Reproducibility of Functional Transcranial Doppler Sonography in Determining Hemispheric Language Lateralization

S. Knecht, MD; M. Deppe, PhD; E.-B. Ringelstein, MD; M. Wirtz, PhD; H. Lohmann; B. Dräger; T. Huber; H. Henningsen, MD

Background and Purpose—Since functional transcranial Doppler ultrasonography (fTCD) allows convenient and fully automated quantification of language lateralization, it seems ideal for longitudinal studies of perfusion changes during deterioration as well as recovery of language functions. However, during serial examinations, the technical, stochastic, and physiological variabilities of cerebral blood flow velocities (CBFV) have to be considered. Therefore, before fTCD is accepted as a tool for evaluation of changes in lateralization in the diseased state, its reliability in healthy subjects needs to be determined.

Methods—We performed fTCD during a word generation task based on a previously validated technique with automated calculation of the averaged CBFV differences in the middle cerebral arteries providing an index of lateralization (LI).

Results—(1) The accuracy of the LI as assessed by the confidence interval was better than 1% of the mean hemispheric difference. (2) On repeated examination, LIs obtained from 10 subjects showed a high test-retest reproducibility (Pearson product moment correlation coefficient $r=0.95$, $P<0.0001$). (3) On 10 repeated assessments of LI in the same subject, no practice effects were detected.

Conclusions—Functional TCD is a suitable and very robust tool for the longitudinal quantitative measurement of cerebral language lateralization. (Stroke. 1998;29:1155-1159.)

Key Words: functional imaging | language | reliability | reproducibility | ultrasonography, Doppler

Recent developments in fTCD have made it possible to noninvasively and quantitatively measure lateralization and time course of brain activation.1–13 The technique of fTCD is based on the linkage of cerebral activation and perfusion, a principle also underlying fMRI and oxygen-15 positron emission tomography.14 Perfusion changes result in corresponding blood flow velocity modulations in the supporting basal intracranial arteries that can be continuously measured by fTCD.

Analysis of cerebral functional lateralization by fTCD as performed in the present study constitutes a fully automated, objective procedure. The quantitative measures obtained by fTCD are not biased by defining variable statistical thresholds, as is often the case in the analysis of fMRI data.15–17 Given the availability and easy applicability of Doppler sonography equipment, fTCD offers a possibility for the noninvasive assessment of lateralization of language and other higher brain functions. Such information would be valuable for the planning of neurosurgical procedures or to conveniently estimate the variability of lateralization of different cognitive functions in the general population. fTCD could also be of interest for the quantitative and longitudinal measurement of partial shifts of cerebral activation during the course of learning procedures or recovery from neurological deficits. For example, during recovery from aphasia, recruitment of areas contralateral to the lesioned hemisphere has been observed.18 Involvement of the unaffected hemisphere has also been demonstrated in other types of cerebral plasticity.19–23

However, for fTCD to reliably detect potential shifts of language lateralization in patients in the course of their disease or recovery, two preconditions need to be fulfilled: (1) functional TCD must be sensitive enough to detect even small changes in hemispheric perfusion differences; and (2) in normal subjects, fTCD must yield a measure of lateralization that is highly reproducible over time and is not subject to random variability, learning, or habituation.

In the present study we investigated the accuracy and reproducibility of fTCD in healthy subjects using a word generation paradigm previously validated by direct comparison with the standard assessment for language lateralization, the transfemoral intracarotid amobarbital injection.12,24

Subjects and Methods

Subjects
Four female and seven male subjects (aged 19 to 32 years) participated in the study after giving informed consent. All were healthy

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volunteers except for one subject (aged 19 years) who suffered from medically intractable epilepsy and who was evaluated for resective surgery. No requests were made for subjects to abstain from nicotine, caffeine, or any medication. Examinations identical in protocol, recording procedures, and data analysis were repeated 1 hour to 1 year after the initial examination for 10 subjects. The intervals between individual examinations are listed in the Table. In addition, another subject was investigated 10 times in approximately equal intervals of 1 week after the initial examination for 10 subjects. The intervals between examinations are listed in the Table. In addition, another subject was investigated 10 times in approximately equal intervals of 1 week after the initial examination for 10 subjects. The intervals between individual examinations are listed in the Table. In addition, another subject was investigated 10 times in approximately equal intervals of 2 months to test for practice effects.

**Protocol**

Assessment of hemispheric language dominance was performed by a word generation task previously validated by direct comparison with another subject was investigated 10 times in approximately equal intervals of 1 week after the initial examination for 10 subjects. The intervals between individual examinations are listed in the Table. In addition, another subject was investigated 10 times in approximately equal intervals of 2 months to test for practice effects.

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**Evaluation of Statistical Differences Between Examinations by t Test**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Exam</th>
<th>Interval</th>
<th>N</th>
<th>LI</th>
<th>$s_I$</th>
<th>$P$</th>
<th>Result</th>
</tr>
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<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>20</td>
<td>−0.66</td>
<td>0.31</td>
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<td>NS</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>mo</td>
<td>20</td>
<td>1.05</td>
<td>0.62</td>
<td>0.5802</td>
<td>NS</td>
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<tr>
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<td>1</td>
<td>12 mo</td>
<td>22</td>
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<td>0.54</td>
<td>0.1689</td>
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<tr>
<td>3</td>
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<td>2 mo</td>
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<td>4.26</td>
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</tr>
<tr>
<td>4</td>
<td>1</td>
<td>14 mo</td>
<td>22</td>
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<td>0.41</td>
<td>0.5313</td>
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<tr>
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<tr>
<td>6</td>
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<td>1 wk</td>
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<td>NS</td>
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<tr>
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<td>1 wk</td>
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<tr>
<td>8</td>
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<tr>
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<tr>
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<tr>
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<td>19</td>
<td>4.49</td>
<td>0.67</td>
<td>0.0564</td>
<td>NS</td>
</tr>
</tbody>
</table>

See “Subjects and Methods” for definitions.

**Reproducibility of fTCD**

**Selected Abbreviations and Acronyms**

- CBFV = cerebral blood flow velocity
- fMRI = functional magnetic resonance imaging
- fTCD = functional transcranial Doppler ultrasonography
- LI = laterality index
- MCA = middle cerebral artery
- TCD = transcranial Doppler ultrasonography

**Functional TCD**

fTCD assessment was performed in a manner previously described in detail. Briefly, a commercially available dual transcranial Doppler ultrasonography device (Multi-Dop T, DWL) was used. The MCAs were insonated at a depth of 50 mm with two 2-MHz transducer probes attached to a headband and placed at the temporal skull windows bilaterally (Figure 1). Details of the insonation technique, particularly the correct identification of the MCA, have been published elsewhere. The spectral envelope curves of the Doppler signal were recorded with a rate of 28 sample points per second and stored for off-line processing with the fTCD-analysis computer program Average.13 Data analysis, ie, reduction of spontaneous signal fluctuations, filtering, and artifact reduction, was performed as previously described in detail.13 Epochs containing CBFV values outside the range of 30% to 200% of the mean flow velocity were rejected from further processing. The remaining data were integrated over the corresponding cardiac cycles, were segmented into epochs that related to the cueing tone, and were then averaged. The epochs were set to begin 15 seconds before and to end 35 seconds after the cueing tone. The mean velocity in the 15-second precueing interval ($V_{mean}$) was taken as the baseline value. The relative CBFV changes ($dV$) during cerebral activation were calculated by the formula $dV(t)=100(V(t)-V_{mean})/V_{mean}$ (formula 1), where $V(t)$ is the CBFV over time.

**Laterality Index**

Figure 1 gives a schematic overview of the procedure by which the LI was obtained. Mathematically the ITCD LI was specified by the formula:
(2) \[ \text{LI} = \frac{1}{N} \sum_{i=1}^{N} \text{LI}^{(i)} \] with \[ \text{LI}^{(i)} = \frac{1}{T_{\text{int}}} \int_{t_{\text{start}}-1/2T_{\text{int}}}^{t_{\text{end}}+1/2T_{\text{int}}} \Delta V^{(i)}(t) \, dt \]

where

(3) \[ \Delta V^{(i)}(t) = dV^{(i)}(t)_{\text{left}} - dV^{(i)}(t)_{\text{right}} \]

is the side-to-side difference between the relative velocity changes of the left and right MCAs of epoch number \( i = 1, \ldots, N \) (\( N \) = number of epochs). \( t_{\text{start}} \) represents the latency of the absolute maximum of

(4) \[ \Delta V(t) = \frac{1}{N} \sum_{i=1}^{N} \Delta V^{(i)}(t) \]

during an interval of 8 to 18 seconds after cueing, i.e., during verbal processing. For integration a time period of \( T_{\text{int}} = 2 \) seconds was chosen.

**Assessment of Accuracy**

Since the LI represents the mean of \( N \) interhemispheric stochastic perfusion differences from repeated runs involving word generation, its confidence interval can be deduced, under the assumption of normal distribution, from the standard error

(5) \[ \sigma_{\text{LI}} = \sigma_{\text{LI}} / \sqrt{N} \]

where

(6) \[ \sigma_{\text{LI}} = \sqrt{\frac{\sum_{i=1}^{10} (\text{LI}^{i} - \overline{\text{LI}})^{2}}{N - 1}} \]

Corresponds to the standard deviation of LI. For example, the 95% confidence region (\( P < 0.05 \)) of LI can be estimated by LI ± 1.96\( \sigma_{\text{LI}} \). The frequency distribution of 165 laterality indices \( \text{LI}^{(i)} \) obtained from 10 repetitions in the same subject was gaussian shaped, supporting the assumption of normal distribution. The results of the Kolmogorov-Smirnov test and Shapiro-Wilk’s W test for detection of deviations from normal distribution accorded with normal distribution. Therefore, \( \sigma_{\text{LI}} \) was regarded as a statistical estimator for the immanent uncertainty of LI, i.e., the predicted accuracy of the applied technique for estimation of language.

**Measures of Reproducibility**

Reproducibility of language lateralization was performed in several ways: First the LI was analyzed on the basis of two measurements in 10 healthy subjects. For test-retest reproducibility, the Pearson product moment correlation coefficient

(7) \[ r = \frac{\sum_{i=1}^{10} (\text{LI}^{i} - \overline{\text{LI}}) (\text{LI}^{2} - \overline{\text{LI}}^{2})}{\sqrt{\sum_{k=1}^{10} (\text{LI}^{1} - \overline{\text{LI}})^{2} \sum_{k=1}^{10} (\text{LI}^{2} - \overline{\text{LI}}^{2})^{2}}} \]

was used.\(^{29} \) LI\(^{i} \) represents the LI of the subject with index \( k = 1, \ldots, 10 \) of the first (\( l = 1 \)) or the second (\( l = 2 \)) examination.

(8) \[ \overline{\text{LI}}^{l} = \frac{1}{10} \sum_{k=1}^{10} \text{LI}^{i} \]

is the corresponding average laterality from the 10 subjects during the first or the second examination. The correlation coefficient \( r \) is based on the correlation between individual LIs from repeated examinations relative to the variances of the indices. Additionally, all measurements were submitted to a Student’s \( t \) test to evaluate whether in any subject a statistically significant difference in the LI was present on repeated examination (Table). LIs from these repeated measurements in 10 different subjects were further evaluated by ANOVA to determine whether the statistically deduced confidence region \( \text{LI}^{(i)} \) for the laterality measure LI describes the true uncertainty in LI. In this case variations in LI during sequential measurements can be explained by the estimated stochastic fluctuations \( \text{LI}^{(i)} \). A two-way factorial design was used with the following factors: subject, examination number, and subject examination number. (The symbol * indicates interactions between factors.) Second, LIs from 10 successive examinations in a single subject were evaluated by a one-way ANOVA (ANOVA with the factor: examination number) to determine whether the statistically deduced confidence region \( \text{LI}^{(i)} \) for the laterality measure LI also describes the true uncertainty in LI. In this case variations in LI of successive measurements should similarly be explainable by the estimated stochastic fluctuations \( \text{LI}^{(i)} \). Additionally, we tested for time-dependent trends in the lateralization, e.g., due to learning or habituation. This was done by linear regression of the 10 subsequent LIs in this subject. The regression function, i.e., the dependence of LI on the number of examinations, as well as its 95% confidence interval, was calculated.

**Results**

**Accuracy**

The accuracy \( \text{SI} \) for detecting relative blood flow velocity differences between the left and right MCA by fTCD varied between 0.3% and 1.0% of the mean for the 20 examinations on the 10 different subjects (Table). The confidence interval \( \text{SI} \) for the 10 repeatedly measured LIs in a single subject varied between 0.55% and 1.1% (mean, 0.79%). On average, the extent of interhemispheric CBFV differences during word generation was approximately 3% in magnitude, ranging from –3.6% (right-dominant subjects) to 4.4% (left-dominant subjects).

**Reproducibility**

Figure 2 demonstrates that the indices of relative language lateralization obtained from 10 subjects were highly reproducible when reassessed 1 hour to 14 months after their first examination (Pearson product moment correlation coefficient: \( r = 0.95, P < 0.0001 \)). On testing for statistical differences between LI on the first and the second examinations by ANOVA (\( P = 0.48 \)) or Student’s \( t \) test (Table), no statistically significant difference (i.e., \( P > 0.05 \)) was found in any individual. Moreover, the correlation analysis of differences between the first and the second examinations consistently revealed no dependence of the time interval (\( \Delta T \)) between examinations and these differences (regression function \( \Delta L = 0.9 - 0.09 \times \Delta T \); correlation \( r = 0.11; P = 0.78 \)).

In none of 10 repeated fTCD examinations in the same subject was a statistically significant variation of the
interhemispheric perfusion difference detected (one-way ANOVA: \( P = 0.8 \)) (Figures 3 and 4). Deviations of successive LIs were within the limits of the standard error \( s_{LI} \). Therefore, variations of the LI over time were considered negligible relative to the statistical variability. Moreover, as Figure 4 shows, there was no trend in lateralization over time suggestive of practice effects (regression function: \( LI = 5.03 - 0.082 \times M; \) index of examination \( M = 1, \ldots, 10 \)).

**Discussion**

This study shows that appropriately performed functional TCD measurements can provide accurate and highly reproducible quantitative information on brain activation. To obtain this accuracy and reproducibility of event-related CBFV changes, data need to be processed in a systematic manner. There are three potential sources of variability: (1) inconsistency of the Doppler sonographic procedure; (2) stochastic variability due to sampling from a “noisy” data set, ie, the spontaneous fluctuations of CBFV; and (3) physiological changes of blood flow due to neuronal activation.

The TCD measurement of the CBFV is dependent on the angle of insonation.\(^3\) Changes of this angle from 0° to 30° by variability of probe position or arterial anatomy can render differences in the calculated, absolute CBFV in the magnitude of up to 15% between examinations or sides. Also, in a narrowed arterial segment incidentally insonated during the test, the absolute velocity increase in blood flow due to cerebral activation would be greater than in a regular segment. This is why flow velocities used for statistical analysis were normalized. \( f \)TCD is a tool for the evaluation of perfusion modulations associated with cerebral activation. It therefore need not exploit absolute but relative changes of CBFV. Flow velocities at rest were set as zero baseline, and CBFV changes during the activated state were expressed as values in percentages relative to this baseline. The use of relative CBFV values eliminated the variability associated with changes in insonation angle or vessel diameter.\(^13\)

Functional CBFV data display a stochastic variability because the averaged CBFV curves represent a limited sample from a signal that is affected by a number of cardiovascular fluctuations such as the heart beat, modulations related to breathing, and other low-frequency modulations such as the B-waves and Mayer waves.\(^29\) These cardiovascular fluctuations are of a considerably greater amplitude (approximately 50% of the mean signal) than the changes related to neuropsychological activation (approximately 3% during word generation). However, by heart cycle integration and subsequent averaging, variability of data can effectively be reduced.\(^13\) An additional procedure to further reduce variability between individual recordings was the evaluation of the relative side-to-side difference in CBFV increase, ie, by subtraction of the relative CBFV increase in the right from the left MCA. By so doing, effects from systemic cardiovascular fluctuations on CBFV were completely eliminated.\(^13\) As demonstrated above, the LI obtained by these mathematical procedures had a low standard error, ie, an accuracy better than 1% of the mean CBFV. With respect to longitudinal studies, we therefore expect to reliably detect shifts in language-related hemispheric perfusion exceeding 1% of the mean CBFV.

Physiological changes over time are another major source of variability between \( f \)TCD examinations. Some of the cardiovascular modulations probably constitute a nonspecific autonomic response to the subjects’ confrontation with the task.\(^30\) The heart beat, for example, shows a biphasic modulation that is paralleled by biphasic CBFV changes.\(^31\) Autonomic responses are subject to habituation and are thus likely to change from one examination to the next. The cerebral hemodynamic response is also influenced by diurnal fluctuations or by substances such as nicotine.\(^32,33\) Since we have not controlled for these aspects, they are likely to add to the variability of CBFV. However, since these factors affect the entire cardiovascular system, they again manifest as bilateral, synchronous changes in CBFV and can also be eliminated by calculating the relative side-to-side difference in CBFV increase.

Practice effects could be another source of physiological variability between examinations. Repeating the same language task several times could reduce cognitive demands or improve performance. Either effect could result in a systematic change of CBFV increases during successive \( f \)TCD...
evaluations. Conceivably, this may even manifest predomi-
nantly unihemispherically. However, in neither the 10 sub-
jects reexamined a second time nor in the single subject
reevaluated 10 times was there any decrease or increase of
the relative side-to-side CBFV increase during word generation.
We do not know whether the repetition of the language task
is in fact associated with a change in cognitive demand or
effort. If it were, one would have to speculate that the stability
of the language LI could be due to a ceiling effect of cerebral
perfusion and blood flow velocity increases. Moderate cog-
nitive effort would thus result in a maximal blood flow
increase within a delimited activated area. Changes in effort
between moderate and maximal strength would subsequently
not change this local effect because regional arterial dilation
and blood flow velocity are already at their maximal level.
Global changes of perfusion associated with changes in effort
would be canceled out because of the side-to-side subtraction
algorithm used in the analysis. Alterations in the language LI
would occur only if the size or the interhemispheric distribu-
tion of the regions with an increased perfusion changed.

In conclusion, perfusional lateralization related to word
generation—despite technical, statistical, and physiological
variability—can reproducibly be quantified by fTCD. Since
the reproducibility of this method falls within the limits of its
predictable accuracy, fTCD in longitudinal studies can be
expected to detect shifts in language lateralizations with an
accuracy of 1% of the mean perfusion in a cerebral vascular
territory. On the basis of these results, future serial studies
with this simple technique will be able to establish the extent
to which interhemispheric functional redistribution plays a
role in the recovery of function.

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