spatial origination of FMT oscillations. Anatomical scans were T1-weighted and collected at the University of North Carolina Biomedical Research Imaging Center using a 3-Tesla Siemens MAGNETOM Prisma scanner (Siemens AG, Berlin, Germany). Source localization was run using the Dynamical Imagine of Coherent Sources beamformer algorithm implemented in the FieldTrip toolbox (35). For each 2-s delay epoch of baseline EEG, we calculated the spectral amplitude for each channel via five-cycle Morlet wavelet convolution at 6 Hz separately for high and low load conditions. Next, we calculated the cross-spectral density matrix to estimate the phase difference and shared amplitude of each channel pair for source localization. The beamformer algorithm was run using a lead field calculated in each participant's anatomical space using skull, skin, and brain tissue estimates calculated using the segmentation function in SPM12 (36). Theta amplitude was source localized into the anatomical space of each participant for each 2-s epoch and then averaged across epochs. The theta amplitude estimates from source localization were not normally distributed. Therefore, the data were log transformed. The data were normalized into the Montreal Neurological Institute (MNI) space using warp estimates calculated from normalizing the anatomical image into the MNI template brain in SPM12. Then, we applied signal normalization using the z-transformation for all voxels within a gray-matter mask in MNI space derived from tissue segmentation estimates provided by the SPM12 toolbox. For display purposes, a pairwise t test between theta amplitude for high and low load was run for every voxel in source space. Analyses were run using custom code written in MatLab using functions from SPM12 and Fieldtrip (see Ref. 37) for another study that used a nearly identical approach).

Statistical Approach

First, three confirmatory analyses were performed to ensure that the experimental manipulations were effective. To confirm that the working memory task was more difficult

for the high load condition, we ran a pairwise Student's *t* test between baseline accuracy (average of both drinks) for high versus low working memory load. To confirm that blood glucose and working memory accuracy increased for the glucose drink relative to water drink, we ran a two-way ANOVA with factors drink and time. These analyses ensured that the glucose drink successfully increased blood glucose levels and that the glucose facilitation effect was observable in the working memory task. Time points with a significant increase in blood glucose level were used in the analysis of working memory accuracy.

Additionally, as participants performed the working memory task over an extended period of time, cognitive effort was hypothesized to be increased over time irrespective of the drink administered. The increase in cognitive effort could manifest as a gradual decrease in accuracy and/or an increase in FMT amplitude with time. Therefore, linear fits were run for both FMT amplitude and accuracy with time. To test for an impact of time on FMT amplitude and accuracy irrespective of drink, the slope of the fit line for both sessions was averaged and tested versus zero using Student's *t* test. To test whether the glucose drink altered the relationship between FMT amplitude or accuracy with time, a pairwise Student's *t* test was run between the slope of the linear fit of each drink. This analysis was also applied to the resting-state FMT amplitude over time to ensure that observed changes were specifically task driven and not the result of changes in the amplitude of background FMT oscillation. Critically, we may not find an effect of glucose drink on the change of FMT amplitude or accuracy over time, but we may still find an immediate effect of glucose drink only on those time points at which blood glucose levels are elevated and not when levels fall.

The primary statistical analysis was whether FMT amplitude increased or decreased as a function of the glucose facilitation effect. Since glucose was hypothesized to reduce cognitive effort regardless of working memory load, a two-way ANOVA of FMT amplitude with factors drink and time was run for high and low working memory load. An increase in FMT amplitude by glucose consumption would provide causal evidence that FMT amplitude tracks with cognitive control, whereas a decrease in FMT amplitude by glucose consumption would provide evidence that FMT amplitude tracks with cognitive effort. This analysis was run for the time points that demonstrated significant increases in blood glucose levels as a result of the intervention. This same analysis was applied for resting-state FMT amplitude to ensure that the effects were specifically task driven. As a control analysis, we hypothesized that this effect would be independent of the gradual increase in FMT amplitude with time. Thus a pairwise Student's *t* test between the slope of the linear fit of FMT amplitude and time was run for glucose versus water consumption with the hypothesis that there would be no difference.

As an exploratory analysis, we ran a Pearson's correlation to assess whether the degree to which glucose modulated accuracy was proportional to its modulation of FMT amplitude. This analysis provides additional evidence that the impact of glucose on task performance and FMT amplitude were related. During this correlational analysis, we captured the rise and fall of blood glucose levels by including all five time points (~15, 45, 75, 135, and 175 min after the drink administration) as opposed to the two-way ANOVA which analyzed only the glucose facilitation effect versus water. The rationale is that this correlation was able to capture the effects of varying glucose levels; thus, the use of all available time points provided a more comprehensive analysis and increased statistical power. This correlation analysis was performed as a fixed effects model given that the low number of participants in our data set due to the COVID-19 pandemic reduced statistical power for a random effects factor. To address the impact of outlier data points on this correlation in our fixed effects approach, we performed a post hoc Spearman's correlation and qualitatively compared the resulting effect size.

All ANOVAs were run using the "lme4" toolbox (38) in R (The R Foundation), and an alpha of 0.05 was considered to be significant. It should be noted that our primary statistical analysis consists of a single two-way ANOVA for the effect of glucose drink on FMT amplitude during the high working memory load condition. Thus multiple comparisons correction is not required for this analysis as this approach strategically reduced comparisons by focusing on time points with a significant increase in blood glucose and a single region of interest that showed a significant increase in FMT amplitude with task demands at baseline. All ANOVAs were run under a fixed effects model due to the low number of study participants, as data collection was suspended prematurely due to the COVID-19 pandemic in the spring of 2020. Therefore, we stress that these results are preliminary, due to the low sample size and resulting use of a fixed effects model.

Code and Data Availability

All scripts and data used in this analysis are available on the Open Science Framework (https://osf.io/vuyjr/).

RESULTS

The goal of our study was to investigate whether the amplitude of frontal-midline theta (FMT) oscillations correspond to cognitive effort or cognitive control using a causal manipulation (glucose drink vs. water) to increase performance in a cognitive control task, presumably by reducing cognitive effort and leaving cognitive control demands fixed. A visuospatial working memory task was used to drive an increase in the amplitude of FMT oscillations in the delay period as a function of the number of items that must be maintained. Task difficulty was titrated in a baseline visit, and participants were screened to be within a normal range of blood glucose level. High-density EEG was acquired during task performance at multiple time points during the rise and fall of blood glucose after drink consumption. Structural MRIs were acquired for source localization of FMT oscillations.

Cognitive Control Demands Increased FMT Amplitude

Participants performed a visuospatial working memory task that consisted of three epochs: encoding of working memory items, maintenance of those items over a delay, and a probe to test for recall accuracy (Fig. 1*A*). Participants were able to perform the task and our titration method was effective at ensuring that performance was in an optimal range

for modulation by the glucose drink: average accuracy across high and low load was 76.03% with a standard deviation of 6.53%. Cognitive control demands were manipulated by the number of items to be held in mind. The manipulation was successful in that participants displayed decreased baseline accuracy for high working memory load (means ± SD) (69.25% ± 9.62%) relative to low working memory load (82.88% ± 6.26%) [$t(8) = 5.080$, $P = 0.000953$, $d = 1.693$] (Fig. 1*B*).

We analyzed the impact of cognitive control demands on neural activity by quantifying the amplitude of theta oscillations during the delay period of the working memory task. The delay period avoids the confounding influence of visual processing. To identify the electrodes in the frontal-midline that displayed increased theta amplitude, we used the contrast of high versus low working memory load in the time points before drink administration. After correction for multiple comparisons, this analysis yielded both an anterior and posterior cluster of electrodes (Fig. 1*C*). Our study was designed to investigate the frontal-midline; thus the electrode cluster over the frontal-midline (FCz and 3 adjacent electrodes) was used as the region of interest for future analyses. Although cognitive control demands drove an increase in theta amplitude over the frontal-midline electrodes in EEG, spectral source localization using individual structural MRI revealed that FMT oscillations originated from bilateral dorsolateral prefrontal cortex (Fig. 1*D*) similar to previous findings (37, 39). The amplitude of theta oscillations for high working memory load was particularly strong in the left middle frontal gyrus, spatially overlapping with previous fMRI studies showing increased activity in the left middle frontal gyrus (40).

Blood Glucose Level Rose and Fell after Glucose Consumption

A 75-g glucose drink was used as a causal manipulation to increase blood glucose levels (Fig. 2*A*). Pairwise t tests revealed that the glucose drink significantly increased blood glucose (multiple comparisons adjusted $P < 0.01$) at three task time points: at ~15 min [$t(8) = 5.268$, adj-$P = 0.000757$,

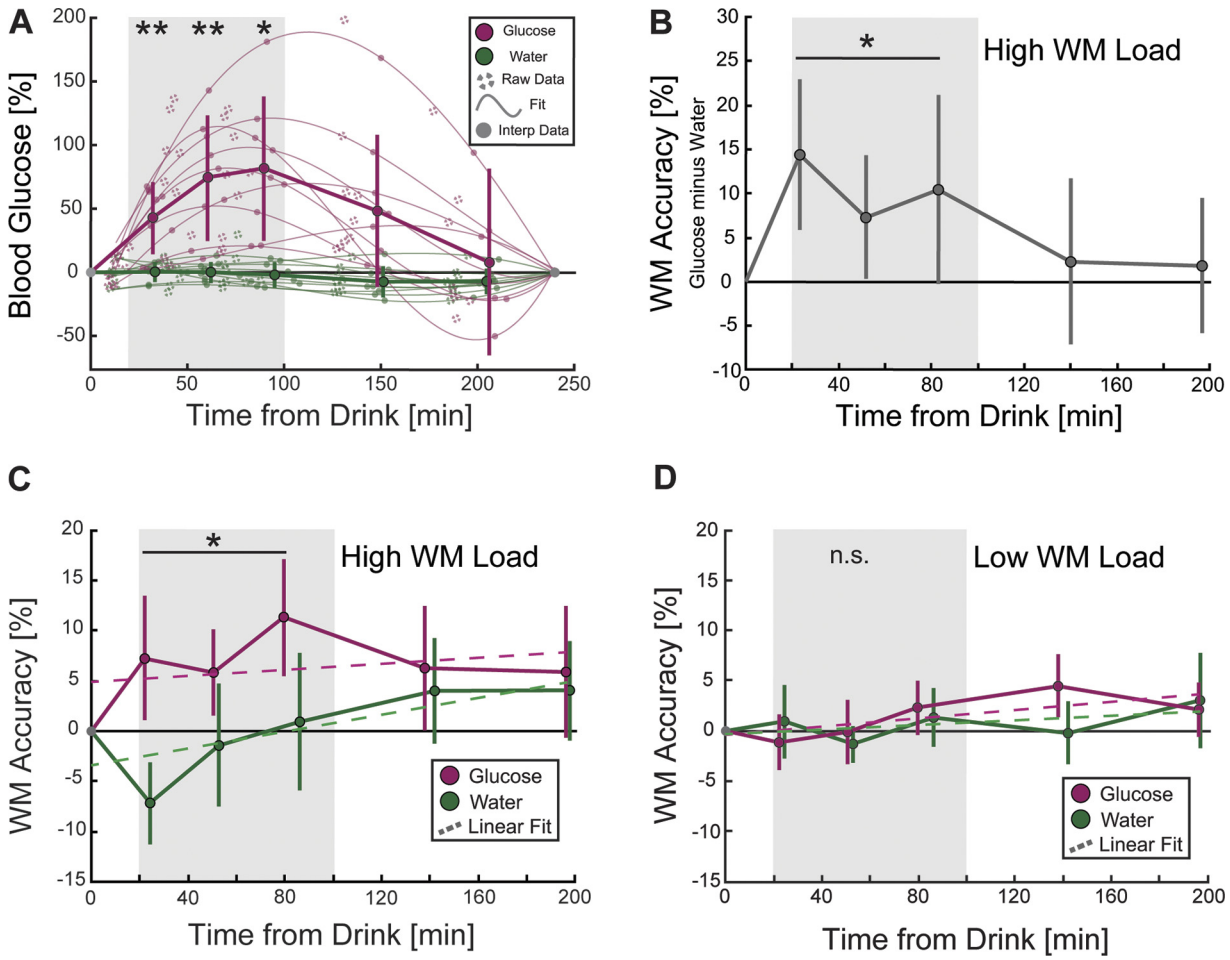

Figure 2. Impact of glucose consumption on blood glucose and task accuracy. *A*: individual traces show the 3rd-degree polynomial fit to the blood glucose level of each participant over time to interpolate blood glucose at the time of the working memory task. An increase in blood glucose level was found for 3 time points after correction for multiple comparisons. *$P < 0.01$, **$P < 0.001$. Error bars are 95% confidence interval. *B*: for the high load condition, participants performed with significantly greater accuracy with the glucose drink relative to water (gray line). Gray box indicates the time points with a modulation of blood glucose level (20 to 100 min). *Significance ($P < 0.05$) for a main effect of drink in two-way ANOVA. Error bars are SE. *C*: comparison of accuracy after the glucose drink (purple) versus water control (green) revealed a significant increase for the time points with elevated blood glucose level (gray box) at $P < 0.05$ tested as the main effect of two-way ANOVA. A linear fit of high load accuracy over time for each drink did not show significant relationship with time (dashed lines). Error bars are SE. *D*: for the low load condition, there was no effect of glucose on accuracy relative to water; n.s, not significant. In addition, there was no relationship between accuracy with time (dashed lines). Error bars are SE. WM, working memory.

$d = 1.756$], 45 min [$t(8) = 5.342$, $P = 0.000693$, $d = 1.781$], and 75 min [$t(8) = 5.026$, $P = 0.00102$, $d = 1.675$] after glucose consumption but not at 135 min [$t(8) = 2.925$, $P = 0.0192$, $d = 0.9749$] or 195 min [$t(8) = 0.761$, $P = 0.4753$, $d = 0.2877$]. Therefore, the initial three time points after glucose consumption were used to analyze the glucose facilitation effect in subsequent analyses.

Glucose Enhanced Working Memory Performance

To confirm the presence of the glucose facilitation effect in our data, accuracy on the visuospatial working memory task for the high and low load conditions was submitted to a two-way ANOVA with factors for drink (glucose or water) and time (first 3 time points). For the high load condition, we found a significant main effect of glucose drink on accuracy [$F(1,52) = 5.562$, $P = 0.0223$, $\eta_p^2 = 0.098$] but no main effect of time [$F(2,51) = 1.210$, $P = 0.2767$, $\eta_p^2 = 0.021$] or interaction effect [$F(2,51) = 0.126$, $P = 0.7236$, $\eta_p^2 = 0.002$] (Fig. 2*B*). The increase in accuracy relative to baseline for glucose versus water was on average 10.70% (±24.02%) such that glucose increased accuracy relative to water for the high load condition (Fig. 2*C*). The average accuracy for the high load conditions was 72.79% ± 7.34% with glucose (+8.15% ± 16.06% with baseline correction) and 67.29% ± 8.00% with water (−2.55% ± 17.04% with baseline correction).

For the low load condition, we did not find a significant main effect of glucose drink on accuracy [$F(1,52) = 0.000$, $P = 0.991$, $\eta_p^2 = 0.000$], time on accuracy [$F(2,51) = 0.446$, $P = 0.507$, $\eta_p^2 = 0.009$], or an interaction effect [$F(2,51) = 0.290$, $P = 0.593$, $\eta_p^2 = 0.006$] (Fig. 2*D*). Therefore, during the low load condition, glucose did not modulate task performance, as the increase in accuracy for glucose versus water was on average 0.0274% ± 5.97%. The average accuracy for the low load conditions was 83.49% ± 8.78% with glucose (+0.36% ± 8.59% with baseline correction) and was 82.86% ± 9.70% with water (+0.33% ± 8.47% with baseline correction). Together, these findings suggest that glucose had a specific impact on task performance only when cognitive control demands were high and more cognitive effort was required.

Task performance may have decreased over time as participants required more cognitive effort. To test for a potential effect of time, a linear fit between task performance and time was run. The average slope of this linear fit was calculated in percentage per hour for the high load (1.09% ± 1.95%) and low load (0.55% ± 1.71%). A *t* test versus zero confirmed that accuracy did not significantly change with time for either load condition averaged across both drinks [high load: $t(8) = 1.671$, $P = 0.1333$, $d = 0.5570$; low load: $t(8) = 0.963$, $P = 0.3638$, $d = 0.3210$]. The glucose drink could have theoretically modulated the change in task performance over time, although this effect was not hypothesized. A pairwise *t* test was run between the slope of the fit for time and accuracy for glucose versus water and found no significant difference for either load condition [high load: $t(8) = -0.064$, $P = 0.9506$, $d = -0.0213$; low load: $t(8) = 0.548$, $P = 0.5989$, $d = 0.1825$]. This analysis provided evidence that the impact of glucose on task performance was not via modulation of the relationship between task performance and time on task but instead was restricted to the window at which blood glucose levels were spiking.

Glucose Decreased FMT Amplitude

The primary outcome of this study was the effect of glucose on the amplitude of FMT oscillations. Two-way ANOVA with factors drink and time found a main effect of glucose on FMT amplitude for both high [−7.26% ± 11.02%; $F(1,52) = 4.547$, $P = 0.0379$, $\eta_p^2 = 0.077$] (Fig. 3, *A* and *B*) and low [−8.57% ± 10.52%; $F(1,52) = 8.263$, $P = 0.00593$, $\eta_p^2 = 0.128$] (Fig. 3*C*) load but not for resting-state [−0.31% ± 0.779%; $F(1,52) = 0.784$, $P = 0.380$, $\eta_p^2 = 0.015$] (Supplemental Fig. S1; see https://doi.org/10.6084/m9.figshare.16438992.v1). Therefore, the impact of glucose on FMT amplitude was only present during the working memory task that required cognitive effort and cognitive control. Post hoc pairwise *t* tests revealed that the glucose drink decreased the amplitude of FMT oscillations for the high load, although not significantly, [$t(8) = 1.975$, $P = 0.08365$, $d = 0.6584$] and significantly for low load [$t(8) = 2.442$, $P = 0.04043$, $d = 0.8140$]. These findings support the interpretation that the amplitude of FMT oscillations reflects cognitive effort because cognitive control, measured as working memory accuracy, increased with blood glucose, but FMT amplitude decreased. While the glucose facilitation effect only increased performance on the more cognitively demanding condition, the suppression of FMT amplitude by glucose across both working memory loads indicated that glucose may have reduced cognitive effort regardless of cognitive demand. However, due to the low sample size, these data are only preliminary and require replication in a larger sample.

Previous research has identified that the amplitude of FMT oscillations increases over time (4) and this signal is thought to indicate an increase in cognitive effort. A two-way ANOVA with factors drink (glucose or water) and time also revealed a nonsignificant time effect for high load [$F(2,51) = 4.003$, $P = 0.0509$, $\eta_p^2 = 0.067$] and a significant time effect for low load [$F(2,51) = 6.014$, $P = 0.01773$, $\eta_p^2 = 0.093$] but no effect for resting state [$F(1,52) = 2.510$, $P = 0.119$, $\eta_p^2 = 0.047$]. These effects indicate that FMT amplitude increased over time when cognitive effort was exerted, but there was no change in background FMT amplitude. An interaction between time and drink was not present in the high load [$F(2,51) = 0.872$, $P = 0.3549$, $\eta_p^2 = 0.015$], low load [$F(2,51) = 0.146$, $P = 0.7043$, $\eta_p^2 = 0.002$], or resting-state [$F(2,51) = 0.431$, $P = 0.515$, $\eta_p^2 = 0.008$]. Thus the impact of glucose on FMT amplitude was specific to the timeframe at which blood glucose increased and did not directly influence the time-dependent rise in FMT amplitude during the working memory task.

To further probe a systematic relationship between FMT amplitude and time, we ran a linear fit of FMT amplitude with time and tested this slope versus zero. A post hoc *t* test comparing the relationship of FMT amplitude and time found a significant positive slope (% per hour) for the low load condition [4.69 ± 4.77% per hour; $t(8) = 2.953$, $P = 0.01833$, $d = 0.9845$] and a nonsignificant positive slope for the high load condition [3.83 ± 5.49% per hour; $t(8) = 2.093$, $P = 0.06966$, $d = 0.6978$]. To test whether glucose had an impact on the relationship between FMT amplitude and time, we ran a pairwise *t* test comparing the slopes for the glucose drink and water. We found no evidence that the slope (in % per hour) for the glucose drink (high load: 3.48% ± 6.45%; low load: 3.56% ± 4.78%) significantly differed from the slope for water (high load: 4.19% ± 7.78%; low load:

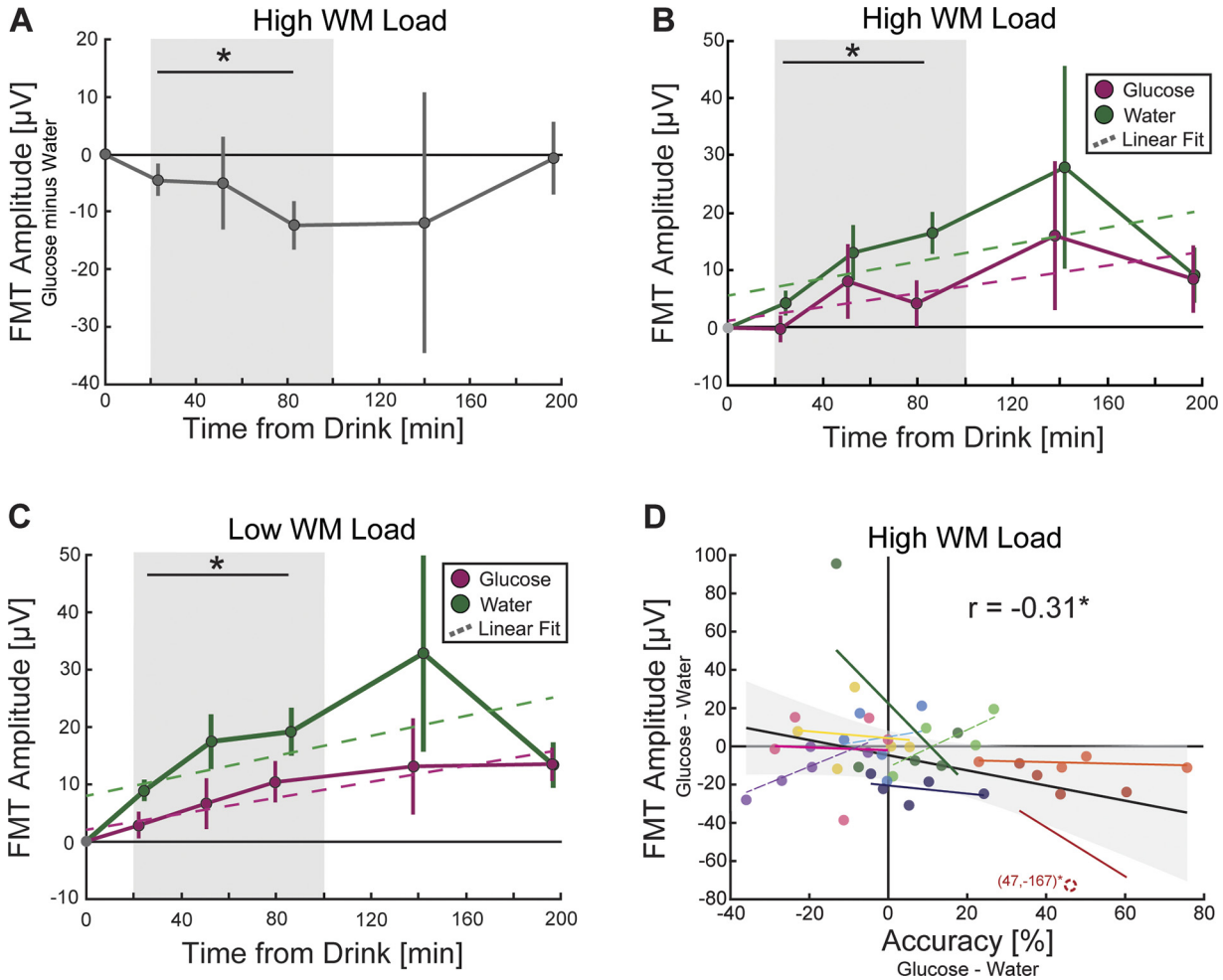

Figure 3. Frontal-midline theta (FMT) amplitude decreased with glucose. *A*: glucose suppressed FMT amplitude during the high load condition. For *A*–*C*, the gray box indicates time points (20 to 100 min) with a significant increase in blood glucose level that were submitted to ANOVA. $*P < 0.05$ in a main effect of drink. Error bars are SE. *B*: for the high load condition, there was a significant main effect of drink when blood glucose was elevated. Trace for the glucose drink (purple) and water (green). Dashed line indicates the trace for linear fit of FMT amplitude with time. The difference between traces was not significant. *C*: suppression of FMT amplitude with glucose was also present in the low load condition. *D*: the change in high load FMT amplitude after glucose consumption was negatively correlated to the change in high load accuracy after glucose consumption for all time points (approximately 15, 45, 75, 135, and 195 min after drink consumption). Each participant is plotted in a different color. Linear regression line is plotted with a 95% confidence interval. $*P < 0.05$ of Pearson correlation. Individual linear fits for each participant are displayed in the matching color. A solid line indicates a negative relationship and a dashed line indicates a positive relationship. An outlier outside of the field of view is indicated using a dashed circle and its coordinates. WM, working memory.

5.07% ± 7.27%) for either level of working memory load [high load: $t(8) = -0.234$, $P = 0.8209$, $d = -0.07800$; low load: $t(8) = -0.618$, $P = 0.5536$, $d = -0.2061$]. This additional analysis provides evidence that glucose has an acute impact on FMT amplitude and does not result in a sustained difference in FMT amplitude over an extended time frame.

An exploratory Pearson correlation was conducted to investigate whether the impact of glucose on FMT amplitude was related to the impact of glucose on working memory accuracy. For the high load condition, we found that FMT amplitude and accuracy were negatively correlated [$r(107) = -0.3054$, $P = 0.04644$] (Fig. 3*D*) such that the degree to which glucose increased accuracy was proportional to decreased FMT amplitude. This relationship was not found for the low load condition [$r(107) = -0.09321$, $P = 0.5522$]. This analysis suggests that participants may have experienced decreased cognitive effort, indexed by decreased FMT amplitude, for all conditions, but reduction in cognitive effort from the glucose drink only translated into improved performance when cognitive control demands were high.

However, it is important to note that the correlation revealed two outliers (−13.15% accuracy and 95.57% FMT amplitude, and 47% accuracy and −167% FMT amplitude). Therefore, we performed a post hoc Spearman's correlation to reduce the influence of these outlier data points. The Spearman's correlation revealed a decrease in effect size [$r(107) = -0.2230$, $P = 0.1502$] as compared with our original Pearson's correlation [$r(107) = -0.3054$]. This indicated that the outliers were partially responsible for the relationship. Although, these outliers contributed to the moderate effect size of our planned exploratory analysis, these data points were both acquired from the 140-min time point. These data fell outside of the range of significant blood glucose increase and therefore did not affect our primary analyses. Finally,

six of the nine participants demonstrated a negative relationship between change in accuracy and change in FMT amplitude from glucose consumption, which suggests that the relationship was relatively consistent. However, this finding should be replicated with a larger sample size to make definitive conclusions; nonetheless, these correlational analyses provide supplemental evidence that the modulation of FMT amplitude and high load accuracy were dependent.

DISCUSSION

The amplitude of FMT oscillations is increased while participants are actively engaged in demanding cognitive control tasks (1, 3, 41, 42). Whether increased amplitude of FMT oscillations corresponds to increased cognitive control (2) or increased cognitive effort (4) is debated (1). Our study utilized the glucose facilitation effect to increase performance of a working memory task presumably by decreasing cognitive effort while maintaining consistent cognitive control demands to dissociate these cognitive processes. First, we confirmed that our visuospatial working memory task evoked FMT oscillations as a function of cognitive control, and this activity was source localized to bilateral prefrontal cortex. Then, a glucose or water drink was administered in a randomized, crossover design and participants performed the working memory task over multiple time points while blood glucose rose and fell. We found that the glucose drink successfully increased blood glucose level and this manipulation resulted in increased cognitive control for the high load condition, suppressed FMT amplitude, and correlation of the impact of glucose on accuracy and FMT amplitude. Recordings during the eyes-open resting-state revealed that there was no impact of glucose consumption on background levels of FMT amplitude. Critically, FMT amplitude increased parametrically with time, consistent with its purported role in cognitive effort, but the relationship between FMT amplitude and time was not impacted by the drink. Altogether, these findings provide preliminary causal evidence in support of the hypothesis that the amplitude of FMT oscillations correspond to cognitive effort. Replication of the effects in this pilot study using a larger sample size is required to draw definitive conclusions regarding the role of FMT oscillations.

The localization of FMT oscillations to bilateral prefrontal cortex is supported by literature that has implicated lateral prefrontal cortex as a principal cognitive control region for working memory encoding, manipulation, and retrieval (8, 40, 43, 44). While FMT oscillations are found in prefrontal electrodes over the midline, source localization has found that central activation can originate from either bilateral activation of homologous regions, as in auditory cortex (45), or by activation in regions directly under the electrodes of interest. Indeed analysis of FMT oscillations have found both bilateral prefrontal localization (37) and a singular cluster in anterior cingulate cortex (46, 47). Research into the neural origin of signals that track cognitive effort often find activation of anterior cingulate cortex as this region is a member of the so-called "pain matrix" (10). Indeed, an early fMRI study that dissociated activation related to task difficulty and working memory processes found that lateral prefrontal cortex tracked with working memory demands, i.e. cognitive control, whereas anterior cingulate cortex tracked with difficulty, i.e. cognitive effort (11). In support of this model, a recent studying using invasive electrocorticography in patients undergoing surgery for intractable epilepsy found task-modulated increases in theta oscillations within the anterior cingulate cortex and in lateral prefrontal cortex, but only activity in lateral prefrontal cortex tracked with working memory task performance (48). In line with this previous research, our localizer found theta oscillations increased in lateral prefrontal cortex as a function of working memory. Although there may be potentially two origins of theta oscillations in frontal cortex, our analysis localized task-evoked theta oscillations to lateral prefrontal cortex.

While Barch et al. (11) investigated task difficulty and found recruitment of anterior cingulate cortex with increased visual degradation, this signal likely arose from increased stimulus/response conflict (49) and did not necessarily arise from increased effortful exertion. The origin of theta as a "cognitive effort" signal may indeed arise from lateral prefrontal cortex. A recent study used continuous theta-burst transcranial magnetic stimulation to inhibit function of the lateral prefrontal cortex and discovered that cognitive effort was decreased as a result (50). Thus lateral prefrontal cortex may allocate limited resources toward tasks that require cognitive effort and this allocation may be communicated by theta-frequency oscillations (33). Nevertheless, the prefrontal cortex is a multimodal association region with diverse connections to the rest of the brain (51). Thus functional connectivity from the lateral prefrontal cortex to the medial temporal lobe and hippocampus (52, 53) might increase during encoding, maintenance, and retrieval of working memory representations. Prefrontal cortical activity might flexibly engage with different brain structures depending on contextual demands.

Prior literature has connected FMT amplitude with cognitive effort by demonstrating increases in FMT amplitude over time during demanding cognitive tasks (4). This trend persists even when accuracy declines over time (54). Therefore, FMT amplitude has been shown to track with cognitive effort independent of change in cognitive control. Similarly, the present study demonstrated that throughout the 3-h experimental session without glucose (control session: water), participants demonstrated an overall stability in task performance. Despite approximately consistent performance on the task, FMT amplitude increased steadily over time suggesting that FMT amplitude could not be attributed to greater working memory capacity. Thus participants presumably compensated for declining metabolic resources throughout the session by expending more cognitive effort (15). The suppression of FMT amplitude after glucose administration, despite increased accuracy on the task, further suggested that FMT amplitude tracked with cognitive effort. If FMT amplitude was merely a correlate of successful working memory, then FMT amplitude would be expected to positively relate with accuracy. Since accuracy was increased by glucose consumption, FMT amplitude should have followed suit; instead, we found that the impact of glucose on high load accuracy and FMT amplitude were negatively correlated. However, it is important to note the fundamental limits to the measurement of cognitive effort as it is an inherently subjective experience. The reduction of cognitive effort with glucose consumption cannot be unequivocally confirmed. Nevertheless, both the observed increase in FMT amplitude over time and decrease

in FMT amplitude with glucose consumption run counter to the direction of cognitive control performance and therefore indicate that FMT oscillations track more closely with cognitive effort than cognitive control.

Both cognitive control and cognitive effort are known to be impaired in psychiatric illness (10, 55, 56). Decreased amplitude of FMT oscillations were observed in patients with depression during cognitive control tasks (57) and spared FMT amplitude is predictive of treatment response in depression to noninvasive brain stimulation (58). Therefore, knowing that the amplitude of FMT oscillations tracks with cognitive effort closer than cognitive control can shape treatment interventions that emphasize increased capacity to exert cognitive effort.

Metabolic accounts of cognitive processes have been criticized for their failure to determine mechanisms for the observed changes in cognition. The present study is similarly limited, as no specific mechanism by which glucose consumption improves cognitive performance was identified. Nonetheless, the glucose facilitation effect may be due to an enhancement of hippocampal activity or a more diffuse increase in glucose stores in the brain (16, 59). In addition, investigations have found that increased cognitive control increased arousal levels that indirectly resulted in glucose expenditure but may not directly impact brain function (10, 60). Future research would be required to dissociate the impact of glucose consumption on physical arousal versus increased resources for neural energy expenditure. Furthermore, the role that insulin plays in restoring normal blood glucose levels can be indexed by the rate of change in glucose after reaching peak glucose levels, and faster restoration of baseline levels is positively related to increased cognitive performance (15, 61). Since insulin proportionally increases with blood glucose and has been shown to independently facilitate delayed memory (62), insulin might have an additive effect where both glucose and insulin contribute to enhanced cognitive performance. Additionally, the administration of glucose has effects on several hormones in addition to insulin, such as ghrelin (63), that could have impacted our findings. It should be further noted that participants underwent a 12-h fasting period before participation that may have influenced other variables. For example, a previous study found that glucose consumption after fasting led to increased cortisol release when faced with psychosocial stress (64). Future research could investigate the effect of glucose consumption on FMT amplitude while dissociating the roles of absolute blood glucose levels, insulin facilitated return-to-baseline rate, and other hormone fluctuations. These investigations could be especially relevant to diabetes research.

Cognitive effort and physical effort are often positively correlated in daily life. However, experimental manipulations have previously been used to selectively drain physical versus cognitive resources. A previous study found that cognitive effort increased FMT amplitude and decreased self-reported cognitive alertness but physical effort had no impact on FMT amplitude and increased cognitive alertness (65). By manipulating blood glucose levels in a crossover design, we provided causal evidence supportive of these previous correlational findings. While we did not manipulate physical effort, we expect that the impact of glucose on brain activity are specific to the theta frequency band during cognitive effort and would not be found with physical effort.

The primary focus of this study was on the amplitude of FMT oscillations. However, recent studies have also begun to explore FMT oscillation peak frequency shifts. These studies have identified causal evidence that participants with theta oscillations with a slower peak frequency have greater working memory capacity (66, 67). Additionally, recent studies found evidence that peak FMT frequency shifts toward lower frequencies under more difficult task conditions (34, 41, 68). These findings relate peak frequency to cognitive control metrics; therefore, it is possible that the peak frequency of FMT oscillations tracks with cognitive control, whereas the amplitude of FMT oscillations tracks with cognitive effort. Future research should aim to dissociate FMT peak frequency and amplitude with regards to how they might relate to cognitive control and cognitive effort.

Altogether, this study provides preliminary causal evidence that the amplitude of FMT oscillations positively tracks with cognitive effort, rather than cognitive control. Again, we emphasize that given the small sample size of this study due to unforeseeable circumstances (COVID-19 pandemic), the preliminary findings of this study should be replicated in a larger sample and that the experimental design can be extended based on the open questions explored here. A power analysis was conducted at the conclusion of our study that found that 21 participants would be necessary to reach 80% statistical power ($1 - \beta > 0.8$) for our primary finding of decreased FMT amplitude in the high load condition ($d = 0.658$) using G*Power with a dependent two-tailed t test. Therefore, a larger sample size is necessary for decisive conclusions. Nonetheless, this pilot study developed a novel paradigm for dissociating cognitive effort and cognitive control. We found preliminary evidence in support of the hypothesis that FMT amplitude tracks with cognitive effort which may be critical to future research. For example, increased amplitude of FMT oscillations during a working memory task was found to be predictive of effective treatment for noninvasive brain stimulation to the lateral prefrontal cortex (58). Our findings suggest that this signal more accurately reflects the ability to exert cognitive effort, and deficits in cognitive effort may underlie some purported deficits in cognitive control.

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DISCLAIMERS

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

DISCLOSURES

F.F. is the lead inventor of Individual Property filed by University of North Carolina. F.F. is founder, shareholder, and chief scientific officer of Pulvinar Neuro, which did not play any role in this study. F.F. has received honoraria from the following entities in the last 12 mo: Sage Therapeutics, Academic Press, Insel Spital, and Strategic Innovation. A.M., J.R., C.W., and J.B.B. have no conflict of interest.

AUTHOR CONTRIBUTIONS

A.M. J.R. C.W. J.B.B., and F.F. conceived and designed research; A.M. and C.W. performed experiments; A.M. and J.R. analyzed data; A.M. J.R. C.W. J.B.B., and F.F. interpreted results of experiments; A.M. and J.R. prepared figures; A.M. and J.R. drafted manuscript; A.M. J.R. J.B.B., and F.F. edited and revised manuscript; A.M. J.R. C.W. J.B.B., and F.F. approved final version of manuscript.

ENDNOTE

At the request of the authors, readers are herein alerted to the fact that additional materials related to this manuscript may be found at https://osf.io/vuyjr/. These materials are not a part of this manuscript and have not undergone peer review by the American Physiological Society (APS). APS and the journal editors take no responsibility for these materials, for the website address, or for any links to or from it.

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