Changes in Intracortical Excitability After Transient Ischemic Attack Are Associated With ABCD² Score

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Background and Purpose—A transient ischemic attack (TIA) is a brief ischemic episode characterized by rapid clinical resolution and not associated with permanent cerebral infarction. Whether changes in intracortical excitability persist and are related to clinical predictors of stroke risk after TIA remains unknown.

Methods—Participants were individuals with clinically resolved motor TIA with no structural lesions and healthy age-matched control participants. Single and paired-pulse transcranial magnetic stimulation was used to measure intracortical excitability. Recruitment curves for percent inhibition and facilitation were used to derive excitability thresholds. Correlations between threshold asymmetries and ABCD² score were performed.

Results—Results showed a significant 3-way interaction with reduced inhibition and enhanced facilitation in the affected compared with unaffected hemisphere after TIA. No significant differences were present in healthy participants. Asymmetries in intracortical inhibition and facilitation were significantly correlated with ABCD² score.

Conclusions—The present study is the first, to our knowledge, to demonstrate altered intracortical inhibition and facilitation in the affected hemisphere after TIA. These changes occurred on average 2 weeks after clinical signs of TIA resolved and in the absence of structural lesions and were not present in healthy age-matched control participants. Furthermore, this study is the first, to our knowledge, to report that changes in intracortical excitability after TIA are associated with ABCD² score. (Stroke. 2011;42:728-733.)

Key Words: ABCD² score • intracortical facilitation • intracortical inhibition • transcranial magnetic stimulation • transient ischemic attack

Persisting changes in cortical excitability occur in the ipsilesional and contralesional cerebral hemispheres after ischemic stroke. Specifically, reduced intracortical inhibition (ICI) is evident in both the affected primary motor cortex and remote regions of the motor network after stroke. These effects are robust in the acute phases of stroke recovery and vary depending on degree of clinical recovery, disease duration, and lesion location. Paired-pulse transcranial magnetic stimulation (TMS) at different interstimulus intervals (ISIs) enables the noninvasive measurement of excitability in inhibitory and facilitatory interneurons. This technique has been extensively used to map neuroplastic changes after stroke; however, less is known about whether changes in intracortical excitability also occur after transient ischemic attack (TIA).

Several key questions remain regarding the nature of intracortical changes after TIA. Only one study has previously examined intracortical circuits in individuals with TIA. Their results showed reduced inhibition and a trend for enhanced intracortical facilitation (ICF) in the TIA-affected hemisphere despite symptom resolution and in the absence of structural abnormalities. However, this work: (1) did not include a comparison group of healthy participants and thus did not determine if intracortical changes were age-related or specific to the effects of TIA; (2) did not examine the potential clinical relevance of these changes; and (3) relied on a method of measuring excitability associated with a high degree of both intra- and intersession variability.

Recently, a new method for indexing ICI and ICF has been developed that enables the identification of excitability thresholds from inhibitory and facilitatory recruitment curves. This method has demonstrated validity and reliability and several investigators have adopted modified versions of this approach in other clinical populations. To date, no study has used this approach to investigate ICI and ICF in individuals with TIA, validated it against a healthy comparison group, or examined the relationship between intracortical changes and clinical variables.

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We used paired-pulse TMS to examine changes in the thresholds for ICI and ICF in both a group of individuals with TIA and a healthy age-matched comparison group. In addition, we tested whether these effects were associated with clinical variables, including the ABCD² score and etiology. We predicted that reduced ICI and enhanced ICF would be present in the affected compared with unaffected hemisphere after TIA. Furthermore, we expected that these changes would be specific to individuals with TIA when compared with healthy participants. Finally, we also predicted that a relationship would be present between the degree of asymmetry in thresholds for ICI and ICF and clinical variables.

### Methods

#### Participants

Thirteen participants with acute TIA affecting unilateral motor or sensorimotor systems (mean age, 66.6 years) and 13 healthy age-matched participants (mean age, 65.8 years) with no history of TIA participated (Table). Participants with TIA were recruited from the Stroke Prevention Clinic at Vancouver General Hospital within 30 days of their most recent event (mean 14.5 days post-TIA; SD, 4.6 days). For individuals in the TIA group, inclusion criteria were diagnosis of TIA with unilateral motor or sensorimotor features and full symptom resolution within 24 hours. For both TIA and healthy participants, exclusion criteria were history of stroke, individual or family history of epilepsy, and/or history of seizures. All participants underwent 1 session of paired-pulse TMS and provided institutionally approved informed consent before participation.

#### Clinical Evaluations

All participants in the TIA group were evaluated by a stroke neurologist in the Stroke Prevention Clinic at Vancouver General Hospital before participation. Diagnoses of TIA with unilateral motor or sensorimotor symptoms were confirmed by the evaluating physician. All TIA participants were clinically resolved and no event-related structural lesions were evident on conventional imaging (eg, CT or MRI). ABCD² scores for each participant in the TIA group were derived using chart information; scores were confirmed by a board-certified stroke neurologist (P.A.T.). For the purposes of this study, participants with TIA were analyzed according to the following etiologic categories: large vessel disease, small vessel disease, cardioembolic, and unknown (Table). Category membership was determined by the diagnosing stroke neurologist and was based on the Trial...
of ORG 10172 in Acute Stroke Treatment (TOAST) criteria incorporating all available clinical and diagnostic imaging information.

Transcranial Magnetic Stimulation
Single and paired-pulse TMS were used to measure intracortical excitability in the primary motor cortices (M1) of both cerebral hemispheres (see Supplemental Data for detailed information on TMS methodology; available at http://stroke.ahajournals.org). TMS was delivered through a figure-of-eight-shaped coil, which delivered focal stimulation to the primary motor cortex (M1; Magstim Co). Motor evoked potentials (MEPs) were recorded using surface electromyography placed over the flexor carpi radialis using LabChart software and a Powerlab amplification/electromyography system (AD Instruments, Colorado Springs, CO).

Using paired-pulse stimulation, a modified recruitment curve protocol was used to assess changes in intracortical excitability, yielding a specific threshold for ICI and ICF for each hemisphere. Conditioning stimuli were randomized and delivered across a range of intensities from 15% to 120% of active motor threshold. To avoid potential facilitatory effects associated with indirect wave interactions in M1, paired-pulse TMS was delivered at ISIs of 2 ms for inhibition and 12 ms for facilitation. For each conditioning stimulus intensity, 8 MEPs were recorded at each ISI for a total of 8 test MEPs and 56 conditioned MEPs at each interval for each hemisphere. Hemisphere and ISI order were counterbalanced across participants.

Data Analyses and Statistical Evaluations
The percent of intracortical inhibition (%ICI) or facilitation (%ICF) produced at each conditioning stimulus intensity was determined by calculating the peak-to-peak amplitude of the conditioned MEP for each trial and expressing this as a percentage of the mean test MEP amplitude. Trials exceeding 2 SDs of the mean peak-to-peak amplitude at each conditioning stimulus intensity were identified and excluded (<5% for each conditioning stimulus at each ISI).

Recruitment curves for %ICI and %ICF, as a function of % active motor threshold, were generated for each participant for both cerebral hemispheres (Supplemental Figure I). Quadratic regression functions were fit to these curves and, using these functions, we then calculated the precise threshold (% active motor threshold) at which the amplitude of %ICI and %ICF exceeded 10% of baseline variability in each hemisphere.

To determine if the thresholds for ICI and ICF differed between individuals with TIA and healthy age-matched participants, threshold values were subjected to a 3-way hemisphere (affected, unaffected)×ISI (2 ms, 12 ms)×group (TIA, healthy) repeated-measures analysis of variance with group entered as a between-subjects factor. Thresholds in the affected hemisphere of the TIA group were compared with thresholds in the nondominant hemisphere of the healthy comparison group. Follow-up 2-way analyses of variance and paired t tests were conducted within each group to evaluate significant contrasts.

Thresholds for ICI and ICF were then used to calculate an interhemispheric asymmetry index (interhemispheric asymmetry index = (ipsilesional − contralesional)−(ipsilesional + contralesional)) designed to provide a measure of the degree of asymmetry in inhibitory and facilitatory thresholds between the hemispheres for each participant with TIA. To determine if changes in the thresholds for ICI and ICF have potential clinical relevance, we correlated asymmetry indices for ICI and ICF with clinical variables, including: ABCD² score, TIA etiology (categories represented numerically), and time from event (days). All analyses were conducted with SPSS for Windows (Version 18.0; SPSS Inc, Chicago IL).

Results
Demographic characteristics for participants with acute TIA affecting unilateral motor or sensorimotor systems (mean age, 66.6 years) and healthy age-matched comparison participants (mean age, 65.8 years) are presented in the Table. The 3-way repeated-measures analysis of variance revealed a significant hemisphere (affected, unaffected)×ISI (2 ms, 12 ms)×group (TIA, healthy) interaction ($F[1,12]=5.90$, $P<0.05$), indicating that a significant difference in thresholds for intracortical excitability between the hemispheres was present for the TIA group compared with the healthy comparison group (Figure 1). Decomposition of the 3-way interaction, using separate hemisphere×ISI 2-way analyses of variance for each group, showed a significant interaction of hemisphere×ISI within the TIA group ($F[1,12]=7.76$, $MSE=9158.70$, $P<0.05$) but no significant interaction for healthy age-matched participants ($F[1,12]=0.47$, $MSE=589.14$, $P=0.51$).
Contrasts revealed that, for TIA participants, the threshold for ICI was significantly greater in the affected compared with the unaffected hemisphere (contrast, \( P<0.05 \)), indicating reduced inhibition in the affected hemisphere after TIA (Figure 1). No significant differences between hemispheres were present for healthy participants (contrast, nonsignificant). By contrast, thresholds for ICF were significantly greater in the unaffected compared with the affected hemisphere after TIA (contrast, \( P<0.05 \)), indicating that facilitation was enhanced in the affected hemisphere after TIA (Figure 1). No differences in ICF were evident for healthy participants (contrast, nonsignificant).

Correlation analyses indicated that asymmetry in the thresholds for ICI and ICF between the affected and unaffected hemispheres were highly correlated \( (r=-0.68, P=0.005) \). In addition, ABCD² score was significantly associated with asymmetry indices for both ICI \( (r=-0.47, P=0.05) \) and ICF \( (r=0.67, P<0.05; \) Figure 2). However, asymmetry indices were not significantly correlated with TIA etiology or time from event (days).

**Discussion**

In the current study, we examined changes in intracortical excitability in individuals with TIA compared with healthy age-matched participants. By identifying specific thresholds for ICI and ICF and correlating clinical variables with the degree of asymmetry between the hemispheres for these thresholds, we report 2 novel findings. First, results indicated that thresholds for ICI and ICF were altered in the affected hemisphere in the TIA group compared with the age-matched
healthy control group (Figure 1). Mean threshold for ICI was increased in the TIA-affected hemisphere compared with the unaffected hemisphere, indicating a decrease of ICI in the affected hemisphere. By contrast, mean threshold for ICF was decreased in the affected hemisphere, indicating an increase in facilitation on the TIA-affected side. The present study is the first, to our knowledge, to include a healthy age-matched comparison group; no differences in thresholds for ICI or ICF were present between the hemispheres of healthy participants, confirming that changes in intracortical excitability were related to TIA. Importantly, these changes occurred in the absence of any acute structural lesions and persisted for up to 2 weeks after the resolution of clinical symptoms beyond the period within which conventional structural imaging can detect ischemic changes after TIA. Second, results showed that the degree of asymmetry in thresholds for both ICI and ICF were significantly correlated with ABCD² scores in individuals with TIA (Figure 2).

Results of this study are consistent with previous findings of reduced ICI in the affected hemisphere after stroke²-⁴ and in individuals with very brief (<1 hour) TIAs.¹⁰ Although Koerner and Meineck¹⁰ showed only a trend for enhanced facilitation, results of the present study showed significant increases in ICF, suggesting that hyperexcitability in the affected hemisphere after TIA involves both inhibitory and facilitatory circuits. These results indicate intracortical excitability is altered even after transient ischemia, potentially through the same mechanisms that affect inhibitory and facilitatory systems after ischemic stroke.

After stroke, changes in cortical excitability are mediated primarily by the interplay between activity in γ-aminobutyric acid (GABA)-ergic and glutamatergic systems.⁸,¹⁷,¹⁸ ICI is modulated by GABAergic neurotransmission and glutamate antagonists modulate ICF.¹⁹,²⁰ After a lesion in the motor cortex, reductions in GABA₅ activity occur both surrounding the infarct² and in remote regions of the motor network through changes in transcallosal neurotransmission.¹,²¹ Motor cortex disinhibition may also be accompanied by the upregulation of glutamatergic function, contributing to peri-lesional hyperexcitability after stroke.²²,²³ Furthermore, increased activation in contralateral motor regions after TIA suggests that even brief ischemia can impact remote motor regions.²⁴ Results from this study suggest that a TIA affecting the motor system may impact GABA and glutamatergic function and that both ICI and ICF contribute to hyperexcitability in the affected hemisphere after TIA.

Several factors may account for the ability to detect changes in ICF not previously shown after TIA. Individuals in the present study were evaluated later post-TIA than in previous work (on average 14 days post-TIA versus less than 7 days post-TIA).¹⁰ It is possible that the changes in the threshold for ICF observed in the present study emerged with time after the ischemic event. After stroke, changes in both ICI and ICF evolve with time.⁴,⁵ In a longitudinal study of poststroke neurophysiology, correlations between intracortical excitability and clinical performance not present in the acute phase emerged at 3 months.²⁵ Furthermore, glutamate transmission is dynamic in the peri-infarct area after ischemia and is upregulated in the later phases of recovery.²⁶ In the present study, increases in ICF in the affected cortex persisted on average 2 weeks after TIA (mean, 14.5 days), potentially reflecting similar changes in recovery from even transient episodes of ischemia. Although these results confirm that the impact of TIA on intracortical excitability persists long after the cessation of clinical symptoms, it remains unclear whether intracortical circuits are permanently altered after TIA. Further investigations are necessary to determine the duration of these effects.

It is also possible that the method of paired-pulse stimulation may account for the facilitatory effects shown here. Koerner and Meineck measured ICI and ICF at a single conditioning stimulus intensity calculated from resting motor threshold,¹⁰ a method that is highly variable.¹¹ By contrast, we measured ICI and ICF across a range of conditioning stimulus values and used active motor threshold for the calculation of absolute conditioning stimulus intensities. This method is reliable and has increased sensitivity to intracortical effects.¹¹ Thus, it is possible that, in part, methodological differences may have allowed us to detect effects not previously reported in individuals with TIA.

Results of this study indicated that asymmetries in the thresholds for ICI and ICF are significantly correlated with ABCD² scores in individuals with TIA. Specifically, increases in ABCD² score were associated with both the degree of reduced inhibition and enhanced facilitation in the affected motor cortex (Figure 2). The clinical profile is of considerable importance in assessing stroke risk after TIA and the ABCD² score is a validated predictor of early stroke risk after TIA.²⁷,²⁸ We show that individuals with higher stroke risk (ie, higher ABCD² scores) showed greater disinhibition, even after transient ischemia. This relationship between altered ICI and ICF after TIA and ABCD² score has not previously been described. These data offer preliminary evidence for the potential importance of intracortical excitability as a neurophysiological marker of stroke risk in individuals with TIA; future work would benefit from the explicit investigation of the potential diagnostic and predictive use of measuring changes in intracortical excitability after TIA.

This study has a number of limitations. TIA participants were selected from an outpatient stroke prevention clinic; thus, it is possible that included participants may have had an event of nonvascular origin. However, because all participants presented with sudden-onset motor or sensorimotor symptoms and TIA diagnoses were confirmed by a stroke neurologist, the likelihood of misclassification was greatly reduced. Values for the ABCD² scores were retrospectively derived in the present study. In addition, although intracortical changes occurred in the absence of structural abnormalities, it is important to note that these findings were based primarily on information from noncontrast CT and MR investigations of the related event. It is possible that with the use of diffusion-weighted imaging, which is sensitive to early ischemic changes,²⁹ some individuals may have shown morphological changes related to the transient event.

The present study is the first, to our knowledge, to show significant changes in both ICI and ICF in the affected hemisphere after TIA compared with healthy age-matched participants. Additionally, this study is the first, to our
knowledge, to demonstrate a significant association between asymmetries in the thresholds for ICI and ICF after TIA and ABCD² score, suggesting that intracortical excitability may provide an alternative source of information to characterize stroke risk after TIA. Results of this study augment the understanding of the impact of TIA on the neurophysiology of the affected cerebral hemisphere and represent an initial investigation into the potential clinical utility of measuring changes in intracortical excitability after TIA.

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Disclosures
None.

References
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SUPPLEMENTAL MATERIAL

Methods

Transcranial Magnetic Stimulation

TMS was delivered via a figure-of-eight shaped coil, which delivers focal stimulation, with a current spread small enough (10 x 10 x 20mm) to stimulate M1 and two Magstim 200 stimulators, connected via a Bistim\textsuperscript{2} Module (Magstim Co., UK). MEPs were recorded using surface electromyography (EMG) placed over the flexor carpi radialis (FCR). EMG data were collected for each trial using LabChart software and a Powerlab amplification/EMG system (AD Instruments, Colorado Springs, CO). The stimulating coil was positioned over the scalp at the optimal site for evoking an MEP in FCR with the handle pointing posterior-laterally laterally at an angle of 45° to the mid-sagittal plane. Motor threshold was determined in steps of 1% absolute stimulator output intensity. Resting motor threshold (RMT) was defined as the lowest intensity to elicit MEPs of at least 50\textmu V in 5 of 10 trials in the relaxed FCR. Active motor threshold (AMT) was the lowest intensity to elicit MEPs of at least 200\textmu V in 5 out of 10 trials, during activation of FCR to 20% of the maximum voluntary contraction.

Single and paired-pulse TMS were used to measure intracortical excitability in the primary motor cortices (M1) of both cerebral hemispheres. Baseline corticomotor excitability was measured by delivering 10 single-pulse stimuli to each M1 with an absolute stimulator output intensity of 115% RMT. In paired-pulse TMS, a supra-threshold test stimulus is preceded by a sub-threshold conditioning stimulus. If the conditioning stimulus precedes the test stimulus by 1-6 ms, the net effect on the resultant motor evoked potential (MEP) is inhibitory and conditioning stimuli at 6-15 ms result in
facilitation of the MEP. The magnitude of these effects is modulated by the relative intensities of the conditioning and test stimuli. Paired-pulse stimulation following a modified recruitment curve protocol was used to assess changes in intracortical inhibition and facilitation. Unlike traditional paired-pulse TMS methods, conditioning stimuli in this protocol were based on AMT instead of RMT and ICI and ICF were measured by varying the intensity of the conditioning stimulus, while maintaining a fixed interstimulus interval (ISI). This enables the generation of recruitment curves for percent ICI and ICF across the entire range of conditioning stimulus intensities, rather than at a single conditioning stimulus, yielding a specific threshold for ICI and ICF for each hemisphere. To avoid potential facilitatory effects associated with indirect (I) wave interactions in the motor cortices paired-pulse TMS was delivered at ISIs of 2ms for inhibition and 12ms for facilitation.

Conditioning stimuli were randomized and delivered across a range of intensities from 15% to 120% AMT; 8 MEPs were recorded for each stimulus intensity, at each interval. This resulted in a total of 8 test MEPs and 56 conditioned MEPs at each ISI, for each M1. The test stimulus was set to the saturation output intensity, defined as the minimum intensity at which increasing stimulator output no longer resulted in an increase in MEP amplitude (~700-1000µV peak to peak). Hemisphere and ISI order were counterbalanced across participants.
Figure Captions

Figure 1. Recruitment curves for a) percent ICI and b) percent ICF in a representative participant with TIA.