Transcranial Magnetic Stimulation-EEG Biomarkers of Poststroke Upper-Limb Motor Function

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Background: Motor evoked potentials obtained with transcranial magnetic stimulation (TMS) can provide valuable information to inform stroke neurophysiology and recovery but are difficult to obtain in all stroke survivors due to high stimulation thresholds. *Objective:* To determine whether transcranial magnetic stimulation evoked potentials (TEPs) evoked using a lower stimulus intensity, below that necessary for recording motor evoked potentials, could serve as a marker of poststroke upper-limb motor function and were different compared to healthy adults. *Methods:* Eight chronic stroke survivors (66 ± 21 years) and 15 healthy adults (53 ± 10 years) performed a motor function task using a customized grip-lift manipulandum. TMS was applied to the lesioned motor cortex, with TEPs recorded using simultaneous high-definition electroencephalography (EEG). *Results:* Stroke participants demonstrated greater hold ratio with the manipulandum. Cluster-based statistics revealed larger P30 amplitude in stroke participants, with significant clusters over frontal ($P = .016$) and parietal-occipital electrodes ($P = .023$). There was a negative correlation between the N45 peak amplitude and hold ratio in stroke participants ($r = -.83$, $P = .02$), but not controls. *Conclusions:* TEPs can be recorded using lower stimulus intensities in chronic stroke. The global P30 TEP response differed between stroke participants and healthy controls, with results suggesting that the TEP can be used as a biomarker of upper-limb behavior.

Key Words: Motor Cortex—transcranial magnetic stimulation—electroencephalography—TMS-evoked potential—stroke—biomarker

Introduction

Stroke can lead to persistent behavioral and motor impairments that impact daily living.[1,2] Despite lengthy periods of rehabilitation and advances in therapeutic approaches to help restore function, many stroke survivors live with persistent impairments and require ongoing community support services.[3] To date, several neuroimaging and neurophysiological techniques have provided valuable information to inform stroke recovery. It is highly likely that information obtained from these techniques will be critical for improving stroke care. Indeed, new insights into stroke physiology provided by these techniques have assisted rehabilitative efforts by identifying treatment approaches, therapeutic targets, and biomarkers of recovery or response to therapy.[4-7] Continual development of these neuroimaging and neurophysiological techniques is a critical catalyst to improving therapeutic interventions or approaches in order to facilitate a more complete recovery of function following stroke.

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One common neurophysiological technique that has received much attention in the stroke literature is transcranial magnetic stimulation (TMS). Single-pulse TMS applied to the motor cortex (M1) is able to evoke a response in a peripheral muscle, termed a motor evoked potential (MEP).[8,9] MEPs provide several critical pieces of information including integrity and excitability of the descending motor pathways that is particularly relevant for stroke. For example, the presence of an MEP after stroke provides some indication that the descending motor pathways are intact, suggesting potential for recovery.[5] Furthermore, following stroke, excitability of the ipsilesional M1 is reduced and the magnitude of this reduction in excitability is correlated with motor impairment.[10,11] As a result, ipsilesional excitability is frequently used as a marker of response to therapy,[12-15] and has been the target of noninvasive brain stimulation interventions.[16,17] Paired-pulse TMS measures have also proven valuable in understanding the cortical mechanisms mediating poststroke impairment and recovery. Paired-pulse measures are able to evaluate the excitability of inhibitory and excitatory neural circuits, that play a critical role in cortical reorganisation processes that facilitate recovery of function.[18-20] Investigation of neurophysiological mechanisms mediating cortical reorganization are crucial for understanding stroke recovery.

While the use of TMS to record MEPs has substantial value in probing the poststroke brain, it does unfortunately limit neurophysiological investigations to the motor cortex. In addition, there are requirements for suprathreshold TMS pulses in order to record MEPs in a peripheral muscle. This limits opportunity to probe corticospinal neurophysiology in stroke survivors where MEPs are not obtainable, or the threshold to obtain a MEP of useful amplitude is high. In these cases, TMS measures using MEPs as a marker are rendered impractical, and critical information of poststroke neurophysiology is not obtainable. Furthermore, MEPs can be influenced by spinal activity and are therefore not a direct measure of cortical excitability. It is likely that new assessments that probe cortical physiology but are not limited by constraints of MEP recordings following TMS may prove highly valuable in stroke.

One possibility is to use a multimodal approach by pairing TMS with electroencephalography (EEG), to provide opportunities to investigate cortical properties including excitation or inhibition, intrinsic oscillatory activity and connectivity of local and distributed networks with high temporal resolution.[21,22] Unlike MEPs, TMS evoked potentials (TEPs) are independent from influence or integrity of descending motor pathways and therefore provide a direct measure of cortical excitability. Importantly, evidence has shown that TEPs can provide information on underlying cortical physiology and may be associated with poststroke impairment. For example, it was recently reported that TEP peak amplitudes are increased and latencies longer in chronic stroke patients compared to healthy adults.[23] Furthermore, a longer latency of the P30 component was associated with less manual dexterity,[23] suggesting this physiological measure may be a functional biomarker. Previous studies have suggested GABA-A postsynaptic receptors may play a role in the generation or modulation of the early TEP components such as the P30 and N45.[24,25] In support, paired-pulse TMS studies have also reported gamma-aminobutyric acid-A (GABA-A) receptor activity is associated with motor function in people with stroke.[26-28] Furthermore, previous work has demonstrated that the presence of the N100 component may have some prognostic value in acute stroke.[29] It may be that the combination of TMS and EEG provides a useful multimodal neurophysiological approach to investigate cortical physiology in people with stroke. However, these previous studies that have reported the P30 and N100 to be markers of stroke function and recovery have evoked TEPs using suprathreshold TMS intensities of 110% and 120% of motor threshold respectively.[23,29] Recently, evidence was provided to indicate that TEPs evoked using lower stimulation intensities could be recorded in people with stroke. A longitudinal study characterized changes in cortical excitability and oscillatory activity by recording TEPs at various time points from subacute to chronic periods after stroke.[30] In addition, the authors report that TMS evoked oscillatory responses early after stroke may be associated with several general function measures including the Berg Balance Scale and National Institute of Health Stroke Scale. While this study provides evidence to suggest TEPs are able to be recorded using lower stimulus intensity in stroke, it remains to be determined whether TEPs evoked using an intensity below that to obtain a reliable MEP are sensitive biomarkers of upper-limb function. Biomarkers of upper-limb function are valuable measures in stroke rehabilitation[7] and further investigation is needed to determine whether the TEP is an appropriate measure. Therefore, we conducted a proof-of-concept study to determine whether TEPs evoked using a lower stimulus intensity, below that necessary for recording MEPs, could serve as a marker of upper-limb behavior following stroke and were different in amplitude or latency compared to healthy adults. We hypothesize that TEPs evoked using a lower stimulus intensity will be associated with upper-limb behavioral outcomes and differ between people with stroke and healthy adults.

Materials and Methods

Participants

Eight chronic stroke survivors (age: 66 ± 21 years, 4 males) and 15 healthy control individuals (age: 53 ± 10 years, 5 males) participated in this study. Inclusion criteria for people with stroke were: (1) first ever ischemic stroke with associated mild to moderate motor impairment, (2) stroke diagnosis confirmed by an experienced neuroradiologist using magnetic

Table 1. *Stroke participant characteristics*

ID	Gender	Age (y)	Time since stroke (mo)	mRS	Lesion volume (cm^3)	Lesion location
1	M	43	34	1	12.84	Left insula, temporo-parietal
2	F	74	30	2	7.33	Right frontotemporo-parietal
3	F	27	26	1	22.13	Left parietal
4	M	67	12	2	0.75	Right parietal-occipital
5	M	76	35	1	2.76	Right temporal
6	F	90	22	2	2.31	Right temporo-parietal
7	M	75	13	2	3.79	Right fronto-temporal
8	F	75	12	2	18.32	Left parietal lobe, insula

Abbreviation: mRS, modified Rankin Scale.

resonance imaging (MRI) or computed tomography (CT) scan at time of acute hospital admission, (3) not currently undertaking any rehabilitation program, (4) 12-36 months after stroke, and (5) Modified Ashworth score less than 4. Exclusion criteria included (1) contraindications to TMS[31] and (2) haemorrhagic stroke or haemorrhagic transformations. Control subjects were recruited if they had (1) no history of stroke, neurodegenerative, or psychiatric disorders, (2) were right handed, (3) no musculoskeletal impairments of the upper-limb, and (4) no contraindications to TMS.[31] Stroke participant characteristics are shown in Table 1. Subjects provided written informed consent in accordance with the World Medical Association Declaration of Helsinki and ethical approval to conduct the study was provided by the University of Adelaide Human Research Ethics Committee (H-2015-250).

Experimental Protocol

This study consisted of a single experimental session of approximately 1.5 hours duration. Participants completed an assessment of upper-limb motor function using a customized grip-lift manipulandum followed by neurophysiological assessments. The modified Rankin Scale was used to provide a measure of disability of the stroke participants.

Magnetic Resonance Imaging

Stroke diagnosis was confirmed with MRI acquired on a Siemens Trio 3T scanner (Siemens, Erlangen, Germany). The imaging protocol consisted of a high-resolution T1-weighted images (repetition time (TR) = 8.6 ms, echo time (TE) = 4 ms, slice thickness = 7 mm), fluid-attenuated inversion-recovery (TR = 9000 ms, TE = 93 ms, slice thickness = 4.5 mm), proton density weighted and T2-weighted brain images (TR = 3890 ms, TE = 15 ms, slice thickness = 4.5 mm). Lesion location was identified by an experienced neuroradiologist and lesion volume was outlined by hand and analyzed using MRIcron (www.mccauslandcenter.sc.edu/crnl/mricron/) similar to a previous study.[32]

Behavioral Outcome Assessment

At the start of each experiment, a sensitive and quantitative assessment of upper-limb motor function was performed using a customized grip-lift manipulandum.[33] The grip-lift

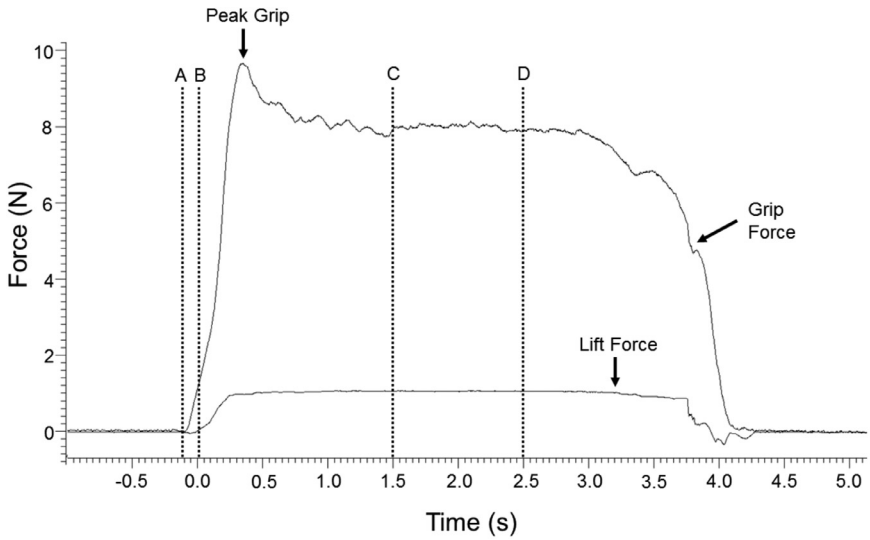

Figure 1. *Example data from an individual grip-lift trial. Dotted vertical lines indicate specific events. (A) onset of grip force, (B) onset of positive lift force, (C-D) a 1 second phase during holding of the grip-lift device as it is held stationary after lifting and is where hold ratio is determined.*

device consisted of 2 force transducers (MLP-10, Transducer Technologies, Temecula, CA) that measured grip and lift forces. Prior to data collection, force transducers were calibrated against known masses (1 N, 2 N, 3 N, 10 N). During each trial, participants placed their index finger and thumb of the paretic hand (or right hand for healthy controls) on 2 polished brass surfaces spaced 35 mm apart. Participants were then required to lift the device off the table with a comfortable, self-selected, grip force to a height of approximately 10 cm, hold it there for 3 seconds, and then lower back down to the table. Participants were instructed to complete the lift task primarily using elbow flexion and were able to practice the task until they were comfortable with the requirements and had achieved a stable level of performance. Ten trials were then collected with data acquisition starting 1 second prior to grip force reaching 1.0 N and ended when the device was returned to the table. Force signals were low pass filtered (100 Hz), sampled at 400 Hz, digitized and stored on computer for off-line analysis. Based on previous studies that reported key measures associated with post-stroke motor function, the grip-lift parameters of interest were peak grip force, hold ratio, correlation maximum, average grip force, and preload duration (see Fig 1).[34-36] Each grip-lift parameter was reported as the average of 10 trials for each participant. The definition of each parameter is shown in Box 1.

Box 1. Grip-lift parameter definition.

Peak grip force—the maximal grip force during the lift phase.
Hold ratio—average grip force to lift force ratio as the device is held stationary after lifting.
Correlation maximum—maximum correlation coefficient obtained when rate of change of grip force (dGF/dt) and lift force (dLF/dt) were cross-correlated, as dGF/dt is shifted in 2.5 ms steps against dLF/dt.
Average grip force—mean grip force during hold phase.
Preload duration—time between onset of grip force and onset of lift force.

Electromyography

Surface electromyography was used to record MEPs from the first dorsal interosseous muscle of the paretic hand (or right hand of healthy controls). Standard skin preparation involved ethanol to clean the skin surface and NuPrep paste to lightly abrade the skin surface. A ground strap was placed around the wrist, and 2 disposable Ag-AgCl surface electrodes were positioned over the first dorsal interosseous muscle in a belly-tendon montage (Ambu, Bellerup, Denmark). Signals were sampled at 5000 Hz (CED 1401; Cambridge Electronic Design, Cambridge, UK), amplified 1000X (CED 1902; Cambridge Electronic Design, Cambridge, UK), filtered (20-1000 Hz) and stored for offline analysis (Signal v4.09, Cambridge Electronic Design, Cambridge, UK).

Transcranial Magnetic Stimulation

Single pulse TMS with monophasic pulse waveform was applied using a 70 mm wing diameter figure-of-eight coil connected to a Magstim 200 stimulator (Magstim Company, Dyfed, UK). The TMS coil was held over the ipsilesional hemisphere (or left hemisphere in healthy controls) at a 45° angle posterolateral to the midsagittal line and generated a posterior-anterior current flow across the hand motor cortex. Using standard protocols the TMS motor hotspot was found by systematically moving the coil to find the optimal position that consistently evoked the largest MEP in the contralateral first dorsal interosseous. Resting motor threshold (RMT) was defined as the minimum TMS intensity needed to elicit a MEP with peak-to-peak amplitude greater than or equal to 50 μV in at least 5 out of 10 trials over M1. A total of 120 TMS pulses (3 blocks of 40) were delivered at RMT with an interstimulus interval of 5 seconds ± 10%.

EEG Recording

Electroencephalography was continuously recorded during application of TMS pulses using PolyBench software (TMSi, Oldenzaal, the Netherlands) to measure TEPs. Signals were acquired by a 64-channel dense-array surface EEG cap with Ag-AgCl electrodes in standard 10-10 positions (Waveguard, ANT Neuro, Enschede, the Netherlands). Head circumference was measured to determine correct cap size. Participants were told to keep their eyes open throughout the experimental process, limit blinking and focus their gaze to a fixed point. Electrical impedance for each electrode was kept below 5 kΩ. Additionally, during TEP recording, headphones playing white noise were provided to participants to reduce auditory artefacts. Signals were sampled at 2048 Hz, amplified 20x, filtered (high pass, DC; low pass, 553Hz), with a ground electrode at AFz and online referenced to the average of all electrodes. Data were stored on a computer for off line analysis.

EEG Pre-Processing and Analysis

All EEG data was exported to MATLAB 8.1.0 (MathWorks, Inc., Natick, MA) for preprocessing with EEGLAB (version 13.6.5b)[37] and the TMS-EEG signal analyser (TESA).[38] The data were epoched (-2000 ms to 2000 ms), baseline corrected (-500 ms to -50 ms) with regard to TMS delivery and bad channels were removed. Data around the TMS pulse was removed (between -2 to 15 ms) before a fast independent component analysis (ICA) was run to remove large amplitude muscle artefacts. Signals were then further filtered (bandpass: 1-80 Hz; bandstop: 48-52 Hz) using the EEGLAB filter function.[37] A second ICA

was conducted for further artefact removal (e.g. stimulus decay, blink/eye movements, auditory evoked potentials, and other noise) before interpolating missing channels. Identified components from each ICA were classified using a semiautomated component classification algorithm (tesa_compselect function) using the TESA toolbox.[38] Similar to previous stroke EEG studies that have group level responses across the whole scalp, cleaned EEG data for participants with a right hemisphere lesion were flipped about the midline so that the lesion appeared in the left hemisphere for all stroke participants.[32,39] The C3 electrode was selected for extracting TEP components given its proximity to the primary motor cortex.[40] TEP peak amplitude and latencies were extracted using the TESA toolbox function tesa_peakanalysis[38] for P30, N45, P55 and N100 peaks. This is an automated function for identification of TEP peaks within predefined time windows. A peak is defined as a data point that is larger/smaller than 5 data points either side. Time windows used for the extraction were relative to TMS delivery (P30, 20ms-40ms; N45, 30ms-60ms; P55, 45ms-75ms; N100, 80ms-150ms). We selected this approach of investigating local peaks within predefined windows following the TMS pulse as this is common within the TEP field.[23,29,40,41] There is evidence to suggest individual TEP peaks measured using this approach represent distinct pharmacological mechanisms (for review, see Darmani and Ziemann [25]).

Statistical Analysis

Data were analyzed using SPSS software (IBM Corp., Released 2016, IBM SPSS Statistics for Windows, Version 24.0, Armonk, NY, USA) and statistical significance was set at $P < .05$. Normality of data was checked using a Shapiro-Wilk test and if required nonparametric statistics were applied. Where appropriate, Bonferroni corrections were applied to correct for multiple comparisons. Participant demographics and neurophysiological characteristics were compared between stroke participants and healthy control subjects with independent t tests for age and RMT, and a chi-square analysis for gender. Behavioral outcomes from the grip-lift manipulandum were compared between stroke participants and healthy control subjects using either independent t tests (peak grip force, hold ratio, correlation max, and average grip) or Mann-Whitney U tests (preload duration). Amplitude and latency for the identified TEP components extracted from the C3 electrode (P30, N45, P55, and N100) were compared between stroke participants and healthy control subjects with either independent t-tests (latency for P30, N45, P55, and N100) or Mann-Whitney U tests (amplitude for P30, N45, P55 and N100). To test if demographics and clinical characteristics were associated with TEPs, correlations were performed between TEP amplitude and latency and RMT, age and lesion volume. Similarly an independent t-test (or Mann-Whitney U test) were performed to determine if gender was associated with TEP amplitude and latency. Global TEP characteristics were investigated by analyzing the TEP response across the scalp at each electrode using nonparametric cluster based permutation statistics. Nonparametric cluster based permutation statistics provides a robust method to protect against multiple comparison errors for analysis across numerous EEG channels and time-points.[42] Clusters were defined as 2 or more neighboring electrodes that had a P value of $< .05$. Monte-Carlo P values were subsequently determined using 5000 iterations. A cluster was deemed significant if the cluster statistic (i.e. the sum of all t-statistics in each cluster) was $P < .025$ when compared to the permutation distribution. Comparisons were performed separately for each TEP peak and responses compared between stroke participants and healthy controls. Finally, to determine whether lower intensity TEPs can serve as a marker of poststroke behavior, Pearson correlation analysis was performed between any behavioral outcome measure that was found to differ between stroke participants and healthy controls and both latency and amplitude of the TEP components extracted from C3 and the identified clusters for the global TEP response.

Results

Participant Characteristics

Participant demographics (age and gender) as well as baseline neurophysiological responses to TMS (RMT) were analyzed to determine differences between stroke and control groups (Table 2). There were no differences between groups for age, gender, and RMT.

Behavioral Outcome Measures

Healthy control participants had lower maximum grip force and hold ratio compared to people with stroke. Following correction for multiple comparisons only hold ratio remained significantly different between healthy control and stroke participants (Table 3). As a result, hold ratio was identified as the measure of interest for correlation with TEP characteristics.

Table 2. *Comparison of characteristics between stroke and control groups*

	Stroke	Control	*Statistic*
Gender (% male)	50%	33%	$X^2_{(1)} = .68$, $P = .44$
Age (y)	53.1 ± 9.7	65.9 ± 2.5	$t_{(8.7)} = -1.67$, $P = .13$
RMT (%MSO)	55.4 ± 8.0	58.8 ± 7.4	$t_{(21)} = -.98$, $P = .34$

Abbreviations: MSO, maximal stimulator output; RMT, resting motor threshold.

Table 3. *Behavioral outcomes for stroke and control participants*

Measure	Stroke	Control	Statistic	Uncorrected *P* value	Corrected *P* value
Max grip (N)	6.4 ± 1.7	4.2 ± 2.1	$t_{(21)} = -2.5$	***P* = .019**	*P* = .095
Hold ratio	6.4 ± 1.8	3.9 ± 2.1	$t_{(21)} = -2.9$	***P* = .009**	***P* = .045**
Correlation nax	.73 ± .06	.8 ± .1	$t_{(21)} = 0.9$	*P* = .36	*P* = 1.0
Avg grip (N)	5.0 ± 1.5	3.4 ± 2.4	$t_{(21)} = -1.8$	*P* = .09	*P* = .45
Preload duration (ms)	103.8 ± 28.9	207.9 ± 265.4	U = 48	*P* = .47	*P* = 1.0

Abbreviations: ms, millisecond; N, Newtons.
Bold indicates statistically significant differences between stroke and control participants.

TMS-Evoked Potentials

The average group TEP traces from C3 are shown in Figure 2. The amplitude of TEP components of P30, N45, P55, and N100 were compared between people with stroke and healthy control participants. The P30 peak was distinguishable in all 15 control participants and 7 of 8 stroke participants. The N45 and P55 peaks were distinguishable in 14 of 15 control participants and all stroke participants. The N100 peak was distinguishable for all control and stroke participants. There were no significant differences in peak amplitudes observed between groups for P30 (U = 45.0, *P* = .63), N45 (U = 52.0, *P* = .82), P55 (U = 34.0, *P* = .15) and N100 (U = 50.0, *P* = .55) (see Fig 2). There were no significant differences observed in latency between groups; P30 ($t_{(20)} = -.14$, *P* = .88), N45 ($t_{(20)} = .55$, *P* = .59), P55 ($t_{(20)} = -.07$, *P* = .94)

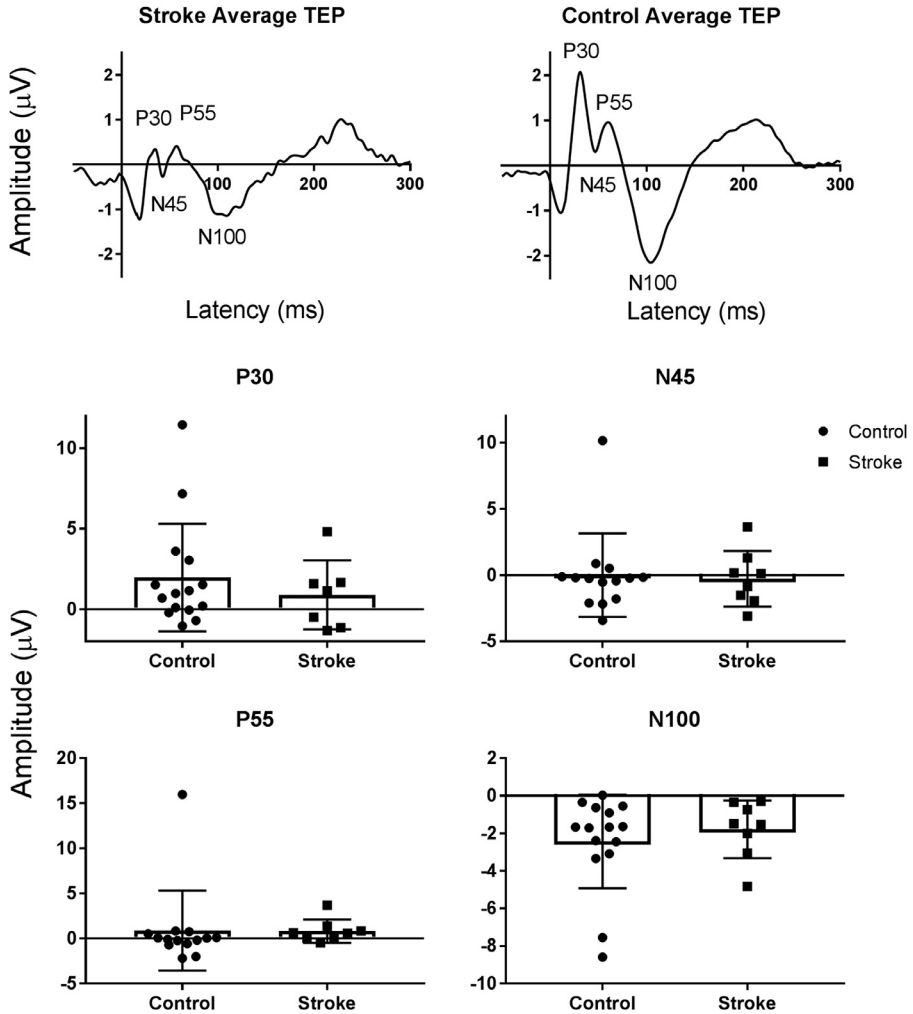

Figure 2. *Stroke and control participant TEP data extracted from C3. Average TEP traces for stroke (top left) and control (top right) participants are plotted. TEP amplitudes (μV) for healthy control and stroke participants are compared for the P30 (middle left), N45 (middle right), P55 (bottom left) and N100 (bottom right) components. There were no statistically significant differences in TEP amplitude between groups. Abbreviation: TEP, transcranial magnetic stimulation evoked potentials.*

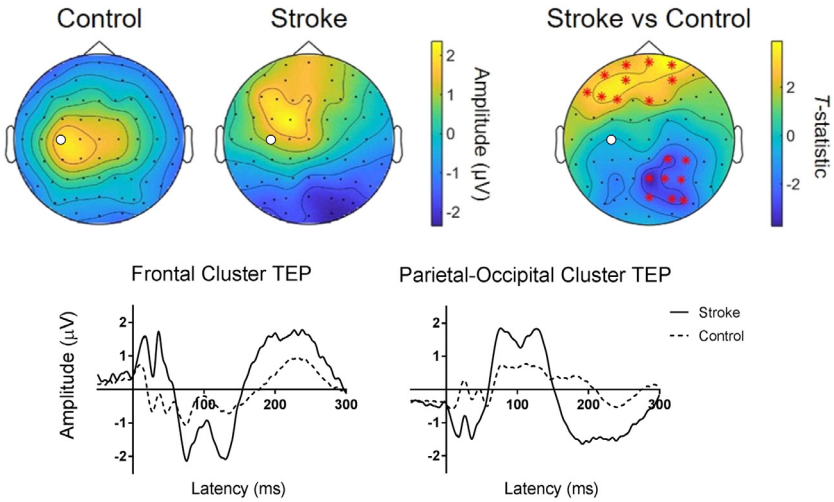

Figure 3. *Cluster analysis of global TEP response across the scalp. Cluster analysis identified 2 clusters for the P30 TEP. Topographic plots represent the distribution of P30 amplitudes in control (top left) and stroke (top middle) participants, and t-statistic values across the scalp comparing P30 amplitudes between groups (top right). Identified clusters (P < .025) are sown with the red asterisks in the top right topographic plot. White circle indicates site of stimulation. The average TEP trace for the frontal electrode cluster (bottom left) and parietal-occipital electrode cluster (bottom right) are provided. Abbreviation: TEP, transcranial magnetic stimulation evoked potentials. (Color version of figure is available online.)*

and N100 ($t_{(21)} = -1.00$, $P = .33$). TEP amplitude and latency were not associated with lesion volume (stroke participants, $P > .11$), age (stroke participants, $P > .15$; healthy adults, $P > .16$), gender (stroke participants, $P > .19$; healthy adults, $P > .12$), or RMT (stroke participants, $P > .17$; healthy adults, $P > .11$) for healthy adults or stroke participants.

Cluster Based Analysis

Cluster based statistics identified 2 significant clusters for P30, with a positive cluster over frontal electrodes ($P = .016$) and a negative cluster over parietal-occipital electrodes ($P = .023$) (Fig 3). Given that the deflection of P30 is positive over frontocentral electrodes and negative over posterior electrodes, both of these clusters indicate an increase in P30 amplitude in stroke participants compared to controls. Topographical plots in Figure 3 demonstrate that, while P30 amplitude in control participants is focused over left central electrodes approximating the stimulated M1, this peak is shifted anterior to the stimulation site in stroke participants. No significant clusters were obtained for N45, P55 or N100 ($P > .05$).

Correlation between Neurophysiology and Behavior

Hold ratio was selected as the behavioral outcome of interest as it was found to be different between stroke participants and healthy controls. We found a negative correlation between the N45 peak amplitude and hold ratio ($r = -.88$, $P = .004$ (uncorrected), $P = .016$ (corrected) Fig 4), but no other significant correlations for P30, P55, or N100 amplitude for stroke participants. There was a significant negative correlation between the N45 latency and hold ratio, however this did not persist after correction for multiple comparisons ($r = -.76$, $P = .027$ (uncorrected) $P = .108$ (corrected)). There were no other significant correlations between TEP latency and hold ratio for stroke

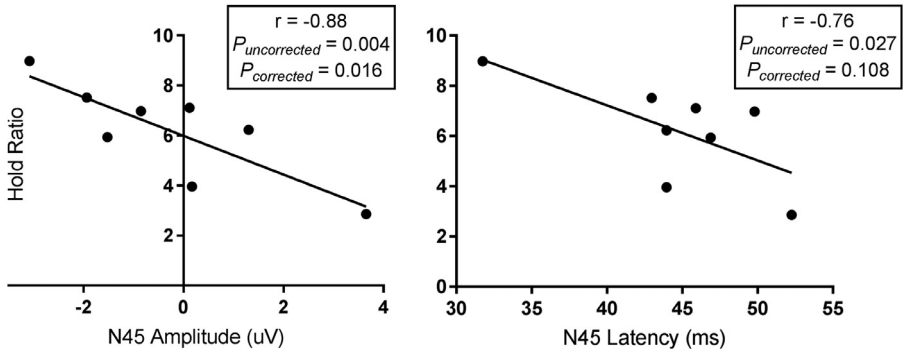

Figure 4. *The amplitude (left) and latency (right) of the N45 TEP component correlated with hold ratio on the grip-lift task for stroke participants. Only amplitude of the N45 component (left) remained significant following correction for multiple comparisons. A higher hold ratio was associated with a more negative deflection of the N45 component. Abbreviation: TEP, transcranial magnetic stimulation evoked potentials.*

participants. Hold ratio was not correlated with any TEP component amplitude or latency measure for healthy adults (all $P > .05$). For participants with stroke, there was a trend for greater positive deflection of the P30 amplitude over the frontocentral cluster and longer hold ratio, however this did not reach significance ($r = .68$, $P = .09$). There was no significant correlation between hold ratio and the parietal-occipital cluster of electrodes for stroke participants, or either cluster for healthy adults (all $P > .05$).

Discussion

The purpose of this study was to determine whether TEPs evoked at a stimulation intensity below that necessary for recording MEPs could serve as a marker of poststroke upper-limb motor function and were different in amplitude or latency compared to healthy adults. Our results indicate that the majority of predetermined TEP components evoked at motor threshold were distinguishable in both stroke participants and healthy adults. Cluster analysis demonstrated a significant difference in the distribution of the P30 component across the scalp, with the response shifted anteriorly in stroke participants compared to healthy adults. The amplitude of the N45 component was associated with performance on a grip-lift manipulandum task for stroke participants. This study provides evidence to suggest that TEPs can be evoked in stroke survivors using lower intensities than those typically used for TMS, while potentially providing valuable data to probe neurophysiology of the poststroke brain that might prove to be a sensitive biomarker of poststroke upper-limb motor function.

There are several lines of evidence to suggest that low intensity TMS applied to the cortex can elicit cortical activity. For example, paired-pulse TMS is a technique to measure intracortical excitability and uses a subthreshold conditioning stimulus to modify the effect of a subsequent test pulse delivered a few milliseconds later.[43] Additionally, several studies have demonstrated that TEPs can be evoked at submotor threshold intensities with the characteristics of the evoked cortical activity similar to that observed for higher intensity stimulation.[44-46] Therefore, it is perhaps not unexpected that we were able to record TEPs in stroke participants using a stimulation intensity below that necessary for evoking MEPs in this study. It could be argued that the lower stimulus intensity used in this study resulted in the P30 peak only being distinguishable in 7 out of 8 stroke participants, and the N45 and P55 peaks distinguishable in 14 of 15 healthy controls. However, previous studies investigating TEPs recorded at the motor cortex in healthy adults have also reported similar difficulty in observing all components in each subject despite stimulation being applied at a higher intensity (120% RMT).[40] We therefore suggest it is unlikely that the lower stimulation intensity used in this study was a major factor in our inability to identify the P30, N45, and P55 components in all participants.

Analysis of the global TEP response across the scalp identified that the P30 component was larger and shifted anteriorly in stroke participants compared to healthy adults. This shift in TEP response may reflect change in network activity as a result of the stroke. There is a growing body of evidence to suggest that increased recruitment of secondary motor areas is observed following stroke.[47] For example, functional MRI has shown greater brain activation in recovered stroke patients compared to healthy adults for a finger-tapping task.[48] Increased activity was observed in both the ipsilesional and contralesional hemisphere. In the ipsilesional hemisphere, the premotor cortex and supplementary motor region both demonstrated greater activity compared to healthy controls.[48] Furthermore, a TMS study has demonstrated that disruption of activity in the ipsilesional premotor cortex using TMS was found to increase reaction time of the paretic hand in well recovered chronic stroke patients, but not healthy controls.[49] Together, these results suggest recruitment of ipsilesional secondary motor areas, including the premotor cortex, can occur in well recovered stroke patients. Although difficult to infer the generators of neural signals recorded at the scalp with EEG, it may be that the anterior shift in the P30 peak for the well recovered stroke participants in this study reflects increased brain activity of secondary motor areas, that could include the ipsilesional premotor cortex.

Critically, this study not only provides evidence that TEPs can be recorded in people with stroke using a lower stimulation intensity than that used previously,[23,29] but there is indication that the neurophysiological data generated by this approach may be a useful marker of motor behavior. Our results indicated that the amplitude of the N45 component may be associated with hold ratio on the grip-lift manipulandum. Using a pharmacological approach, a previous study that also evoked TEPs at motor threshold demonstrated that GABA-A receptor activity contributes to the N45 component following motor cortex stimulation.[50] Therefore, our results imply that a greater negative deflection at approximately 45 milliseconds following the TMS pulse, indicative in part of greater GABA-A receptor activity, is associated with a higher grip force to lift force ratio during the static hold phase. It is of note that hold ratio was significantly higher in stroke participants compared to healthy controls in this study. This strategy of increasing grip force during a static hold may be a compensation technique to prevent the object from slipping and is attributed to altered sensorimotor inputs or processing.[35,36] In support of this finding, paired-pulse TMS investigating GABA-A excitability also suggested increased inhibition is associated with greater poststroke upper-limb impairment. [27, 28]

To build upon these preliminary findings, an extension of this work may be to investigate TEPs in stroke survivors where MEPs are unable to be evoked. While the presence of a MEP following TMS applied to the ipsilesional M1 has

positive predictive value for recovery of function as it indicates an intact descending motor pathway,[4,5,51] the inability to evoke a MEP does not directly preclude recovery. The ipsilesional hemisphere may be able to reorganise to help restore function in some stroke survivors without MEPs, but where integrity of the corticospinal tract had not passed a certain threshold of disruption.[5,51-53] Currently we are unable to probe the cortical neurophysiology of those stroke survivors where MEPs are not obtainable, despite evidence that some may go on to experience reasonable functional recovery. Using TMS-EEG, the TEP may provide insight to cortical neurophysiology in those where MEPs are difficult or impossible to obtain. However, determining stimulation parameters in stroke participants where MEPs cannot be obtained is an additional challenge that requires consideration before experimental work can begin. One approach could be to set stimulation intensity relative to the contralesional motor cortex. The advantage of this approach is that it would result in an individualized stimulation intensity for each participant reflective of their cortical neurophysiology. An alternative approach may be to use a standardized stimulation intensity based on percentage of stimulator output rather than individual thresholds. However a limitation to this technique is that previous work has shown the TEP varies in a nonlinear fashion for different stimulation intensities relative to motor threshold.[44] As a result, this approach to standardize stimulation may not account for individual differences in cortical physiology. Accordingly, we favor an approach to base stimulation intensity relative to the contralesional motor threshold.

There are limitations to this study that should be acknowledged. Most notably, the sample sizes were relatively small and stroke participants were relatively higher functioning. Both of these limitations are a reflection of our intention to conduct a proof-of-concept investigation. Studies should now progress towards larger samples with more impaired stroke survivors. Furthermore, neuronavigation was not used to determine the TMS motor hotspot in this study. While the use of neuronavigation and anatomical brain imaging may improve the consistency and precision with which TMS is applied, previous studies have shown no differences in the variability and reproducibility of MEPs elicited by navigated and nonnavigated TMS to M1.[54] Therefore, we are confident that the lack of neuronavigation in this study would have had minimal influence on TEPs.

Conclusion

In summary, our findings suggest that the TEP is most likely able to be obtained in people with stroke using stimulation intensities lower than that used previously and below that necessary for recording MEPs. Given the common occurrence of high motor thresholds or inability to obtain MEPs from stimulation applied to the ipsilesional hemisphere, the TEP may provide a unique opportunity to probe cortical physiology in this subgroup of stroke survivors. Importantly, we provide evidence that even at lower stimulation intensities, it appears the global TEP response across the scalp differs between healthy adults and stroke participants. Furthermore, TEP components may be used as a biomarker of behavior with the N45 amplitude found to be associated with abnormal sensorimotor integration. Future studies should continue to explore the use of TEPs in stroke survivors where MEPs are difficult or impossible to obtain.

Declaration of Competing Interest

None of the authors have potential conflicts of interest to be disclosed.

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