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# NON-INVASIVE ASSESSMENT OF CEREBRAL BLOOD FLOW CHANGES DURING COMPLEX ACTIVATION PARADIGM

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**Abstract**

Background: The aim of this study was to cross-validate functional transcranial Doppler (FTCD) and brain single photon emission computed tomography (SPECT) using complex activation paradigm and to test the feasibility of FTCD in complex neuroactivation research.

Methodology: The study was performed in the group of 60 healthy, right-handed subjects. Cerebral blood flow velocity (CBFV) was measured in both middle cerebral arteries (MCA) during baseline and during computer game interaction, using FTCD. Identical stimulus and response patterns were used in the subgroup of 15 subjects that underwent brain SPECT. Quantitative assessment of results was done to detect the percentage variation between the two measurements.

Results: A statistically significant increase of cerebral blood flow (CBF) was detected by FTCD and brain SPECT ($p<0.0001$). In comparison between the two methods, statistically significant association ($r=0.5608$, $P=0.030$, coefficient of determination $r2 = 0.3154$) was detected only for CBFV increase in the right MCA and for the right-sided cerebral blood perfusion (CBP) increase, indicating that these two methods only partially measure the same characteristics associated with activation of specific brain areas.

Conclusion: In comparison with SPECT, FTCD is not sufficiently sensitive method for evaluation of CBF changes during complex activation paradigm. Our study represents negative evidence and stand against the common belief that FTCD is as good as other neuroimaging methods used for CBF measurements during neuroactivation. Therefore, FTCD might not be a reliable and suitable method for evaluation of CBF changes during complex neuroactivation paradigm.

**Keywords**

• Cerebral blood flow • Neurovascular coupling • Neurosonology • SPECT • TCD

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## 1. Introduction

Functional transcranial Doppler sonography (FTCD) represents a complementary neuroimaging tool for measuring cerebral blood flow (CBF) changes that occur during neural activation [1,2]. It might be applied in neuroactivation research in medical settings that are not equipped with functional neuroimaging methods [3]. Since its introduction, FTCD has substantially contributed to the clarification of the hemispheric organization of cognitive, motor, and sensory functions because it is based on a close coupling between regional CBF changes and neural activation [4-7]. Due to a pulse-wave Doppler technology and continuous registration of CBF in cerebral basal arteries, FTCD offers an excellent temporal resolution in comparison to other neuroimaging techniques [8,9]. Although different in nature from other neuroimaging procedures and accordingly limited for spatial resolution, TCD offers the advantage of being noninvasive and repeatable as clinically indicated [10]. By contrast, due to the technical demands brain single-photon emission computed tomography (SPECT) is not widely used for neuroactivation research. The ability of brain SPECT to detect regional CBF variations in different conditions has favored the investigation of different cerebral functions in both, the normal and the abnormal brain [11-14]. Interventional SPECT detects CBF changes induced by specific sensory, motor, or cognitive stimuli (neuroactivation) or by specific drugs (pharmacologic intervention). It requires the performance of at least two SPECT scans: one under baseline conditions and the other during the task, or under a pharmacologic effect. These two studies may be performed on separate days or on the same day using the split-dose technique. When the two SPECT studies are compared, obtaining the identical tomographic slices may be a problem, which has been addressed by 3D software [15,16]. For proper comparison of the two studies, quantification or subtraction techniques are used on slices reconstructed identically. Reference test–retest values in normal subjects are available for interpreting quantitative results (e.g. percentage of regional CBF changes) to recognize the percentage variation between two studies on the same subject under the same baseline conditions. Cognitive tasks represent a major difficulty to overcome, so tasks are usually carefully selected, because no other cerebral activity than the target one is desirable [10,11,17]. The purpose of this study was cross-validation of FTCD and brain SPECT in non invasive assessment of neurovascular coupling and CBF changes during complex activation paradigm in healthy volunteers. The objectives of the study were: 1) to determine the correlation of cerebral blood flow velocity (CBFV) changes measured with FTCD and cerebral blood perfusion (CBP) changes measured with brain SPECT during cerebral activation; 2) to evaluate inter-hemispheric CBFV changes during

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activation by FTCD and 3) to evaluate inter-hemispheric CBP changes during activation by Tc-hexamethylpropyleneamine oxime ($^{99m}$Tc-HMPAO) SPECT; 4) to determine the correlation of age and gender and relative changes of CBF during activation.

## 2. Experimental Procedures

The study group consisted of 60 healthy, right-handed volunteers: 32 men, aged 31.7 years (29.9-33.5) [5.0] {average value, (95% CI), [SD]} and 28 women, aged 31.2 years (29.4-33.2) [4.7]. Subjects who participated in the study were volunteers. Study protocol was approved previous to commencement by the responsible institutional Ethics committee of University Hospital Centre "Sestre milosrdnice". The study procedures were carried out in accordance with the ethical standards of the Helsinki Declaration of 1975 and revised version of 1983, and in compliance with good clinical practice guidelines. In all subjects, bilateral simultaneous TCD measurement of CBFV in both MCA was performed. Measurements were completed using MultiDop X4 TCD device with incorporated software MDX TCD-8 (DWL Elektronische Systeme GmbH, Sipplingen, Germany), according to the standard TCD insonation protocol [18]. Details of the insonation technique, particularly the correct identification of the MCA, have been published elsewhere. Functional measurements of CBFV in cm/s were carried out during 5-minute baseline and during 5-minute activation periods. Both MCA were insonated simultaneously at a depth of 50 mm with two 2-MHz transducer probes, attached to a headband and placed at the temporal skull bone windows. Subgroup of 15 subjects: men N=7 (46.7%), aged 31.7 years (27.2-36.2) [4.9] {average value, (95% CI), [SD]} and women N=8 (53.3%), aged 30.1 years (25.7-34.5) [5.3] underwent brain SPECT split-dose neuroactivation procedure with identical stimulus and response patterns that were used in TCD procedure. Measurements were performed using Multispect device (Siemens Medical Solutions, Erlangen, Germany) after intravenous injection of a radiopharmaceutical tracer at a dose of 740 MBq (20 mCi) $^{99m}$Tc-HMPAO, during 5-minute baseline. The same SPECT procedure including activation task in duration of 5 minutes was performed the next day. Images were derived in 64 transaxial projections during 360 degrees rotation, in a step and shoot mode, at every 3 to 5 degrees. Estimated spatial resolution was 7-15 mm. The regional CBF was detected by means of hyperperfusion indices in hyperperfusion areas of the temporal lobe and/or parietal lobe referent to cerebellum. The regional CBF increase in hyperperfusion areas was calculated in percentage.

### 2.1 Activation paradigm

Subjects underwent an activation task performing a commercial computer game of car-driving simulation (Swedish Touring Car Championship 2, Interactive Game Ó 2001, Digital Illusions Electronic Arts Inc.). The activation model enclosed elements of visual-spatial orientation, sustained attention, and visual-motor components. The game was performed using functional keys on the computer keyboard, which was adjusted for precise steering. The skill or precision of the task performance was not taken into account.

### 2.2 Data processing

Quantitative evaluation of brain activation was performed by calculating the relative increase of mean CBFV for both MCA and by calculating relative CBP increase in cortical regions of interest. Mean CBFV in 5 minute pre-activation interval was taken as the baseline value ($CBFV_{baseline}$). Relative CBFV change (DCBFV) during cerebral activation was calculated using the formula: $DCBFV = 100 \times (CBFV_{activation} - CBFV_{baseline} / CBFV_{baseline})$; where $CBFV_{activation}$ is the mean CBFV value over 5-minute period of activation. Relative CBFV change was calculated in percentage. Mean CBP value in the 5-minute pre-activation interval was taken as the baseline value ($CBP_{baseline}$). Relative CBP increase (DCBP) was calculated using the formula: $DCBP = 100 \times (CBP_{activation} - CBP_{baseline} / CBP_{baseline})$; where $CBP_{activation}$ is the mean CBP value over 5 minute period of activation. Relative CBP was calculated in percentage. Visual and semi-quantitative analysis of CBP changes were completed by a human rater analyzing coronal, transaxial and sagittal projections and regional indices of CBP in analogue regions of interest.

### 2.3 Statistical analysis

Power analysis provided a sample size of 60 respondents (n=60) considering the level of significance a=0.05 and the expected output of 70%. Statistical analysis was performed using the statistical software package Statistics for Windows, version 6.0 (Stat Soft Inc., Tulsa, OK). Analyzed parameters were: mean CBFV in cerebral arteries and regional CBF, using regional CBF parameters. Size changes of measured parameters were expressed as a percentage of basal values. As preliminary analysis showed that the measured dependent variables are distributed normally (Kolmogorov-Smirnov test), an appropriate descriptive statistics (mean, 95%-percent confidence intervals and standard deviation) were used and for the analysis parametric tests (Student's t -test for dependent and independent samples and analysis of variance) were used. Categorical variables are shown as number and percentage. Connection between certain variables was parsed by linear regression analysis. The level of significance of a=0.05 was determined as statistically significant.

## 3. Results

Demographic data of study subjects are listed in Table 1. It is evident that there is no difference in distribution by gender between the main groups and subgroups of subjects that underwent SPECT (P=0.6787). It is also evident that there were no differences in age distribution of respondents between the main group and subgroup of subjects that underwent SPECT (P=0.6688); nor among subgroups by gender (all comparisons P>0.05), indicating that the study sample was well balanced with regard to the demographic characteristics.

### 3.1 Quantitative assessment of CBF changes

Table 2 shows mean CBFV values in both MCA, at baseline and during activation; and relative increase of mean CBFV calculated for both MCA. It is evident that there is a small but statistically significant difference in CBFV between left and right MCA, at baseline and during activation, in favor of the right side of 57.8 : 59.0, $P < 0.0017$ and 62.1 : 63.6, $p<0.0001$, respectively. On both

sides, there was statistically significant increase of CBFV in activation compared to CBFV at baseline, with the relative increase (SD) of 7.5% (2.2) /p<0.0001/ in the left MCA and 7.9% (2, 8) /p<0.0001/ in the right MCA. Table 3 shows semi-quantitative values of CBP at baseline and during activation, at both sides, and the relative increase in CBP. There is no statistically significant difference in CBP between left and right side (0.951: 0.956, P = 0.7009). On both sides, there was statistically significant increase of CBP during activation compared to baseline, with the relative increase (SD) of 1.98% (0.99) /p<0.0001/ on the left side and 2.94% (1.21) / p<0.0001/ on the right side. On the left side, confidence intervals for the mean CBP values at baseline and during activation are overlapping, in contrast to the right side.

Table 1. Characteristics of subjects according to the subgroups (N=60).

| | | Subject who underwent FTCD (N=60) | Subject who underwent 99mTc-HMPAO SPECT (N=15) | *P*-value |
|---|---|---|---|---|
| Male, number (%) | | 32 (53,3) | 7 (46,7) | 0,6787* |
| Age, mean value, (95% CI), [SD] | | 31,5 (30,2-32,7) [4,8] | 30,9 (28,1-33,6) [5,0] | 0,6688* |
| Age, mean value, (95% CI), [SD] | Male | 31,7 (29,9-33,5) [5,0] | 31,7 (27,2-36,2) [4,9] | 0,9999* |
| | Women | 31,2 (29,4-33,2) [4,7] | 30,1 (25,7-34,5) [5,3] | 0,5737* |
| | *P*-value | 0,6926** | 0,5563** | |

FTCD – functional transcranial Doppler, 99mTc-HMPAO SPECT – technetium-99m-hexamethyl-propylene-amine-oxime single photon emission computed tomography, 95% CI – 95%-confidence interval, SD – standard deviation.
*P-value for comparison between FTCD and SPECT group, Student-t test for comparison of age, $\chi^2$ tests for comparison of gender distribution.
**P-value for comparison of gender differences within FTCD and SPECT group, Student t-test.

Table 2. Mean CBFV values in left and right MCA, at baseline and during activation, and relative increase of CBFV calculated for both MCA (N=60).

| | Left MCA | Right MCA | *P*-value* |
|---|---|---|---|
| Mean CBFV at baseline (95% CI) [SD], cm/s | 57,8 (57,2-58,3) [2,1] | 59,0 (58,5-59,4) [1,9] | <0,0017 |
| Mean CBFV during activation (95% CI) [SD], cm/s | 62,1 (61,5-62,6) [2,1] | 63,6 (63,2-64,0) [1,5] | <0,0001 |
| *P*-value** | <0,0001 | <0,0001 | |
| Relative increase of CBFV – Activation/baseline (95% CI) [SD], % | 7,5 (6,9-8,1) [2,2] | 7,9 (7,2-8,6) [2,8] | |

CBFV-cerebral blood flow velocity, MCA – middle cerebral artery, FTCD – functional transcranial Doppler, 95% CI – 95% Confidence interval, SD – standard deviation
*P-value for comparison of left and right MCA, Student-t test.
**P-value for comparison of values at baseline and during activation, Student t-test for dependent samples.

Table 3. Mean CBP values during baseline and activation; and relative CBP increase at left and right side (N=15).

| | Left side | Right side | *P*-value* |
|---|---|---|---|
| Mean value of CBP at baseline (95% CI) [SD] | 0,951 (0,939-0,964) [0,023] | 0,956 (0,946-0,966) [0,017] | 0,7009 |
| Mean value of CBP during activation (95% CI) [SD] | 0,970 (0,960-0,980) [0,018] | 0,984 (0,975-0,993) [0,016] | 0,2165 |
| *P*-value** | <0,0001 | <0,0001 | |
| Relative increase of CBP-activation/baseline (95% CI) [SD], % | 1,98 (1,43-2,53) [0,99] | 2,94 (2,27-3,61) [1,21] | |

CBP-cerebral blood perfusion, $^{99m}$Tc-HMPAO SPECT - technetium-99m-hexamethyl-propylene-amine-oxime single emission computed tomography, 95% CI – 95% confidence interval, SD – standard deviation
*P-value for comparison of the left and right side, Student-t test. **P-value for comparison of values at baseline and during activation, Student t-test for dependent samples.

### 3.2 Correlation between changes of CBFV and regional CBP indicators during cortical activation

Table 4 shows the correlation coefficients of regional cerebral hemodynamic parameters: CBFV in the left and the right MCA as well as indicators of regional CBP. There is a strong statistically significant linear relationship that is exactly proportional to CBFV in both MCA, between the baseline and during activation (r= 0.8337, r=0.5944, P<0.001, respectively). The correlation is even more pronounced for the CBP on both sides (r=0.9256, r=0.7687, P<0.001, respectively). It is also evident that there is a significant linear correlation inversely proportional to CBFV in both MCA, between baseline and relative changes of CBFV in activation compared to baseline (r= -0.3925, r= -0.6752, P<0.01, P<0.001; respectively). Comparable correlation coefficients were also obtained for the ratio of CBP at baseline and relative CBP change in activation; left side (r= - 0.7021, P<0.001) and right side (r= - 0.4521, P=0.091), but the correlation coefficient for the right side was not statistically significant. Table 4 and Figure 1 show that the only statistically significant correlation between CBFV in left

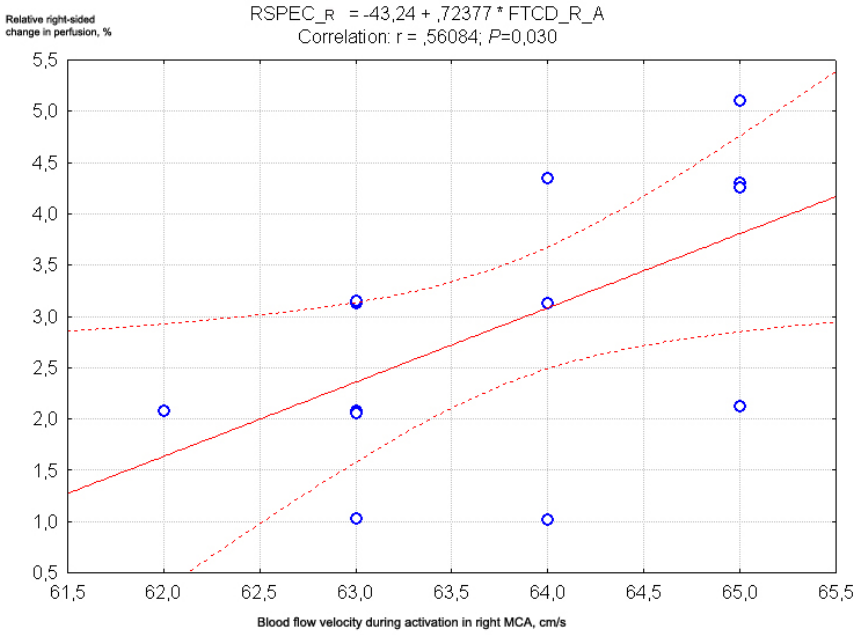


Figure 1. Scatter diagram for the correlation of CBFV in right MCA during activation and relative right-sided CBP change (N=15).
Legend: CBFV-cerebral blood flow velocity, CBP-cerebral blood perfusion, FTCD – functional transcranial Doppler, MCA – middle cerebral artery, 99mTc-HMPAO SPECT – technetium-99m-hexamethyl-propylene-amine-oxime single emission computed tomography, RSPEC_R – relative change of right-sided cerebral perfusion, FTCD_R_A – FTCD of right MCA during activation. The full line represents the direction of linear regression. Dashed lines represent 95% CI.

Table 4. Correlation coefficients of CBFV in left and right MCA and regional CBP indices at left and at right side (N=15).

| | FTCD_L_B | FTCD_L_A | RTCD_L | FTCD_R_B | FTCD_R_A | RTCD_R | SPEC_L_B | SPEC_L_A | RSPEC_L | SPEC_R_B | SPEC_R_A |
|---|---|---|---|---|---|---|---|---|---|---|---|
| FTCD_L_B | | | | | | | | | | | |
| FTCD_L_A | 0,83371# | | | | | | | | | | |
| RTCD_L | -0,39248** | 0,18045 | | | | | | | | | |
| FTCD_R_B | 0,45194# | 0,45545# | -0,05261 | | | | | | | | |
| FTCD_R_A | 0,31174* | 0,39582** | 0,09851 | 0,59435# | | | | | | | |
| RTCD_D | -0,26462* | -0,19048 | 0,15642 | -0,67516# | 0,19144 | | | | | | |
| SPEC_L_B | 0,05139 | -0,02049 | -0,07550 | -0,30756 | -0,22130 | 0,19839 | | | | | |
| SPEC_L_A | 0,10181 | 0,09057 | -0,00773 | -0,27398 | -0,25687 | 0,14678 | 0,92559# | | | | |
| RSPEC_L | 0,05671 | 0,21557 | 0,17446 | 0,22338 | 0,05708 | -0,19607 | -0,70212# | -0,38053 | | | |
| SPEC_R_B | 0,11169 | -0,22977 | -0,36755 | -0,10331 | -0,31701 | -0,05172 | 0,21601 | 0,11687 | -0,32175 | | |
| SPEC_R_A | 0,13950 | -0,15024 | -0,31095 | -0,18771 | 0,05557 | 0,22190 | 0,04236 | -0,07376 | -0,24983 | 0,76866# | |
| RSPEC_R | 0,02623 | 0,14395 | 0,12814 | -0,09793 | 0,56084* | 0,38217 | -0,27420 | -0,28356 | 0,14710 | -0,45213 | 0,22292 |

CBF-cerebral blood flow, CBFV-cerebral blood flow velocity, CBP-cerebral blood perfusion, FTCD_L_B – FTCD of left MCA at baseline, FTCD_L_A - FTCD of left MCA during activation, RTCD_L – relative change in left MCA, FTCD_R_B – FTCD of right MCA at baseline, FTCD_R_A – FTCD of right MCA during activation, RTCD_R – relative change in right MCA, SPEC_L_B – left-sided cerebral perfusion at baseline, SPEC_L_A – left-sided cerebral perfusion during activation, RSPEC_L – relative left-sided cerebral perfusion change, SPEC_R_B – right-sided cerebral perfusion at baseline, SPEC_R_A – right-sided cerebral perfusion during activation, RSPEC_R – relative right-sided perfusion change. *P<0, 05 **P<0, 01 # P<0,001

and right MCA and regional CBP was obtained for the relationship between CBFV in the right MCA during activation and the relative CBP change on the right side (r=0.5608, P=0.030), with a coefficient of determination $r^2 = 0.3154$.

### 3.3 Assessment of interhemispheric differences during cortical activation

Data presented in Table 2 show that there are statistically significant CBFV differences in left and right MCA, during baseline and activation. The relative increase in mean [SD] CBFV is almost equal on both the left and right side (7.5% [2. 2] in left MCA and 7.9% [2.8] in right MCA, P=0.3328), and it was not statistically significant, although it was slightly higher in right MCA than in left MCA. Analysis of variance for repeated measurements according to the side, as an independent variable, showed the same results (F=1.5382, P=0.2174). There is also statistically significant linear relationship between CBFV in activation between the left and right MCA (r=0.3958, P <0.01, Table 4), but not for the relative increase of CBFV between the left and right side (r=0.1564, P=0.233). An average (95% CI) difference of relative CBFV increase in right MCA compared to left MCA was 0.42% (-0.44 to 1.28). Cerebral perfusion values showed statistically significant difference in relative CBP increase [SD] at left and right side (1.98% [0.99] on the left side and 2.94% [1.21] on the right side, P=0.0225), although there were no statistically significant CBP differences detected on the left and right side, in baseline and during activation (Table 3). Analysis of variance for repeated measurements according to the side, as an independent variable, showed the same results (F=6.0708, P=0.0202). An average (95% CI) difference in relative CBP increase on the right side compared to the left side was 0.96% (0.16 to 1.76); and the difference that was greater than the lower limit of the confidence interval (>0.16%) on the right side was found in 9 (of 15) respondents.

### 3.4 Assessment of age and gender differences and relative changes in CBF during cortical activation

There was no statistically significant correlation found between the ages and following parameters: CBFV in the left and right MCA at baseline and during activation, and relative CBVF changes in the left and right MCA (N=60); and CBP on the left and right side during baseline and activation, and relative changes in CBP on the left and right side (N=15). The FTCD correlation coefficients ranged from r= -0.1218 to r=0.1194 (P>0.35), and for SPECT from r= -0.3592 to r=0.1541 (P>0.18). Table 5 shows CBF values of hemodynamic parameters: CBFV in the left and right MCA, at baseline and activation, and relative CBFV changes in the left and right MCA; and indicators of regional CBP on the left and right side, during baseline and activation, and relative CBP changes on the left and right side, according to the gender of respondents. A statistically significant difference was not detected for any of the measured parameters, between men and women (P>0.10).

## 4. Discussion

Functional TCD enables CBFV examination in real time and allows repeated measurements that may contribute to the greater reproducibility. Brain SPECT has a good spatial resolution and allows anatomical localization of cortical areas

Table 5. Relative CBF changes, according to gender, measured by FTCD and SPECT.

| Parameter | Male (N=32) | Female (N=28) | *P*-value* |
|---|---|---|---|
| FTCD_L_B, SV (95% CI) [SD], cm/s | 57,8 (57,0-58,6) [2,3] | 57,8 (57,0-58,5) [1,9] | 0,9935 |
| FTCD_L_A, SV (95% CI) [SD], cm/s | 62,2 (61,5-63,0) [2,1] | 61,9 (61,1-62,7) [2,1] | 0,5979 |
| RTCD_L, SV (95% CI) [SD], % | 7,73 (6,83-8,62) [2,48] | 7,18 (6,44-7,93) [1,92] | 0,3531 |
| FTCD_R_B, SV (95% CI) [SD], cm/s | 58,9 (58,2-59,6) [1,9] | 59,0 (58,3-59,8) [1,9] | 0,7444 |
| FTCD_R_A, SV (95% CI) [SD], cm/s | 63,8 (63,2-64,3) [1,5] | 63,3 (62,7-63,9) [1,5] | 0,2429 |
| RTCD_R, SV (95% CI) [SD], % | 8,39 (7,37-9,42) [2,83] | 7,32 (6,25-8,39) [2,76] | 0,1434 |
| | Male (N=7) | Female (N=8) | |
| SPEC_L_B, SV (95% CI) [SD] | 0,941 (0,922-0,961) [0,021] | 0,960 (0,942-0,978) [0,021] | 0,1155 |
| SPEC_L_A, SV (95% CI) [SD] | 0,963 (0,948-0,978) [0,016] | 0,976 (0,961-0,991) [0,017] | 0,1506 |
| RSPEC_L, SV (95% CI) [SD], % | 2,29 (1,37-3,21) [1,00] | 1,70 (0,90-2,51) [0,96] | 0,2686 |
| SPEC_R_B, SV (95% CI) [SD] | 0,954 (0,932-0,977) [0,024] | 0,958 (0,950-0,965) [0,009] | 0,7328 |
| SPEC_R_A, SV (95% CI) [SD] | 0,986 (0,965-1,006) [0,022] | 0,983 (0,974-0,991) [0,010] | 0,7195 |
| RSPEC_R, SV (95% CI) [SD], % | 3,31 (1,77-4,85) [1,67] | 2,61 (2,14-3,08) [0,56] | 0,2808 |

CBF-cerebral blood flow, CBFV-cerebral blood flow velocity, CBP-cerebral blood perfusion, FTCD_L_B – FTCD of left MCA at baseline, FTCD_L_A - FTCD of left MCA during activation, RTCD_L – relative change in left MCA, FTCD_R_B – FTCD of right MCA at baseline, FTCD_R_A – FTCD of right MCA during activation, RTCD_R – relative change in right MCA, SPEC_L_B – left-sided cerebral perfusion at baseline, SPEC_L_A – left-sided cerebral perfusion during activation, RSPEC_L – relative left-sided change of cerebral perfusion, SPEC_R_B – right-sided cerebral perfusion at baseline, SPEC_R_A – right-sided cerebral perfusion during activation, RSPEC_R – relative right-sided change of cerebral perfusion. **P*-value for comparison according to the gender, Student t-test.

supplied by a certain cerebral blood vessel that are activated by functional testing. Cerebral blood flow velocities obtained by TCD are measured in the basal cerebral arteries, thus an increase of CBFV in smaller vascular branches that supply small cortical areas can not always be detected and visible. Therefore, regional activation of small cortical areas within the much larger MCA territory will not cause major changes in blood flow of the MCA as measured by TCD [19]. Therefore, regional activation of small cortical areas detected by SPECT, FMRI, or PET, may not be identified by FTCD. However, previous studies have shown that FTCD is a sensitive method with good time resolution for selective measurement of CBF during brain activation [20-23]. In previous FTCD studies, statistically significant differences based on gender of respondents were not observed [24,25], which is also consistent with the results of this study. This study did not show any statistically significant correlation between age and the following parameters: CBFV in both MCA at baseline and during activation, and relative changes of CBFV in both MCA. Previous studies examined the influence of age and gender on brain perfusion [25], using $^{99}$mTc-HMPAO and 99mTc-EDC SPECT [26-28]. In this study, 15 subjects underwent $^{99}$mTc-HMPAO SPECT testing, at baseline and during brain activation. No significant correlation between age relative CBP changes at both sides was detected. The lack of correlation might be explained by the small size of the study sample. Previous studies of brain activation have confirmed the association of cognitive activity, increased CBF and metabolism [29-33]. Different methods for the functional imaging of the brain can be used complementary. FTCD results have proven CBFV increases during visual stimulation and comparison between FTCD and brain SPECT confirmed that changes in CBFV reflected changes in regional CBF [34]. In this study, a statistically high and significant linear relationship exactly proportional to CBFV in both MCA between baseline and activation was detected. The relationship was even more pronounced for CBP. A statistically significant difference in CBFV was detected for both MCA between baseline and activation, and relative CBFV increases were similar at both sides. A statistically significant linear correlation of CBFV was detected during activation in both MCA but a correlation was not found for the relative CBFV between the left and right MCA. A small but statistically significant difference of CBFV between the left and right MCA was found during baseline and activation. A significant linear and inversely proportional correlation between CBFV in both MCA between baseline and activation and relative changes of CBFV was also found. Correlation values between baseline and activation showed an inverse correlation, which may imply that the higher the value at rest, the smaller the relative increase in activation. The correlation coefficient for the ratio of perfusion at baseline and relative changes in perfusion was statistically significant for the left side, but the correlation coefficient was not statistically significant for the right side. During baseline and activation there was no statistically significant difference in CBP according to the side, however a statistically significant difference in relative CBP increase was detected. A detected right-sided CBP increase in 9 subjects could corroborate the hypothesis of hemispheric lateralization for visuospatial functions in right-handed subjects. In this study, a significant correlation between baseline and activation for both sides and for the method of measurement was detected ($P<0.001$) with significant but inverse correlation with the relative changes. These results could indicate that there is a certain upper plateau during activation and that the same was reached (meaning that the activation model was properly selected). The results of this study show a significant difference during activation between the left and right hemispheres measured by SPECT in relation to FTCD because SPECT directly measures the location of cerebral activation while FTCD measures CBFV in basal cerebral arteries. We could conclude that quantitative differences between FTCD and SPECT are due to the methodologies themselves [19], although both methods showed statistically significant bilateral increase of CBP (SPECT) and CBFV (FTCD) during activation. Neither of the two methods revealed a dependence of results according to the age or gender. Only for CBFV in the right MCA during activation and the relative right-sided CBP change, a statistically significant association between the two methods was detected ($r=0.5608$, $P=0.030$, coefficient of determination $r^2 = 0.3154$), again corroborating the hypothesis of right-sided lateralization fro visuospatial functions. Potential reasons for that might include activation of remote brain areas in the right somatosensory regions and in structures related to visual attention and motivation [35]. In fMRI studies in healthy subjects, spatial attention has been associated with the inferior parietal cortex, particularly in the right hemisphere [36-38]. Regarding the integration of spatial attention with motivation, the spatial attention network has been shown to significantly interact with limbic system [39]. As no correlation between CBFV in left MCA during activation and relative left-sided CBP change was detected, a possible explanation might include down regulation of other sensorimotor systems that, in turn, may reflect our insufficient knowledge upon basic mechanisms of sensorimotor interactions [19]. Limitation of this study might further include the lack of measurement of the vessel diameter of both MCA. However, previous FTCD studies accepted an assumption about the constancy of the blood vessel diameter during activation. Based on results of previous studies, we could conclude that FTCD reflects CBF changes only in non-complex and rather circumscriptive brain activation patterns, such as during visual stimulation. In this study, FTCD has not demonstrated to be sufficiently sensitive method for measuring CBF changes during complex activation paradigm. Results of our study thus suggest that FTCD is not as good as SPECT for CBF measurement; hence it should not be applied as a reliable method in future neuroactivation studies.

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