The Cerebellum (2019) 18:489–499
https://doi.org/10.1007/s12311-019-01013-9

ORIGINAL PAPER

Cerebellar Theta Frequency Transcranial Pulsed Stimulation Increases Frontal Theta Oscillations in Patients with Schizophrenia

Arun Singh[1] · Nicholas T. Trapp[2,6] · Benjamin De Corte[3,6] · Scarlett Cao[4] · Johnathon Kingyon[4,6] · Aaron D. Boes[5,6] · Krystal L. Parker[2,6]

Published online: 1 March 2019

Abstract

Cognitive dysfunction is a pervasive and disabling aspect of schizophrenia without adequate treatments. A recognized correlate to cognitive dysfunction in schizophrenia is attenuated frontal theta oscillations. Neuromodulation to normalize these frontal rhythms represents a potential novel therapeutic strategy. Here, we evaluate whether noninvasive neuromodulation of the cerebellum in patients with schizophrenia can enhance frontal theta oscillations, with the future goal of targeting the cerebellum as a possible therapy for cognitive dysfunction in schizophrenia. We stimulated the midline cerebellum using transcranial pulsed current stimulation (tPCS), a noninvasive transcranial direct current that can be delivered in a frequency-specific manner. A single 20-min session of theta frequency stimulation was delivered in nine patients with schizophrenia (cathode on right shoulder). Delta frequency tPCS was also delivered as a control to evaluate for frequency-specific effects. EEG signals from midfrontal electrode Cz were analyzed before and after cerebellar tPCS while patients estimated the passage of 3- and 12-s intervals. Theta oscillations were significantly larger following theta frequency cerebellar tPCS in the midfrontal region, which was not seen with delta frequency stimulation. As previously reported, patients with schizophrenia showed a baseline reduction in accuracy estimating 3- and 12-s intervals relative to control subjects, which did not significantly improve following a single-session theta or delta frequency cerebellar tPCS. These preliminary results suggest that single-session theta frequency cerebellar tPCS may modulate task-related oscillatory activity in the frontal cortex in a frequency-specific manner. These preliminary findings warrant further investigation to evaluate whether multiple sessions delivered daily may have an impact on cognitive performance and have therapeutic implications for schizophrenia.

Keywords Cerebellum · Neuromodulation · Noninvasive stimulation · Cognitive task · Schizophrenia

Introduction

Schizophrenia is a severe, chronic, and disabling mental illness that affects 1% of the US population [1]. Deficits in cognitive and executive functions include impairments in working memory, attention, planning, and timing, which are currently untreatable and significantly decrease quality of life [2–6]. A biological correlate to the cognitive impairment seen in schizophrenia is electroencephalographic (EEG) abnormalities in the frontal cortex [7–9]. Previous work demonstrated dysfunctional working memory and executive function in subjects with schizophrenia is associated with abnormal activity within a functionally connected network including the temporal lobe and cerebellum [10, 11].

Work from our lab has shown that patients with schizophrenia have attenuated frontal low-frequency rhythms concurrent with impaired interval timing performance and the two are

Arun Singh and Nicholas T. Trapp are co-first authors.

✉ Krystal L. Parker
krystal-parker@uiowa.edu

[1] Department of Neurology, University of Iowa, Iowa City, IA 52242, USA

[2] Department of Psychiatry, University of Iowa, 169 Newton Road, 2336 PBDB, Iowa City, IA 52242, USA

[3] Neuroscience Graduate Program, University of Iowa, Iowa City, IA 52242, USA

[4] University of Iowa Carver College of Medicine, Iowa City, IA 52242, USA

[5] Department of Pediatrics, Neurology and Psychiatry, University of Iowa, Iowa City, IA 52242, USA

[6] Iowa Neuroscience Program, University of Iowa, Iowa City, IA 52242, USA

correlated, such that greater attenuation of theta rhythms corresponds with worse task performance [9]. A similar profile of underestimating the passage of time and attenuated theta oscillations follows D1 dopamine-antagonist infusions in a region of the rodent frontal cortex [9]. This parallels a finding in human schizophrenia patients: there is a homologous area in the frontal cortex of patients with schizophrenia reported to have a decreased binding potential for D1 dopamine [12], lending support to the importance of this mechanism for timing performance. Further, we showed that optogenetic stimulation of another node in the network recruited for timing performance, the cerebellum, could rescue theta frequency oscillations in the frontal cortex and normalize timing performance [9].

Currently, there are no therapies that reliably improve cognitive dysfunction in schizophrenia; new therapeutic options are desperately needed to mitigate the disabling burden of this disease. Although traditionally associated with motor function, there is also a prominent role of the cerebellum in cognitive function [13–18]. Previous clinical and neuroimaging reports have provided evidence of cerebellar pathology in schizophrenia patients with cognitive deficits and psychosis [19–22], which may be involved in abnormal cerebello-thalamo-cortical circuitry concurrent with cognitive and motor deficits [23, 24]. According to the traditional view, discrete circuits between the basal ganglia and cerebellum with motor areas of the cerebral cortex underlie motor performance, while other circuits between the basal ganglia and cerebellum functionally linked with prefrontal cortices associate more strongly with cognitive functions [25]. There is emerging evidence that the cerebellum can be a neuromodulation target not only for motor control but also for cognitive abnormalities in patients [26, 27].

Although neuromodulation (such as transcranial direct current stimulation; tDCS) of frontal cortical areas in healthy participants and patients may enhance cognition, stimulation of the cerebellum has received less attention [28, 29]. In 2008, however, Ferrucci et al. reported changes in cognitive performance in healthy individuals after cerebellar tDCS [30]. Based on these findings, one could hypothesize that cerebellar stimulation may be able to rescue frontal theta brain rhythms essential for normal cognition, thus correcting physiologic abnormalities that impair function and performance. Indeed, emerging evidence suggests that cerebellar stimulation may hold promise for improving cognitive problems in schizophrenia [31, 32], though it is not known whether this is mediated by enhanced frontal theta rhythms. We aim to extend this work by evaluating whether noninvasive approaches of cerebellar neuromodulation can enhance cognitive function by modifying frontal brain oscillations in patients with schizophrenia.

Here, we investigate whether noninvasive transcranial cerebellar stimulation, specifically transcranial pulsed current stimulation (tPCS), influences frontal cortex theta oscillations. TPCS is a relatively safe and inexpensive way to pass electrical current noninvasively through the skull in a frequency-specific manner that may influence neuronal activity and connected brain circuitry [33]. Using this methodology, we tested the hypothesis that theta frequency cerebellar tPCS stimulation would (1) augment midfrontal theta frequency oscillations in patients with schizophrenia and (2) influence timing performance. We also tested the effect of delta frequency cerebellar tPCS to evaluate whether any changes in frontal oscillations were frequency-specific relative to the stimulation. Results showed that theta but not delta frequency cerebellar tPCS rescued low-frequency activity in the midfrontal region, yet neither frequency of stimulation improved timing performance.

Materials and Methods

Human Subjects

Nine patients with a DSM-IV diagnosis of schizophrenia (7 men, 2 women) were recruited from the Iowa Longitudinal Database. Subjects were recruited from a database of patients that had diagnoses confirmed by a board-certified psychiatrist at the University of Iowa. Data from the nine age-, sex-, and education-matched healthy control subjects performing the interval timing task without stimulation were included for comparison [9]. All participants were determined to have the decisional capacity to provide informed consent, resided within 100 mi of Iowa City and were able to independently travel to the University of Iowa Hospitals and Clinics. Written informed consent was obtained from every subject and all research protocols were approved by the University of Iowa Human Subjects Review Board. Medication status was not altered for any of the patients. Subject demographics and scores on cognitive tasks are described in Table 1.

Interval Timing Task

Timing is a well-characterized capability of the cerebellum; however, it is typically associated with sub-second timing [34]. Our lab has recently shown that the cerebellum and frontal cortex participate in supra-second processing and timing [9]. Previous studies have demonstrated that estimating the passage of time is a reliable way of eliciting frontal theta activity [35]. While EEG recordings were being acquired, participants performed an interval timing task before and after cerebellar tPCS. The interval timing task involved the appearance of a "3-s" or "12-s" text cue on the computer screen that indicated both the interval of time to estimate and the start of the trial (Fig. 1). The subject would make a motor indication of their estimation of the elapse of the specified timing duration by pressing the keyboard space bar. Participants received feedback about their response time after 20% of the trials. The task was self-paced, and the participants were instructed not to count time in their head during the task. The interval timing

Table 1 Demographics and cognitive scores for patients with schizophrenia and controls

	Control	Schizophrenia		
		Pre-tPCS	Post-delta tPCS[#]	Post-theta tPCS[#]
Sex	9 (3 F)	9 (3 F)	8 (3 F)	9 (3 F)
Age	47.9 (6.8)	45.8 (2.9)	45.2 (3.1)	46.1 (2.2)
Education (years)	15 (0.6)	12.8 (0.9)	12.9 (0.76)	12.9 (0.7)
MOCA	28 (0.6)	23.8 (1.5)*	24.7 (0.8)	24.4 (1.2)
TMT	26.9 (3.4)	81.9 (28.7)*	90.0 (20.1)	59.2 (15.1)
VF	45.6 (3.1)	29.2 (4.9)*	29.9 (4.4)	29.6 (2.6)
Digit	20.4 (1.1)	14.7 (1.4)*	15.2 (1.1)	14.3 (0.8)

The data are presented as mean ± SEM. *$p < 0.05$ control vs schizophrenia pre-tPCS. [#] Following stimulation in either delta or theta frequencies, all cognitive measures remained significantly different from controls, i.e., there was not an improvement in their cognitive function. All controls were age and sex matched to a patient. *MOCA*, Montreal Cognitive Assessment; *TMT*, Trails Making Test; *VF*, verbal fluency

task contained 80 total trials with 3-s and 12-s intervals presented in random order.

Each subject's time estimates for the two intervals were fit with Gaussian distributions, using Matlab (fitdist.m), and performance was quantified two ways using the mean and standard deviation [36]. First, we subtracted the mean from the actual instructed interval, providing a measure of timing accuracy. Larger and smaller values correspond to systematic under- and over-estimation of time, respectively. While subjects can be highly accurate with respect to average response times, their estimates can vary substantially from trial-to-trial. Therefore, we also measured timing precision. Timing precision (taken as the standard deviation of response times) decreases linearly with the interval being timed, a form of Weber's law often referred to as the "scalar property" of interval timing [37]. Therefore, we evaluated precision by dividing the standard deviation by the mean response time, defined as the "coefficient of variation" (CV). Larger and smaller values correspond to lower and higher precision, respectively. Furthermore, in both humans and a variety of other species, the coefficient of variation remains constant, regardless of the interval being timed [38].

Previous findings in healthy controls indicate that midfrontal theta activity (4–8 Hz) following trial start is essential for accurate timing performance [9, 35, 39]. This cue-locked theta activity is attenuated in patients with schizophrenia and Parkinson's disease [9, 35]. Based on these results, a time-frequency region of interest (tf-ROI) was derived from our previous work and was constrained to 0 to 1.0 s following cue. In addition to this highly implicated a priori tf-ROI, we applied cluster-based permutation correction on the full time-frequency analysis in order to identify any other consistent changes between groups. This method involved thresholding the size of the statistical cluster (for 1000 permutations of group labels) and picked the one-dimensional cluster mass (at the 95th percentile) as the threshold for chance occurrence.

Cerebellar tPCS

Figure 1 displays the experimental setup. A single session of noninvasive brain stimulation was delivered via a battery-driven stimulator (MindAlive, Inc., Oasis Pro) with anodal pulsed current stimulation of the cerebellar vermis applied via surface conductive rubber electrodes. The vermal location was defined by the location 1 cm below the external occipital protuberance or highest and point of the largest projection in the occipital bone is referred to as the inion ([40]; Fig. 5). The cathodal electrode was placed on the right shoulder [41–44]. Stimulation duration was 20 min, with 1-mA peak-to-peak amplitude at either delta ($n = 8$) or theta ($n = 9$) frequencies. Delta frequency cerebellar tPCS was used as a control to evaluate the frequency-specific effects of cerebellar modulation of the frontal cortical oscillations during timing performance. This may be optimal as an "active sham"; stimulation causes a "buzzing" or "tingling" sensation at the stimulation site, which would be absent with a purely sham stimulation condition [45]. Additionally, using delta frequency cerebellar tPCS as a control frequency confirmed that general characteristics of physical stimulation were comparable across all conditions. Delta and theta stimulation session order was randomized and separated by 1–3 months. Although we did not test the stimulator before we applied stimulation, we did record EEG activity during stimulation and could see the artifact induced by stimulation to verify that stimulation was being delivered at the correct frequency.

EEG Recording and Analysis

EEG recording was collected before ("pre") and after ("post") cerebellar tPCS. EEG was recorded on a Nihon Kohden system with a sampling rate of 500 Hz [46]. EEG was recorded from 21 channels based on the 10–20 system (Fz, Cz, Pz, F3/4, C3/4, P3/4, F7/8, T3/4, T5/6, O1/2, M1/2), as well as left-

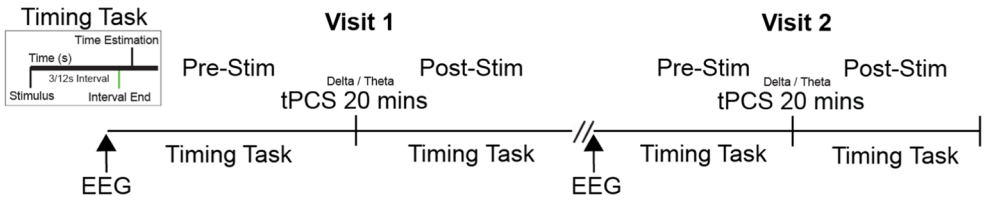

Fig. 1 Experimental timeline. Delta and theta stimulations were randomized in visit 1 and 2

eye vertical electrooculography (VEOG) and ground (forehead). The lead that is traditionally placed at the FP1 location was relocated to 1 cm below the inion bone on the cerebellar midline while FP2 was placed 1 cm to the right of FP1. Traditional 10-mm EEG-type passive electrodes were used to collect signals from the scalp. We applied EEG conductive gel (Covidien 30806734 Ten20) to hold the electrodes in place to conduct the signal with higher signal-to-noise ratio. This approach was selected to match our previous EEG datasets that described differences in low-frequency rhythms between patients with Parkinson's disease and controls [35]. Impedance of all electrodes was below 5 kΩ. Continuous data were re-referenced to the mathematical average of the two mastoid channels, yielding a total of 21 scalp EEG channels. Signals were segmented on the basis of the stimulus (cue) onset (− 2 to 6 s for 3-s trials; − 2 to 18 s for 12-s trials), from which the cue-locked segments were isolated. Eye blinks and horizontal eye movements were removed by hand using independent component analysis and EEGLab [47]. Afterwards, EEG signals were then re-referenced to an average reference. Previous studies have associated cognitive impairment with changes in midfrontal regions; therefore, we selected midfrontal Cz electrode for the main analysis [9, 35, 39]. We also analyzed the midline cerebellar electrode, the right cerebellar electrode, and the electrode above the right cerebellar lead to evaluate how cerebellar delta\theta tPCS influenced activity at and around the site of stimulation.

Power spectral analysis of "pre" and "post" cerebellar tPCS EEG signals was computed from the "pwelch" method. Signals were transformed into the power spectrum domain (using pwelch method: 256-point window size). Furthermore, a frequency range of 1–50 Hz was selected to compute relative power spectrum to abate the inter-recording variation. Here, we exported the mean relative power at delta (1–4 Hz) and theta (4–8 Hz) frequency bands.

Time-frequency analysis was computed by multiplying the fast Fourier transformed (FFT) power spectrum of single-trial EEG data with the FFT power spectrum of a set of complex Morlet wavelets. These complex Morlet wavelets are defined as a Gaussian-windowed complex sine wave: $e^{i2\pi tf}e^{-t\hat{}2/(2\times\sigma\hat{}2)}$, where t is time and f is frequency (which increases from 1 to 50 Hz in 50 logarithmically spaced steps). This equation defines the cycle of each frequency band, increasing from 3 to 8 cycles between 1 and 50 Hz and taking the inverse FFT. Ultimately, this computational method converts signal into time-domain convolution and results in estimates of instantaneous power (the magnitude of the analytic signal). The power value was normalized by conversion to a decibel (dB) scale ($10*\log10(power_t/power_{baseline})$), allowing a direct comparison of effects across frequency bands [46]. The baseline for each frequency was calculated based on average power from − 0.3 to −0.2 s prior to the onset of the stimulus. Each epoch was then cut in different lengths for visualization purpose (3-s full trial, − 2 to 5 s; 12-s full trial, − 2 to 14 s; also, around 3-s and 12-s cue, − 0.5 to 1.0 s time from cue). Time-frequency plots were analyzed from electrode Cz in delta (1–4 Hz) and theta (4–8 Hz) frequency bands in accordance with well-established prior hypotheses [35, 39].

Statistical Analyses

For binary comparisons between "pre" and "post" tPCS, we used paired t tests with an alpha level of 0.05. Paired t tests were performed to compute the statistical differences before ("pre") and after ("post") the tPCS for behavioral, clinical data measures, and task-related frequency bands power. We applied repeated measure analysis of variance (ANOVA) followed by pairwise comparison using SPSS for spectral power in the targeted ROIs (tf-ROI) of 3-s and 12-s data for delta and theta tPCS separately. Spearman's correlation analysis was performed to analyze the correlation between clinical scores and reaction time/tf-ROIs.

Results

Midfrontal Activity and Behavioral Response in Control Subjects

Healthy control subjects have increased delta frequency power in the midfrontal electrode Cz at the onset of cue in the interval timing cognitive task (Fig. 2 a; [9]). However, patients with schizophrenia have attenuated midfrontal theta frequency power around the time of cue (Fig. 2 b). Patients with schizophrenia also had significantly increased variation in the time estimation (3-s and 12-s interval timing tasks) as compared with control subjects, specifically in timing efficiency and the coefficient of variation (Fig. 2 c–f). These results are previously published and are the foundation for the hypothesis that midfrontal activity is necessary for timing performance on an interval timing task [9].

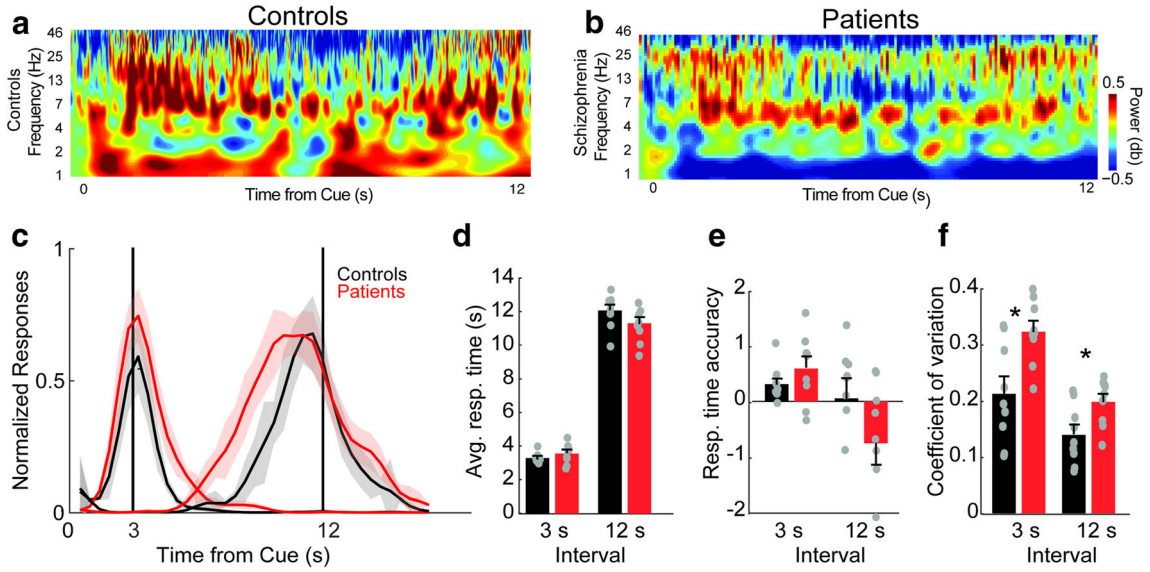

Fig. 2 Spectral analysis of electrode Cz signal in healthy control and schizophrenia patients during interval timing tasks. **a**, **b** Time-frequency spectrograms show increased delta frequency power around cue in the interval timing task in control subjects as compared with schizophrenia patients. **c** Quantification of response histograms reveals variations in the measurements of timing, including less variations in average response time (**d**), more variations in timing efficiency as defined by the number of responses occurring around 3 s (2–3 s) and 12 s (11–12 s) (**e**), and a larger coefficient of variation (**f**). These data have been described previously in [9]. *$p < 0.05$

Midfrontal Activity in Patients

Spectral analysis of delta and theta EEG data was computed before and after tPCS to examine the effect of stimulation on midfrontal region Cz during performance of the timing task in patients with schizophrenia. Over the entire 12-s interval, delta (Fig. 3 a and b) and theta frequency tPCS (Fig. 3 e and f) did not significantly alter the relative power of delta and theta frequency bands in the midfrontal cortex at electrode lead Cz. This is further confirmed by time-frequency analyses of the midfrontal electrode that show no significant changes in power after delta tPCS subtracted from power before, across

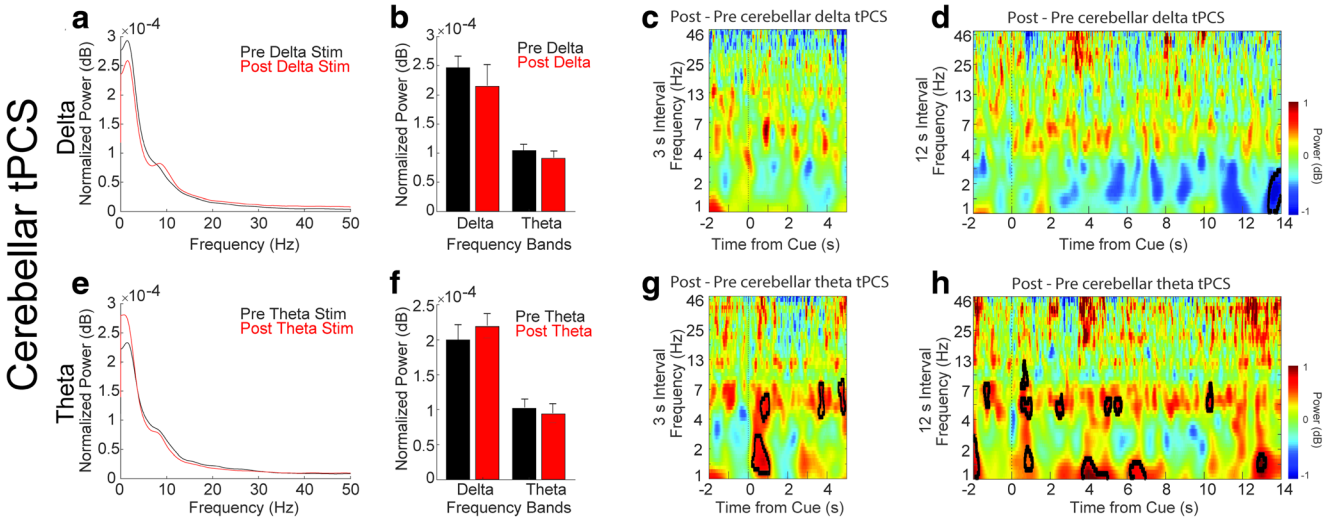

Fig. 3 Spectral analysis of electrode Cz signal before and after cerebellar tPCS during interval timing tasks. There are no significant differences in relative power delta and theta frequency bands at electrode Cz in the midfrontal cortex following delta (**a**, **b**) and theta frequency tPCS (**e**, **f**). **c**–**h** Time-frequency spectrograms after theta frequency tPCS show significant changes in power (increased in red with permutation-corrected statistical significance $p < 0.05$ outlined in black lines) around 3 s (**g**) and 12 s (**h**) cue events at time 0 s (note that the *x*-axes are scaled and shortened to 5 s to include only the time of the trial so that the intertrial intervals and next trial starts are not included in the image for 3 s trials). Increased power was more prominent in lower frequency bands (1–8 Hz; delta/theta frequency band). This effect was specific for theta frequency stimulation as delta frequency tPCS did not significantly alter power at any time points throughout the interval on either the 3-s (**c**) or 12-s (**d**) task

Table 2 Repeated measure ANOVA tests (within subject variables, 3-s and 12-s tasks) were applied on tf-ROI power values to compute the difference between pre- and post-delta or pre- and post-theta tPCS

	tf-ROI, 0–0.5 s		tf-ROI, 0.5–1.0 s	
	1–4 Hz	4–8 Hz	1–4 Hz	4–8 Hz
Delta tPCS	$F = 0.01$; $p = 0.99$	$F = 0.3$; $p = 0.6$	$F = 0.25$; $p = 0.6$	$F = 1.9$; $p = 0.2$
Theta tPCS	$F = 1.3$; $p = 0.3$	$F = 1.3$; $p = 0.3$	$*F = 10.9$; $p = 0.01$	$*F = 7.9$; $p = 0.02$

$*p < 0.05$ represents statistical significance

both the short (Fig. 3 c) and long (Fig. 3 d) intervals. Interestingly, theta tPCS significantly increased both delta and theta oscillatory activity around the time of the cue for both 3-s and 12-s trials (Fig. 3 g and h—permutation-corrected statistical significance $p < 0.05$ outlined in bold lines).

Our previous work reports the importance of increased activity immediately following the onset of the cue (0–0.5 s) for accurate timing performance [9, 39]. Here, we find that delta frequency tPCS did not modulate the activity at 3-s and 12-s cue-related tf-ROIs at electrode Cz in patients with schizophrenia: 1–4 Hz, 0–0.5 s from cue, (see Table 2; Fig. 4 a and b) and 4–8 Hz, 0.5–1.0 s from cue (see Table 2; Fig. 4 a and c).

Theta frequency tPCS also did not change the activity at 3-s and 12-s cue-related tf-ROIs 1–4 Hz, 0–0.5 s from cue (see Table 2; Fig. 4 d and e). However, theta frequency tPCS increased the activity significantly at 3-s and 12-s cue-related tf-ROIs 1–4 Hz and 4–8 Hz, 0.5–1.0 s from cue (see Table 2; Fig. 4 d and f). We also performed skewness tests on tf-ROIs to measure the normality. We found that the absolute values of skewness were < 1 m, which confirm data normality.

Notably the increased theta was not entirely consistent with that of previous reports from healthy controls as it was delayed in onset by 0.5 s. Although our analyses focused on electrode Cz, several electrodes showed significant power increases as indicated by the large black diamonds on the topo plots ($p < 0.05$: Fig. 4 d and f). Interestingly, theta frequency tPCS reinstated theta oscillations (4–8 Hz) in the midfrontal region. Previous reports have confirmed the role of increased midfrontal activity at 4–8 Hz in the improvement of cognitive performance in patients with cognitive deficits [48, 49]. Therefore, current results suggest the potential benefits of theta tPCS with improved stimulation parameters to induce behavioral changes in patients with schizophrenia and other diseases associated with cognitive impairment. This effect was specific to theta frequency as cerebellar delta tPCS did not induce any significant changes in frontal activity (Fig. 4 a–c).

Further, we analyzed the electrodes which were located at the site of stimulation (1 cm below the inion bone), 1 cm to the right of the stimulation site, and a site to the right and superior to the stimulation location to evaluate the effects of tPCS on cue-evoked theta frequencies. Cue-evoked power at theta frequencies at the site of stimulation and 1 cm to the right of stimulation tended to increase after theta but not delta tPCS (Fig. 5 a and b). Analyses of the electrode above and to the right of the stimulation site did not show any changes in power after delta\theta tPCS (Fig. 5 c). These results indicate that lasting effects of theta cerebellar tPCS activity were localized only to the frontal cortex and there were no local persistent changes at the cerebellar site of stimulation.

Interval Timing Performance in Patients

To further explore how the cerebellum may modulate the frontal cortex to support timing (and possibly cognitive function), schizophrenia patients performed an interval timing task before and after delta ($n = 8$) or theta ($n = 9$) tPCS. At baseline, patients with schizophrenia underestimated the short interval ($p = 0.043$) and overestimated the long interval, although the latter effect only had a trend towards significance ($p = 0.09$). This resembles a pattern of regression to the mean referred to as a "migration effect," which is also observed in Parkinson's patients [50]. Further consistent with Parkinson's data, subjects showed larger coefficients of variation (CVs = standard deviation/mean of the response times) for the short interval than the long, indicating that they did not conform to the "scalar property" of interval timing. In other words, the variability of the response times did not grow linearly with mean response time, as one would expect constant CVs for both durations if this were the case. However, neither delta nor theta stimulation significantly altered these patterns (Fig. 6 a–d).

Cognition in Patients

Although unlikely that a single session of tPCS could alter performance on core cognitive tasks, we compared pre-post tPCS baseline scores for cognitive measures including the Montreal Cognitive Assessment, Trail Making Task, verbal fluency, and digit span on 7 of the schizophrenia patients included in this study. There were no significant changes in cognitive function following a single session of tPCS (see Table 1).

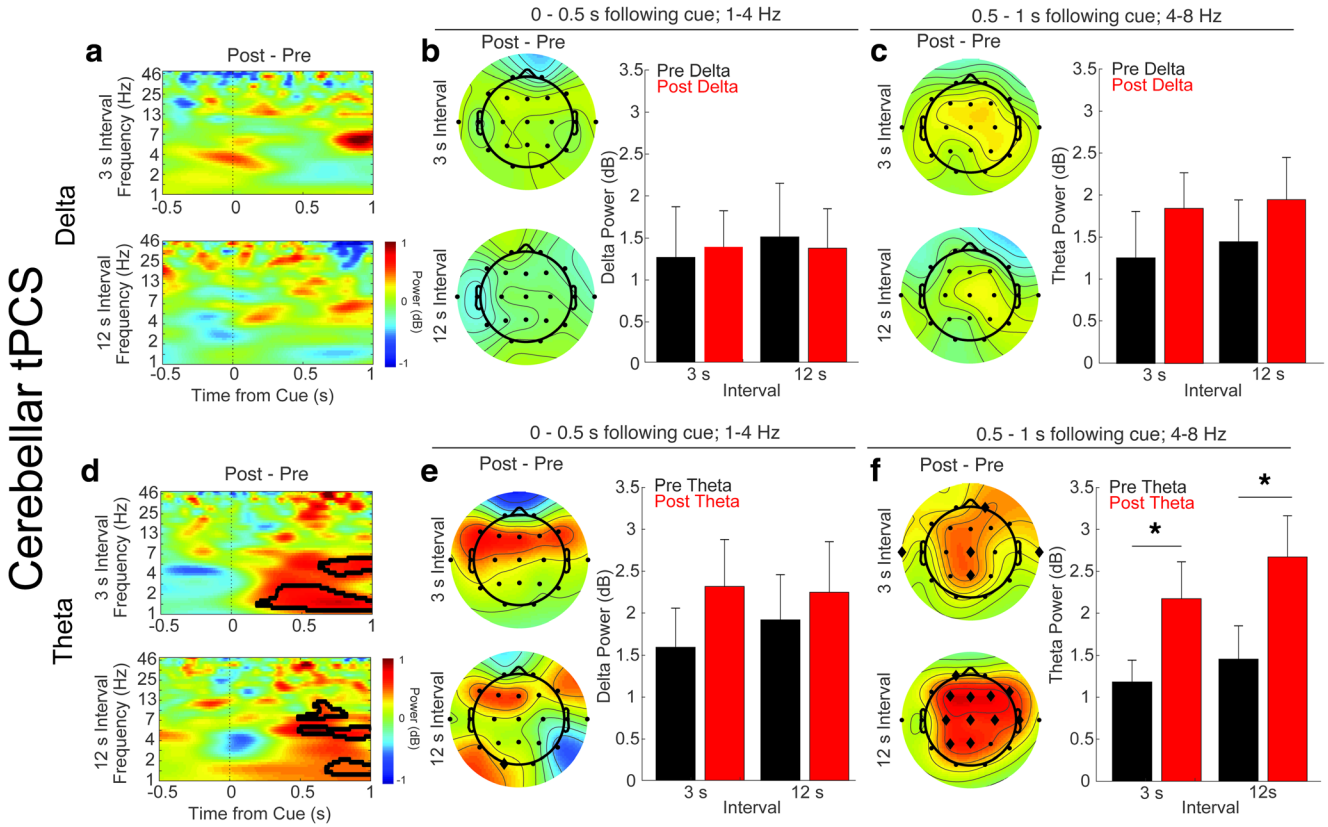

Fig. 4 Cue-evoked low-frequency activity increases significantly at tf-ROIs following theta frequency tPCS. **a** tf-ROI 3-s and 12-s cue-related midfrontal activity (at Cz) did not modulate following delta tPCS around 3-s and 12-s cues **b** at 1–4 Hz, 0–0.5 s from cue, or **c** at 4–8 Hz, 0.5–1.0 s from cue. **d** However, tf-ROI 3-s and 12-s cue-related midfrontal activity (at Cz) increased around 3-s and 12-s cues after theta tPCS (Permutation-corrected statistical significance $p < 0.05$ outlined in bold lines). **e** Increased power was observed in delta frequencies (1–4 Hz), 0–0.5 s from cue (non-significant; $p > 0.05$), and **f** in theta frequencies (4–8 Hz), 0.5–1.0 s from cue (significant, $^*p < 0.05$). Although the analyses focused on electrode lead Cz, there were several significantly increased electrodes on the scalp topography of tf-ROIs following theta tPCS that could have been analyzed for both time points (**e**, **f**, left—statistically significant electrodes $p < 0.05$ indicated by large diamonds). Figure 4 a, d are "zoom-ins" of Fig. 3 c, d, g, h

Discussion

We demonstrate that 20 min of 1-mA theta-range tPCS of the midline cerebellum can alter power in time-frequency spectrograms in the lower frequency (delta and theta) range during an interval timing task. This effect was not seen with delta-range tPCS, suggesting that this is a frequency-specific effect of tPCS. These changes were most notable in the Cz (midfrontal) and prefrontal tf-ROIs, which is consistent with our hypothesis that midline cerebellar stimulation would alter frontal cortex EEG activity. This is also consistent with previous literature utilizing other forms of low-frequency cerebellar stimulation to modulate frontal EEG activity during a timing task [9]. Some literature suggests that pulsed stimulation protocols are effective at entraining the dominant brain rhythm, which is often task- and region-specific. Indeed, there is some evidence that in other tasks, such as associative learning tasks, behavior is associated with theta synchronization between the cerebellum and prefrontal cortex regions [16]. Thus, it is possible that theta-range tPCS was effective when delta-range stimulation was not due to driving already-dominant cerebellar theta rhythms that are active and synchronized in the execution of a timing task [9]. Additionally, although theta-range tPCS altered low-frequency EEG activity, why this was not specific to theta-range activity remains unclear. The localization of induced EEG changes to the midfrontal regions is promising as this is a region previously implicated in cognitive timing tasks and abnormal in patients with cognitive impairment [9, 35, 39].

Despite the tPCS frequency-dependent changes in time-frequency spectrograms noted in this study, there were no notable changes in task performance behavior. There are several possible explanations for the lack of behavioral modifications. First, it is possible that the stimulation parameters were not robust enough to drive a change in brain activity significant enough to modify behavior on a task that probes a core cognitive process like timing. Studies of tPCS show a duration-dependent effect of the stimulation, with 20 min of stimulation often leading to the longest-lasting effects [33]. However, whether this is optimal for a clinical population of

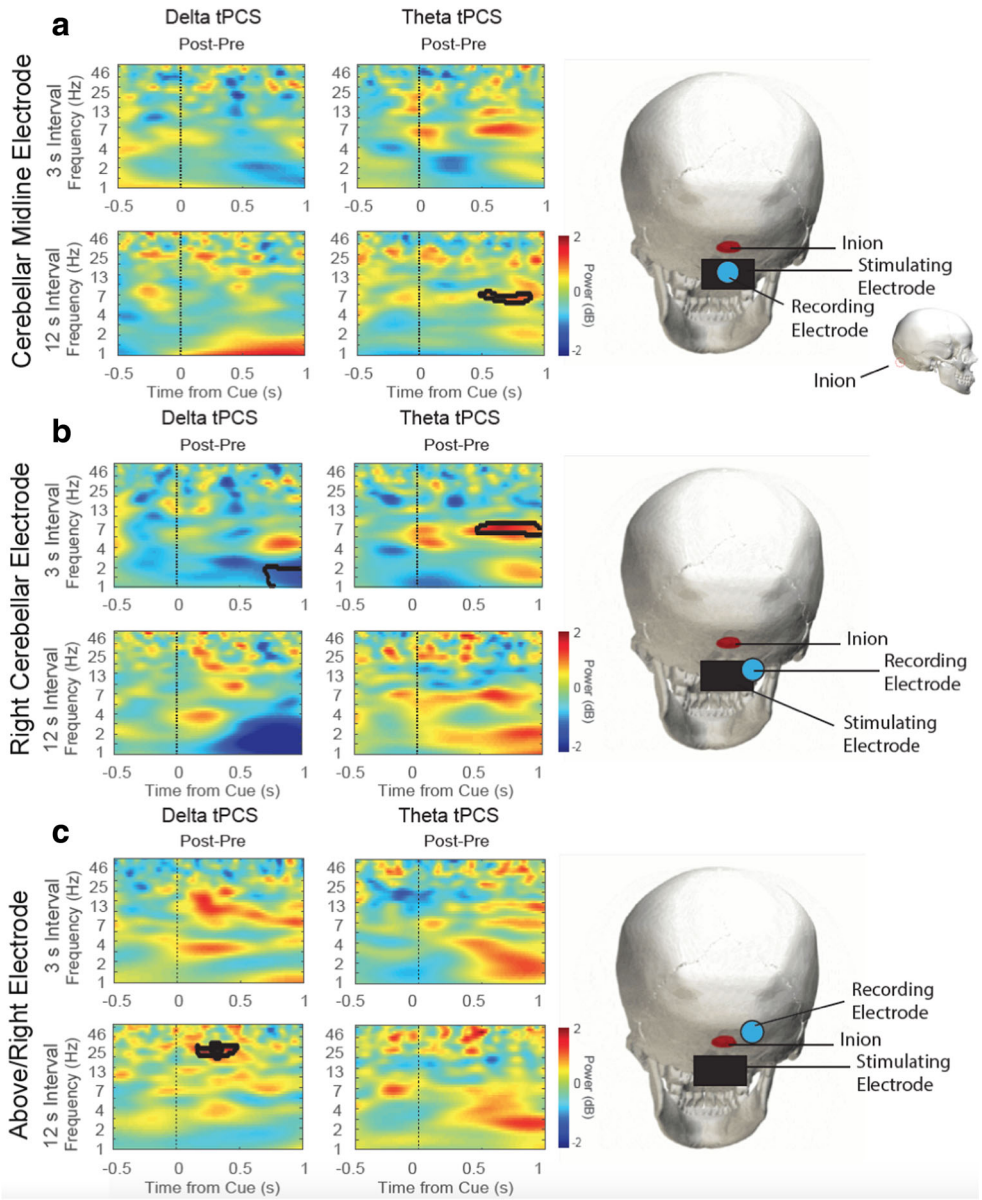

Fig. 5 Cue-evoked activity at cerebellar electrodes is not modulated by cerebellar theta frequency tPCS. **a** There was no effect on cue-related activity at the site of cerebellar stimulation as measured by the midline electrode following delta tPCS on 3-s and 12-s trials (left side). Theta tPCS significantly increased cue-related activity in theta frequencies (4–8 Hz) on 12-s trials (black outlined; $p < 0.05$) while 3-s trials only trended to increase ($p > 0.05$) (right side). **b** Similar to cerebellar midline electrode, delta frequency stimulation did alter cue-related theta frequency activity at the right cerebellar electrode. Theta frequency tPCS significantly increased theta frequency activity for 3-s trials (black outlined; $p < 0.05$) while there was only a trend for 12-s trials. **c** The electrode above and to the right of the site of cerebellar stimulation did not show changes in theta frequencies after delta or theta tPCS. Increased power in red with permutation-corrected statistical significance $p < 0.05$ outlined in black lines

patients with schizophrenia with abnormal low-frequency frontal EEG rhythms is unknown. Although some studies will show EEG changes from a single session of stimulation lasting up to 50 min [33], other studies, especially those focusing on entrainment of specific EEG rhythms with brain stimulation, have shown that effects may last only minutes after the stimulation is stopped, or cease with stimulation offset [51]. It is possible that repeated stimulation sessions, or sessions with different stimulation parameters, could have induced longer-lasting or more robust EEG changes that may have ultimately led to behavioral changes. Future studies could explore this question with longer stimulation protocols.

The EEG findings identified in this study beg the question of the specific mechanism of action through which the cerebellar tPCS is altering frontal regions. The neurophysiologic data for cerebellar tPCS is limited. Transcranial magnetic stimulation (TMS) studies using paired associative stimulation paradigms have detected changes in motor cortex plasticity after cerebellar TMS, with pharmacological manipulations demonstrating that these effects are dependent on N-methyl-D-aspartate (NMDA) receptor activity. Transcranial direct current stimulation (tDCS) studies with similar anodal and cathodal placement to this study have modeled the electrical fields induced and suggest the posterior aspect of the cerebellum can be affected by the stimulation [52]. Despite this evidence, some research has drawn into question whether a low electrical current, such as that utilized in this study (1 mA) can actually induce electrical changes in the brain that would be clinically or physiologically relevant [53].

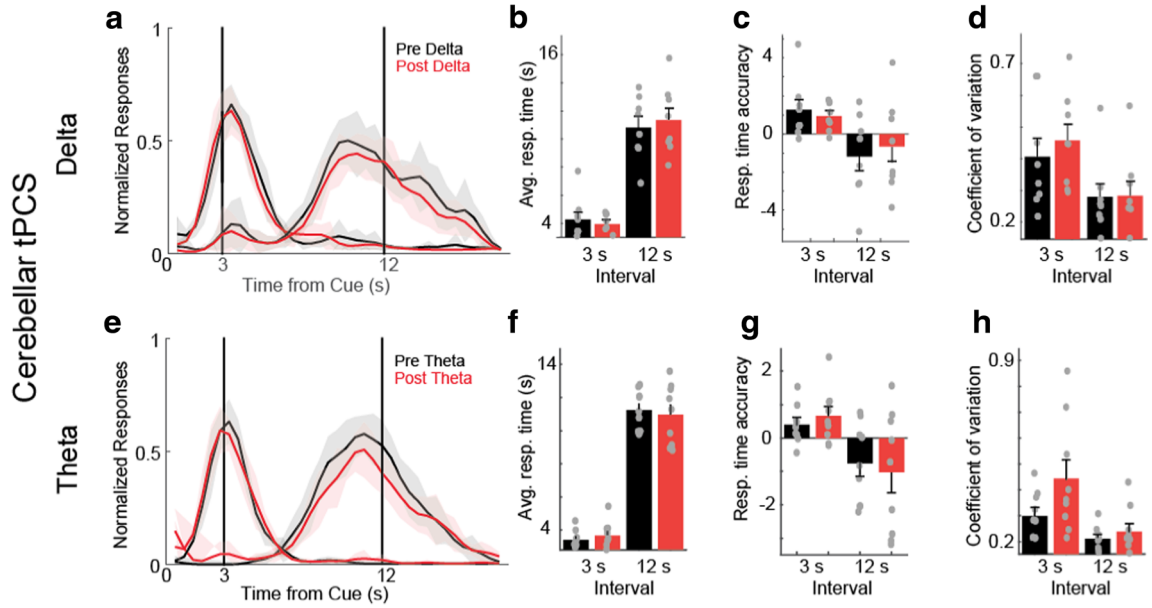

Fig. 6 Single-session delta or theta frequency cerebellar tPCS does not influence interval timing performance. **a** Delta frequency cerebellar stimulation did not influence interval timing on 3-s or 12-s trials. This is indicated by similar average response histograms and overlapping standard error bands of 40 trials before (black) and 40 trials after (red) 20 min of stimulation. Quantification of these response histograms reveals no significant differences for measures of timing, including average response time (**b**), timing efficiency as defined by the number responses occurring around 3 s (2–3 s) and 12 s (11–12 s) (**c**), and coefficient of variation (**d**). **e** Likewise, theta stimulation did not influence interval timing performance as shown by similar average response histogram curves and as quantified by insignificant average response times (**f**), response time accuracy (**g**), and coefficient of variation (**h**)

Although this remains an open question, it invites other potential physiologic explanations for the changes noted here, as well as in volumes of previous noninvasive electrical stimulation research. Another potential mechanism may include direct stimulation of peripheral nerves triggering a "bottom-up" central nervous system response to stimulation [54]. Whether peripheral or cranial nerve stimulation at a rhythmic frequency could entrain neuronal elements remains to be seen. Based on the organization of the cerebellum where the hemispheres slightly cover our targeted vermis, it is likely that stimulation influenced the hemispheres in addition to the vermis. The vermis was targeted based on previous reports of improvement of cognitive function using cerebellar transcranial magnetic vermal stimulation. Going forward, we will investigate the spread of stimulation and changes in the deep nuclei using electrophysiology in rodents to clarify the spread and pathways influenced by midline cerebellar stimulation.

Despite several unanswered questions, this study demonstrates that it is possible to use frequency-specific tPCS over the cerebellum to alter low-frequency brain rhythms during a timing task in a population of patients with schizophrenia. Although this did not lead to behaviorally relevant changes in this patient sample, it should prompt future studies looking at methods for optimizing this modulatory effect. As other studies have demonstrated compromised cerebello-frontal activity in schizophrenia and that low-frequency stimulation of the cerebellum can "rescue" low-frequency deficits in the frontal cortex with associated behavioral improvements, this is a line of research worthy of further pursuit and exploration. Transcranial PCS itself is a highly understudied technology, and the research of its application to the cerebellum in healthy or diseased populations is sparse.

Future studies should include looking at different stimulation parameters (e.g., different current intensities, polarities, and/or electrode locations) or attempting to replicate these findings with other stimulation modalities, such as transcranial magnetic stimulation, which can also be pulsed in frequency-specific patterns (e.g., theta burst stimulation) and may induce a more robust neuromodulatory effect with increased spatial precision. Indeed, there have been two promising studies of using theta-range transcranial magnetic stimulation of the midline cerebellum to improve cognitive and executive functioning in populations of patients with schizophrenia, suggesting some potential clinical benefit to this technique [31, 32]. This effect may be facilitated by cerebellar transcranial magnetic stimulation increasing functional connectivity between the cerebellum and non-motor cortical nodes within the default mode network, as has been previously reported [55]. Schizophrenia remains a major source of morbidity worldwide, and research searching for new treatments and therapies to address the disabling cognitive impairments of the disease should be a priority for clinician scientists and mental health researchers.

Author Contributions K.L.P. designed the research; K.L.P. and S.C acquired data; K.L.P., A.S., B.D., and J.K. analyzed data; K.L.P., N.T.T., A.D.B., B.D., and A.S. wrote the manuscript; and all authors provided feedback.

Funding Information K.L.P has received generous funding to complete this research from the Brain & Behavior Foundation Young Investigator NARSAD Award, The Nellie Ball Research Trust, and NIMH K01 MH106824, the University of Iowa Department of Psychiatry, and the Iowa Neuroscience Institute.

Compliance with Ethical Standards

Written informed consent was obtained from every subject and all research protocols were approved by the University of Iowa Human Subjects Review Board.

Competing Interests The authors declare that they have no competing interests.

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. McGrath J, Saha S, Chant D, Welham J. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev. 2008;30:67–76. https://doi.org/10.1093/epirev/mxn001.
2. Andreasen NC, Paradiso S, O'Leary DS. "Cognitive dysmetria" as an integrative theory of schizophrenia: a dysfunction in cortical-subcortical-cerebellar circuitry? Schizophr Bull. 1998;24:203–18.
3. Carroll CA, O'Donnell BF, Shekhar A, Hetrick WP. Timing dysfunctions in schizophrenia span from millisecond to several-second durations. Brain Cogn. 2009;70:181–90.
4. Green MF, Harvey PD. Cognition in schizophrenia: past, present, and future. Schizophr Res Cogn. 2014;1:e1–9. https://doi.org/10.1016/j.scog.2014.02.001.
5. Ho BC, Nopoulos P, Flaum M, Arndt S, Andreasen NC. Two-year outcome in first-episode schizophrenia: predictive value of symptoms for quality of life. Am J Psychiatry. 1998;155:1196–201.
6. Ward RD, Kellendonk C, Kandel ER, Balsam PD. Timing as a window on cognition in schizophrenia. Neuropharmacology. 2011;62:1175–81. https://doi.org/10.1016/j.neuropharm.2011.04.014.
7. Andreasen NC, O'Leary DS, Flaum M, Nopoulos P, Watkins GL, Boles Ponto LL, et al. Hypofrontality in schizophrenia: distributed dysfunctional circuits in neuroleptic-naïve patients. Lancet. 1997;349:1730–4.
8. Carter CS, Perlstein W, Ganguli R, Brar J, Mintun M, Cohen JD. Functional hypofrontality and working memory dysfunction in schizophrenia. 2014. Available at: http://ajp.psychiatryonline.org/doi/10.1176/ajp.155.9.1285 [Accessed March 27, 2015].
9. Parker KL, Kim Y, Kelley RM, Nessler AJ, Chen K-H, Muller-Ewald VA, et al. Delta-frequency stimulation of cerebellar projections can compensate for schizophrenia-related medial frontal dysfunction. Mol Psychiatry. 2017;22:647–55.
10. Andreasen NC, Pierson R. The role of the cerebellum in schizophrenia. Biol Psychiatry. 2008;64:81–8. https://doi.org/10.1016/j.biopsych.2008.01.003.
11. Repovs G, Csernansky JG, Barch DM. Brain network connectivity in individuals with schizophrenia and their siblings. Biol Psychiatry. 2011;69:967–73. https://doi.org/10.1016/j.biopsych.2010.11.009.
12. Abi-Dargham A, Mawlawi O, Lombardo I, Gil R, Martinez D, Huang Y, et al. Prefrontal dopamine D1 receptors and working memory in schizophrenia. J Neurosci. 2002;22:3708–19.
13. Koziol LF, Budding D, Andreasen N, D'Arrigo S, Bulgheroni S, Imamizu H, et al. Consensus paper: the cerebellum's role in movement and cognition. Cerebellum. 2013. https://doi.org/10.1007/s12311-013-0511-x.
14. Schmahmann JD. From movement to thought: anatomic substrates of the cerebellar contribution to cognitive processing. Hum Brain Mapp. 1996;4:174–98. https://doi.org/10.1002/(SICI)1097-0193(1996)4:3<174::AID-HBM3>3.0.CO;2-0.
15. Schmahmann JD. Dysmetria of thought: clinical consequences of cerebellar dysfunction on cognition and affect. Trends Cogn Sci (Regul Ed). 1998;2:362–71.
16. Schutter DJLG, van Honk J. An electrophysiological link between the cerebellum, cognition and emotion: frontal theta EEG activity to single-pulse cerebellar TMS. Neuroimage. 2006;33:1227–31. https://doi.org/10.1016/j.neuroimage.2006.06.055.
17. Stoodley CJ. The cerebellum and cognition: evidence from functional imaging studies. Cerebellum. 2011;11:352–65. https://doi.org/10.1007/s12311-011-0260-7.
18. Strick PL, Dum RP, Fiez JA. Cerebellum and nonmotor function. Annu Rev Neurosci. 2009;32:413–34. https://doi.org/10.1146/annurev.neuro.31.060407.125606.
19. Jurjus GJ, Weiss KM, Jaskiw GE. Schizophrenia-like psychosis and cerebellar degeneration. Schizophr Res. 1994;12:183–4. https://doi.org/10.1016/0920-9964(94)90076-0.
20. Nopoulos PC, Ceilley JW, Gailis EA, Andreasen NC. An MRI study of cerebellar vermis morphology in patients with schizophrenia: evidence in support of the cognitive dysmetria concept. Biol Psychiatry. 1999;46:703–11.
21. Sandyk R. Psychotic behavior associated with cerebellar pathology. Int J Neurosci. 1993;71:1–7.
22. Tavano A, Grasso R, Gagliardi C, Triulzi F, Bresolin N, Fabbro F, et al. Disorders of cognitive and affective development in cerebellar malformations. Brain. 2007;130:2646–60. https://doi.org/10.1093/brain/awm201.
23. Andreasen NC, O'Leary DS, Cizadlo T, Arndt S, Rezai K, Ponto LL, et al. Schizophrenia and cognitive dysmetria: a positron-emission tomography study of dysfunctional prefrontal-thalamic-cerebellar circuitry. Proc Natl Acad Sci U S A. 1996;93:9985–90.
24. Rüsch N, Spoletini I, Wilke M, Bria P, Di Paola M, Di Iulio F, et al. Prefrontal-thalamic-cerebellar gray matter networks and executive functioning in schizophrenia. Schizophr Res. 2007;93:79–89. https://doi.org/10.1016/j.schres.2007.01.029.
25. Middleton FA, Strick PL. Basal ganglia and cerebellar loops: motor and cognitive circuits. Brain Res Rev. 2000;31:236–50. https://doi.org/10.1016/S0165-0173(99)00040-5.
26. Ferrucci R, Cortese F, Bianchi M, Pittera D, Turrone R, Bocci T, et al. Cerebellar and motor cortical transcranial stimulation decrease levodopa-induced dyskinesias in Parkinson's disease. Cerebellum. 2016;15:43–7. https://doi.org/10.1007/s12311-015-0737-x.
27. Grimaldi G, Argyropoulos GP, Boehringer A, Celnik P, Edwards MJ, Ferrucci R, et al. Non-invasive cerebellar stimulation-a consensus paper. Cerebellum. 2014;13:121–38. https://doi.org/10.1007/s12311-013-0514-7.
28. Fregni F, Boggio PS, Nitsche M, Bermpohl F, Antal A, Feredoes E, et al. Anodal transcranial direct current stimulation of prefrontal cortex enhances working memory. Exp Brain Res. 2005;166:23–30. https://doi.org/10.1007/s00221-005-2334-6.
29. Jo JM, Kim Y-H, Ko M-H, Ohn SH, Joen B, Lee KH. Enhancing the working memory of stroke patients using tDCS. Am J Phys Med Rehabil. 2009;88:404–9. https://doi.org/10.1097/PHM.0b013e3181a0e4cb.
30. Ferrucci R, Marceglia S, Vergari M, Cogiamanian F, Mrakic-Sposta S, Mameli F, et al. Cerebellar transcranial direct current stimulation

impairs the practice-dependent proficiency increase in working memory. J Cogn Neurosci. 2008;20:1687–97. https://doi.org/10.1162/jocn.2008.20112.
31. Demirtas-Tatlidede A, Freitas C, Cromer JR, Safar L, Ongur D, Stone WS, et al. Safety and proof of principle study of cerebellar vermal theta burst stimulation in refractory schizophrenia. Schizophr Res. 2010;124:91–100. https://doi.org/10.1016/j.schres.2010.08.015.
32. Garg S, Sinha VK, Tikka SK, Mishra P, Goyal N. The efficacy of cerebellar vermal deep high frequency (theta range) repetitive transcranial magnetic stimulation (rTMS) in schizophrenia: a randomized rater blind-sham controlled study. Psychiatry Res. 2016;243:413–20. https://doi.org/10.1016/j.psychres.2016.07.023.
33. Vasquez A, Malavera A, Doruk D, Morales-Quezada L, Carvalho S, Leite J, et al. Duration dependent effects of transcranial pulsed current stimulation (tPCS) indexed by electroencephalography. Neuromodulation. 2016;19:679–88. https://doi.org/10.1111/ner.12457.
34. Ivry RB, Spencer RM. The neural representation of time. Curr Opin Neurobiol. 2004;14:225–32. https://doi.org/10.1016/j.conb.2004.03.013.
35. Parker KL, Chen K-H, Kingyon JR, Cavanagh JF, Naryanan NS. Medial frontal ~4 Hz activity in humans and rodents is attenuated in PD patients and in rodents with cortical dopamine depletion. J Neurophysiol. 2015. https://doi.org/10.1152/jn.00412.2015.
36. Rakitin BC, Gibbon J, Penney TB, Malapani C, Hinton SC, Meck WH. Scalar expectancy theory and peak-interval timing in humans. J Exp Psychol Anim Behav Process. 1998;24:15–33.
37. Gibbon J, Church RM, Meck WH. Scalar timing in memory. Ann N Y Acad Sci. 1984;423:52–77.
38. Buhusi CV, Meck WH. What makes us tick? Functional and neural mechanisms of interval timing. Nat Rev Neurosci. 2005;6:755–65. https://doi.org/10.1038/nrn1764.
39. Parker KL, Chen K-H, Kingyon JR, Cavanagh JF, Narayanan NS. D1-dependent 4 Hz oscillations and ramping activity in rodent medial frontal cortex during interval timing. J Neurosci. 2014;34:16774–83. https://doi.org/10.1523/JNEUROSCI.2772-14.2014.
40. Gülekon IN, Turgut HB. The external occipital protuberance: can it be used as a criterion in the determination of sex? J Forensic Sci. 2003;48:513–6.
41. Bocci T, Santarcangelo E, Vannini B, Torzini A, Carli G, Ferrucci R, et al. Cerebellar direct current stimulation modulates pain perception in humans. Restor Neurol Neurosci. 2015;33:597–609. https://doi.org/10.3233/RNN-140453.
42. Bocci T, Ferrucci R, Barloscio D, Parenti L, Cortese F, Priori A, et al. Cerebellar direct current stimulation modulates hand blink reflex: implications for defensive behavior in humans. Phys Rep. 2018;6. https://doi.org/10.14814/phy2.13471.
43. Ferrucci R, Giannicola G, Rosa M, Fumagalli M, Boggio PS, Hallett M, et al. Cerebellum and processing of negative facial emotions: cerebellar transcranial DC stimulation specifically enhances the emotional recognition of facial anger and sadness. Cognit Emot. 2012;26:786–99. https://doi.org/10.1080/02699931.2011.619520.
44. Ferrucci R, Brunoni AR, Parazzini M, Vergari M, Rossi E, Fumagalli M, et al. Modulating human procedural learning by cerebellar transcranial direct current stimulation. Cerebellum. 2013;12:485–92. https://doi.org/10.1007/s12311-012-0436-9.
45. van Driel J, Sligte IG, Linders J, Elport D, Cohen MX. Frequency band-specific electrical brain stimulation modulates cognitive control processes. PLoS One. 2015;10:e0138984. https://doi.org/10.1371/journal.pone.0138984.
46. Cohen MX. Analyzing neural time series data: theory and practice (issues in clinical and cognitive neuropsychology). The MIT Press; 2014.
47. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J Neurosci Methods. 2004;134:9–21. https://doi.org/10.1016/j.jneumeth.2003.10.009.
48. Cavanagh JF, Frank MJ. Frontal theta as a mechanism for cognitive control. Trends Cogn Sci. 2014;18(8):414-2.
49. Singh A, Richardson SP, Narayanan N, Cavanagh JF Mid-frontal theta activity is diminished during cognitive control in Parkinson's disease. Neuropsychologia. 2018;117:113-122.
50. Malapani C, Deweer B, Gibbon J. Separating storage from retrieval dysfunction of temporal memory in Parkinson's disease. J Cogn Neurosci. 2002;14:311–22. https://doi.org/10.1162/089892902317236920.
51. Ozen S, Sirota A, Belluscio MA, Anastassiou CA, Stark E, Koch C, et al. Transcranial electric stimulation entrains cortical neuronal populations in rats. J Neurosci. 2010;30:11476–85. https://doi.org/10.1523/JNEUROSCI.5252-09.2010.
52. Parazzini M, Rossi E, Ferrucci R, Liorni I, Priori A, Ravazzani P. Modelling the electric field and the current density generated by cerebellar transcranial DC stimulation in humans. Clin Neurophysiol. 2014;125:577–84. https://doi.org/10.1016/j.clinph.2013.09.039.
53. Vöröslakos M, Takeuchi Y, Brinyiczki K, Zombori T, Oliva A, Fernández-Ruiz A, et al. Direct effects of transcranial electric stimulation on brain circuits in rats and humans. Nat Commun. 2018;9:483. https://doi.org/10.1038/s41467-018-02928-3.
54. Underwood E. How the body learns to hurt. Science. 2016;354:694. https://doi.org/10.1126/science.354.6313.694.
55. Halko MA, Farzan F, Eldaief MC, Schmahmann JD, Pascual-Leone A. Intermittent Theta-burst stimulation of the lateral cerebellum increases functional connectivity of the default network. J Neurosci. 2014;34:12049–56. https://doi.org/10.1523/JNEUROSCI.1776-14.2014.