A Truly Simultaneous Combination of Functional Transcranial Doppler Sonography and $H_2{}^{15}O$ PET Adds Fundamental New Information on Differences in Cognitive Activation Between Schizophrenics and Healthy Control Subjects

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Working memory deficits are a cardinal feature of the pathophysiology of schizophrenia. Lesion studies and functional blood flow–dependent imaging methods with coarse temporal resolution, such as PET and functional MRI (fMRI), tend to paint a fairly static picture of the cortical regions involved. In contrast, functional transcranial Doppler sonography (fTCD) provides a high temporal resolution. Truly simultaneous fTCD–fMRI is not yet possible for technical reasons, but $H_2{}^{15}O$ PET and fTCD can be used really simultaneously. However, this combination has not yet been used for cognitive activations in schizophrenia. We therefore investigated the extent to which there are both spatial (PET) and temporal changes (fTCD) in the activation patterns of schizophrenic patients. **Methods:** Eleven clinically stable chronic schizophrenic, right-handed patients and 10 healthy, right-handed control subjects, matched for age, sex, education, and intelligence quotient, participated in the study. We selected stable chronic schizophrenic patients who could perform a working memory task (N-back task) as well as healthy volunteers to exclude the possibility of imaged artifacts due to poor performance. All subjects were examined with a truly simultaneous fTCD–$H_2{}^{15}O$ PET combination under cognitive activation. **Results:** Schizophrenic patients activate a significantly larger cortical volume for adequate task performance ($P < 0.05$), but with a significantly lower blood flow increase in this volume ($P < 0.01$), than do control subjects. Furthermore, they cannot significantly increase blood flow velocity during the time course of cognitive activation as control subjects do. There were only significant correlations between neuropsychologic performance and imaging parameters (fTCD changes, PET blood flow changes) in control subjects (all $r \geq |0.65|$; $P < 0.05$), but no significant correlations in schizophrenics (all $r < |0.3|$; $P > 0.4$). **Conclusion:** We demonstrated that schizophrenic patients exhibit qualitative differences in the spatial and temporal resolution of cognitive processing. All facts could be interpreted as a sign of alternative, less efficient problem-solving strategies in schizophrenia that lead to the working memory deficits observed during the further course of this disease. Truly simultaneous fTCD–PET can be used in neuroscience to add fundamental new information on spatial and temporal cognitive activation behavior to understand the true physiologic nature of the disease-specific differences of mental illnesses that are seen as disorders of the mind arising in the brain.

Key Words: PET; functional transcranial Doppler sonography; functional MRI; schizophrenia; working memory

J Nucl Med 2003; 44:671–681

Neuroimaging studies have provided direct evidence of frontal malfunction in schizophrenia, but the results have been inconsistent and controversial. In studies of patients at rest, hypofrontality has been an inconsistent finding (*1*). Recently, we investigated a comparatively large number of neuroleptic-naive, actively psychotic, first-episode schizophrenic patients at rest before and after treatment. The results clearly showed that in such patients different positive symptoms correlated exclusively with either cerebral hyper- or hypoperfusion (*2*,*3*). With transcranial Doppler sonography (TCD) we determined that such schizophrenics show altered cerebral blood flow velocity (CBFV) in the anterior, middle, and posterior cerebral arteries (ACA, MCA, PCA) (*4*). After psychopathologic improvement, regional CBF and CBFV normalized (*2*–*4*).

Deficits in working memory, a brain system that provides temporary storage and manipulation of the information nec-

Received Jul. 11, 2002; revision accepted Nov. 27, 2002.

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essary for complex cognitive tasks such as language comprehension, learning, and reasoning, have been reported to be a cardinal feature of the pathophysiology of schizophrenia (*5*–*7*). Poor performance on working memory tests in schizophrenia is a strong predictor of poor community outcome and impairment in skills learning (*6*). Postmortem studies have shown abnormalities of cortical areas within the working memory network, including the prefrontal, cingulate, and temporal cortices (*5*). However, working memory activation paradigms have been reliable in showing prefrontal hypofunction in schizophrenia, but these results have been challenged as artifacts of poor performance (*1*). We therefore selected clinically stable chronic schizophrenic patients who could perform a working memory task (N-back task) as well as healthy volunteers to understand the physiologic nature of the postulated differences in cognitive activation between schizophrenics and control subjects.

Lesion studies and coarse temporal resolution imaging methods, such as PET and MRI, tend to paint a fairly static picture of the involved cortical regions (*8*). In contrast, the high temporal resolution of functional transcranial Doppler sonography (fTCD) (*9*) provides a dynamic picture of subsecond changes in working memory effects over the course of individual trials (*10*). Furthermore, because all of these methods (PET, functional MRI [fMRI], fTCD) determine blood flow–dependent imaging parameters as surrogate parameters of neuronal activity, they are comparable. In principle, fTCD and PET can be used simultaneously. However, to our knowledge, there is no report in the literature of this combination being used for cognitive activation in schizophrenic patients. A truly simultaneous fTCD–fMRI combination is not yet possible for technical reasons because the paramagnetic components of fTCD probes do not allow an artifact-free simultaneous fTCD–fMRI acquisition.

We therefore examined schizophrenic patients and healthy control subjects with a truly simultaneous combination of fTCD and $H_2{}^{15}O$ PET under working memory activation to investigate the extent to which there are both spatial (PET) and temporal (fTCD) changes in the activation patterns of schizophrenic patients and how these are influenced by the corresponding neuropsychologic performance.

Because results of a recent PET study (*11*) indicate a cinguloparietal dysfunction underlying the impairment of working memory control processes in schizophrenia, and because in our pretests of this study for the methodologic development of a truly simultaneous PET–fTCD acquisition we found pronounced differences between schizophrenics and control subjects in the supply area of the ACA, we focused on a comparison of PET and simultaneous fTCD of the ACA. However, to our knowledge, there is no report in the literature of fTCD of the ACA in schizophrenia and of the role of fTCD of the ACA in working memory in healthy subjects. Therefore, a truly simultaneous PET–fTCD acquisition can close this gap because PET can serve to cross-validate the findings of fTCD in the ACA.

MATERIALS AND METHODS

Subjects

Eleven clinically stable chronic schizophrenic, right-handed patients (DSM-IV 295.60 (*12*); 7 male, 4 female; mean age, 30.6 ± 11.9 y; intelligence quotient [IQ], 110.1 ± 13.2) and 10 healthy, right-handed comparison subjects, matched for age, sex, education, and IQ (6 male, 4 female; mean age, 30.7 ± 10.7 y; IQ, 111.1 ± 12.3) participated in the study. The subjects with schizophrenia were clinically stable, medicated outpatients without clear delusions, hallucinations, disorganized speech, or grossly disorganized or catatonic behavior and with persistent negative symptoms or 2 or more mild symptoms meeting the DSM-IV criterion A for schizophrenia (e.g., unusual ideas) (*12*). We thereby avoided an artificial influence of strongly pronounced positive symptoms (*2*,*3*) on rCBF measurements under working memory stimulation. Furthermore, the neuropsychologic performance in the working memory task (N-back task) of all schizophrenic patients did not differ significantly from that of healthy control subjects. Control subjects were excluded for any lifetime axis I disorder, first-degree family history of psychotic disorder, substance abuse within 6 mo, neurologic illness, previous head trauma, or mental retardation. All participants gave written informed consent and were paid for their participation. This study was performed using a protocol approved by the local ethics committee.

Activation Paradigm

Subjects were scanned under working memory conditions of the N-back task (*5*,*13*). This task has been previously shown to produce activation in a cortical network, including the same regions involved in the Wisconsin Card Sorting Test, and to reveal similar pathophysiologic characteristics in patients with schizophrenia (*14*,*15*). Subjects observed random sequences of single numbers at the center of a display. They pressed a button as quickly as possible when the number presented was the same as the number 2 back in the sequence. As a reference condition (0-back), they pressed a button whenever the number 3 appeared. Stimuli were presented for 1,500 ms with a 1,500-ms interstimulus interval. Time parameters and the number of critical stimuli were identical across both conditions. Neuropsychologic performance (reaction times to on-screen stimuli) was stored for later analysis. A 2-back condition, in which subjects respond according to a number seen 2 stimuli before, requires continuous updating of the mental set and the use of working memory (*14*). However, both conditions require that subjects encode and evaluate each stimulus and respond to targets (*13*). The 2-back condition further requires that subjects maintain the identity and order of 2 previous numbers and continuously update this representation. Therefore, in a subtraction design (2 back minus reference condition), all common functional components that are conceptually independent of working memory processes were not considered (*13*).

fTCD

Using a 2-MHz–pulsed Doppler device (Pioneer TC4040, software version 2.40; Nicolet EME, Kleinostheim, Germany), the intracranial flow patterns of both anterior cerebral arteries (ACA, A2) were investigated simultaneously in the healthy subjects and patients during working memory tasks. In the preparation phase of this study, every subject underwent MRI (Magnetom SP63; Siemens Medical Systems, Hoffman Estates, IL; 3-dimensional FLASH (3D-FLASH) sequence; voxel size, $1 \times 1 \times 1$ mm^3; echo

time [TE], 5 ms; repetition time [TR], 40 ms; flip angle [FA], 40°) to exclude morphologic abnormalities and rare variants of the circle of Willis. Both ACAs were insonated transtemporally above the zygomatic arch, and CBFV was measured at a depth of 70–80 mm. In a recent study on 20 healthy control subjects, we showed a high test–retest reproducibility for locating the ACA as well as good insonation ($0.90 < r < 0.95$; $P < 0.0001$) (*4*). Motion artifacts were avoided by fixing both fTCD probes to the subject's head in addition to the PET head holder (*3,16,17*) using a special fTCD head frame (Spencer Mark 500; Spencer Technologies, Seattle, WA). We did not assess other factors influencing CBFV, such as P_{CO_2}, blood pressure, and heart rate frequency during activation, because we recently showed that cognitive tasks result in only minor changes of these factors (*18*). The start of cognitive activation was recorded on-line with an electronic marker signal, and CBFV acquisition was done truly simultaneously with PET.

TCD measures the cerebral blood flow velocity (CBFV) in a vessel. Here, CBFV refers to the mean flow velocity (V_{mean}). This is an average of all velocities—which are detected simultaneously in real time—along the parabola-shaped laminar flow front, where velocity is greatest at the center of the vessel. The various velocity components V_i are weighted with their respective Doppler frequency W_i, which varies in proportion with the number of red blood cells flowing at that velocity. The sum of the weighted component velocities is then divided by the sum of all intensities that are proportional to the total number of red blood cells present in the cross-section of the vessel. This is given by the formula:

$$V_{mean} = SUM(W_i \times V_i)/SUM(W_i),$$

where i stands for the 256 frequencies. (This is comparatively high and very accurate, given that the usual number is 64 or 128.) Therefore, because the flow velocities are directly related to the CBF, fTCD measures the relative CBF changes in a vessel. During carotid artery and intracranial aneurysm surgery in humans, Newell et al. (*19*) used electromagnetic flowmetry to directly measure blood flow in the internal carotid artery (ICA) and compared it with the TCD-measured CBFV in the ipsilateral MCA. Because the ICA supplies mostly the MCA area, flow fluctuations in the 2 vessels should be nearly identical. During artificially induced hypotension in patients, both parameters did indeed show highly similar changes compared with the initial values before hypotension ($r = 0.995$; $P < 0.001$; $y = 5.6 + 0.94x$). These findings agree with those of Aaslid et al. (*20*) and, therefore, it could be shown that CBFV is proportional to CBF.

The spectral envelope curves of the Doppler signal were recorded with a comparatively high rate of 256 sample points per second (*21*) and stored for off-line processing with a special fTCD analysis computer program (*18*). Using a specially designed, computer-aided integration procedure (*18*), the mean CBFV was calculated on-line for each heart cycle from the original TCD envelope curve. The CBFV curves during the initial resting phase, which were defined as baseline, were normalized to 1; the trials of each task were averaged separately for each subject. Mean CBFV changes were then related to the corresponding baseline values. The average curves of each subject were later used for statistical analysis. The reason for averaging was to systematically amplify event-dependent changes and to eliminate random variations. In prestudy tests, we determined that 4 scans for each condition generated a sufficient signal-to-noise ratio for both PET and fTCD in healthy control subjects as well as schizophrenic patients.

Test conditions followed in the order 0-back (reference condition) to 2-back (activation condition): For each task, the following course in PET and fTCD was kept, as shown in Figure 1.

PET

For 3D rCBF–PET measurements, $H_2{}^{15}O$ was synthesized with an ODS 111 cyclotron (Siemens/CTI, Knoxville, TN). The PET data were acquired on an ECAT EXACT PET 922/47 scanner (Siemens/CTI). To correct for photon attenuation, a 12-min transmission scan (3 ^{68}Ge sources) at the beginning of the session was done. Motion artifacts were avoided by immobilizing the subject's head in a special head holder system (*3,16,17*). An intravenous bolus injection of 550 MBq (15 mCi) $H_2{}^{15}O$ was administered for each scan. The success of the $H_2{}^{15}O$ PET technique to localize statistically significant changes in rCBF is dependent on factors such as the activity level injected and the magnitude of the flow change. Undetectable changes may occur if insufficient activity is injected, leading to high levels of statistical noise, or the task performed results in only small changes in blood flow (*22*). It was recently shown that for a working memory task (the N-back task), the peak counting-rate performance for an ECAT EXACT HR+ scanner (Siemens/CTI) is approached at injected activity levels of $H_2{}^{15}O$ around 550 MBq (15 mCi) (*22*). Activation began at injection and lasted for 80 s. Twenty seconds after injection, a 60-s emission scan was acquired, and reconstruction of 47 attenuation-corrected slices of 3.375 mm each in a 128 × 128 matrix with a pixel size of 1.702 mm was done. Because there were 4 scans per condition, a total of 8 measurements of rCBF was done.

Data Analysis

The differences in rCBF between the 2 conditions were analyzed both groupwise (all patients vs. all healthy control subjects) and individually using statistical parametric mapping (SPM99b software; Wellcome Department of Cognitive Neurology, Institute of Neurology, University College London, London, U.K.). The realigned images were normalized to the Talairach space (*23*) in the first case (groupwise analysis) and to the individual, nonster-

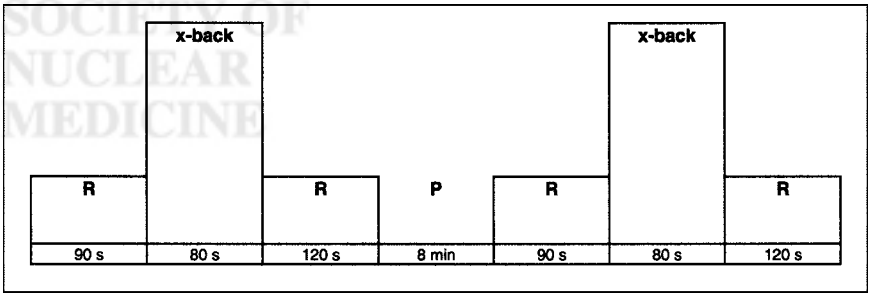

FIGURE 1. Schematic illustration of time course of PET and fTCD measurements. Test conditions followed in order 0-back (reference condition) to 2-back (activation condition): R = rest with fTCD acquisition but without activation; x-back = simultaneous PET-fTCD acquisition under 0-back (reference condition) or 2-back task; P = pause without any acquisition for isotope washout (PET).

eotactic brains (i.e., the actual acquired, averaged PET images) to preserve the local size of activation or deactivation volumes in the second case (individual analysis). The normalized images were smoothed with a 12-mm full width at half maximum gaussian kernel and clusters with significantly ($P < 0.001$; minimum cluster size, 30 voxels; voxel size, $2 \times 2 \times 2$ mm^3) changed rCBF extracted in both cases.

In the comparison of the 2 groups (groupwise analysis), contrasts were modeled within SPM99b, which show activations or deactivations for 1 group or simultaneously both groups as well as contrasts, where 1 group activates or deactivates more than the other.

Overlaying the individual SPMs with a recent (about 1 wk before PET) individual MRI (Siemens Magnetom SP63; 3D-FLASH-sequence; voxel size, $1 \times 1 \times 1$ mm^3; TE, 5 ms; TR, 40 ms; FA, 40°) for each subject, activated or deactivated clusters belonging to the supply area of the ACA were identified (individual analysis). The cluster volumes for significant activations and deactivations as given by SPM analysis results sections were further computed. As activations in PET are connected with CBF increases and deactivations with CBF decreases, the differences between the volumes (volumes of significant activations minus volumes of significant deactivations) were computed. rCBF differences were calculated by projecting the clusters onto the averaged PET images of each task and computing the mean rCBF difference in the significant clusters for activation ($rCBF_{activation}$ minus $rCBF_{reference\ condition}$) and deactivation ($rCBF_{reference\ condition}$ minus $rCBF_{activation}$). For reasons of simplicity and to enable group comparisons, the global mean perfusion in every individual brain was scaled to 50 mL/100 g/min. Figure 2 gives an example of identification and delineation of such activation or deactivation clusters.

Because the height of the fTCD signal differences ($CBFV_{activation}$ minus $CBFV_{reference\ condition}$) in the ACA should both depend on the size of the activated or deactivated clusters and on the averaged height of rCBF change within these clusters (multiple linear regression of fTCD signals with size of clusters and height of perfusion changes as predictors showed that both predictors contribute significantly to the fTCD signal; $P < 0.0005$; $r = 0.92$; adjusted $r^2 = 0.83$), the product of these values was calculated for each cluster and summed over all clusters belonging to the ACA area, giving a volume-weighted perfusion change (volume-weighted perfusion change of significant activation clusters minus volume-weighted perfusion change of significant deactivation clusters).

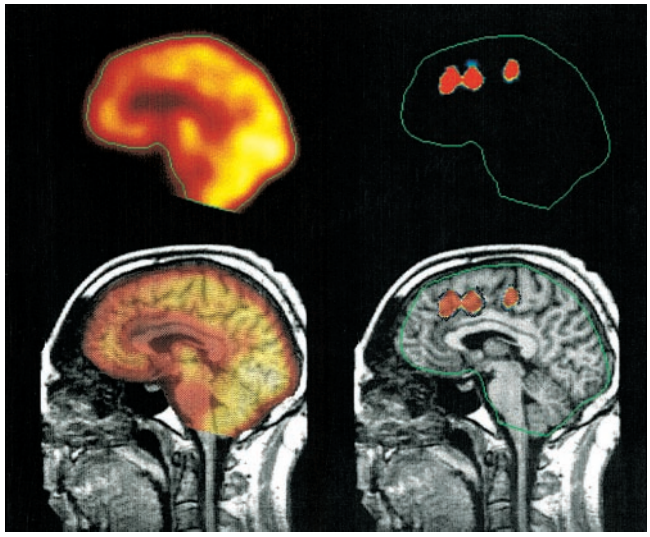

FIGURE 2. Illustration of 1 subject's significant activation clusters. (Top left) Sagittal averaged PET image of 4 scans under activation condition (2-back task) for contour finding (green). (Bottom left) Overlay of averaged PET image onto individual MRI dataset. (Top right) Identification and delineation of significant activation clusters (2-back minus reference condition; $P < 0.001$; minimum cluster size, 30 voxels; voxel size, $2 \times 2 \times 2$ mm^3) by SPM99b analysis. For better visualization, these clusters were projected into contour (green) of PET image here. Note that individual SPM analysis was done by normalization to individual, nonstereotactic brains (i.e., averaged PET images) and not to Talairach space (*23*) to preserve local size of activation volumes for each subject. Cluster volumes are given by SPM analysis; rCBF differences are calculated by projecting clusters onto averaged PET images of each task and computing mean rCBF differences in clusters ($rCBF_{activation}$ minus $rCBF_{reference\ condition}$). Same procedure was done for significant deactivation clusters (reference condition minus 2-back). (Bottom right) Overlay of PET contour (green) and significant activation clusters onto individual MRI dataset for individual anatomic localization. These activation clusters lie in supply area of ACA.

Statistical Analysis

Differences between patients and control subjects in the volume (mm^3) of all activated and deactivated clusters (volume of activated clusters minus volume of deactivated clusters), in rCBF change (mL/100 g/min) of all activated and deactivated clusters (rCBF change of activated clusters minus rCBF change of deactivated clusters), in volume-weighted rCBF changes (mm^3 × mL/100 g/min) of all activated and deactivated clusters, and in the mean fTCD signal ($CBFV_{activation}$ minus $CBFV_{reference\ condition}$) of the ACA were tested with *t* tests for independent samples after normal distribution of the parameters was ensured (Shapiro–Wilks test, Lilliefors test; $P > 0.05$). Differences in the neuropsychologic performance (reaction times, error rates) under 2-back activation between the 2 groups were tested using the Mann–Whitney *U* test. Regression analyses of volume differences (volume of activated clusters minus volume of deactivated clusters) and volume-weighted rCBF changes versus mean fTCD changes ($CBFV_{activation}$ minus $CBFV_{reference\ condition}$) of the ACA were done for schizophrenics and control subjects both together and separately. Next, the regression slopes of volume differences and of volume-weighted rCBF changes versus mean fTCD changes for the 2 groups were compared. Furthermore, Spearman correlation coefficients instead of Pearson's correlations were obtained for volume differences, volume-weighted rCBF changes, and mean fTCD changes to neuropsychologic performance in schizophrenic patients and control subjects altogether and separately because neuropsychologic performance values were not normally distributed. Finally, in the averaged Doppler curves, the regression slope of the fTCD signals during the activation period was calculated and compared for schizophrenics versus control subjects to determine differences in temporal behavior of cognitive activation between both groups.

RESULTS

Compared with healthy control subjects, schizophrenic patients showed no significant difference in neuropsychologic performance in the 2-back task (reaction times,

536.0 ± 89.1 ms vs. 501.4 ± 99.7 ms, $P > 0.2$; error rates, 0.34 ± 0.46 vs. 0.27 ± 0.27, $P > 0.2$).

Under working memory activation (2-back task minus reference condition) in PET, healthy control subjects as well as schizophrenic patients showed significant bilateral activations in the frontoparietal areas as well as in the cerebellum (Fig. 3). However, it appears that schizophrenic patients showed more extended significant activations in the supply area of the ACA.

Figure 4 shows SPM projections of those clusters onto a standard template, where schizophrenic patients activated significantly more than healthy control subjects. Schizophrenic patients showed significantly more activations in the supply area of the ACA (Brodmann areas 9 + 10) and in the left temporal areas than healthy control subjects. Table 1 shows all significant brain regions, the stereotactic coordinates, and the *Z* scores corresponding to voxels of peak activity for this contrast (patients [2-back minus reference condition] minus control subjects [2-back minus reference condition]).

Figure 5 shows the fTCD curves at rest and during cognitive stimulation (2-back task) to illustrate the temporal behavior during cognitive activation for healthy control subjects and schizophrenic patients. It becomes evident that healthy control subjects showed bilaterally a continuously increasing CBFV after an initial peak under activation, whereas schizophrenic patients did not increase the CBFV after the initial peak. Instead, they showed a different, slightly decreasing CBFV under activation, as evidenced by the slightly negative regression slope of the fTCD signals.

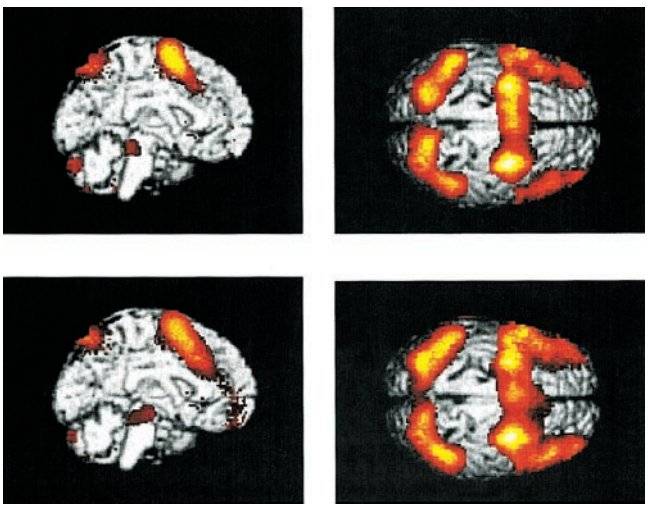

FIGURE 3. SPM projections onto standard template of significant activation clusters from control subjects and schizophrenic patients for working memory activation (2-back task) minus reference condition. (Top row) Significant activations (2-back task) bilateral frontal, parietal, in cerebellum and brain stem (mesencephalon) in healthy control subjects (SPM99b; $P < 0.001$; all $Z > 4.0$) on sagittal (left) and top (right) views. (Bottom row) Significant activations (2-back task) bilateral frontal, parietal, in cerebellum and brain stem (mesencephalon) in schizophrenic patients (SPM99b; $P < 0.001$; all $Z > 4.0$) on sagittal (left) and top (right) views. Activations in supply area of ACA (sagittal image, left) appear to be more extended compared with those of healthy control subjects (Table 2).

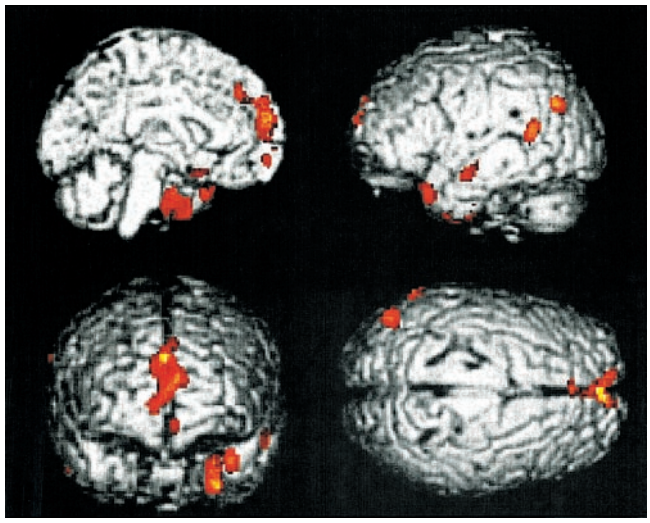

FIGURE 4. SPM projections onto standard template of clusters, where schizophrenic patients activated significantly more than healthy control subjects. Sagittal (top left), lateral (top right), front (bottom left), and top (bottom right) views. Schizophrenic patients show significantly more activations in supply area of ACA (Brodmann areas 9 + 10) and left temporal areas than healthy control subjects (contrast: patients [2-back minus reference condition] minus control subjects [2-back minus reference condition]; SPM99b; $P < 0.001$; all $Z > 3.5$).

Statistical Comparison of PET and fTCD Data

Table 2 shows the comparison of different PET parameters and fTCD changes in the supply area of the ACA under working memory stimulation (2-back task minus reference

TABLE 1
Brain Regions Where Schizophrenic Patients Activated Significantly More than Healthy Control Subjects Under Working Memory Stimulation

Brain region (BA)	*x*	*y*	*z*	*Z* score
Frontal (supply area of ACA)				
Right superior frontal gyrus (BA 9)	6	62	30	3.79
Left superior frontal gyrus (BA 10)	−4	60	−8	3.52
Left middle frontal gyrus (BA 10)	−2	58	18	3.73
Right middle frontal gyrus (BA 10)	8	68	4	3.62
Temporal				
Left superior temporal gyrus (BA 38)	−36	18	−28	3.92
Left superior temporal gyrus (BA 22)	−60	−54	14	3.91
Left angular gyrus (BA 39)	−46	−72	30	3.94
Left limbic lobe, uncus (BA 36)	−28	−6	−32	3.83
Left inferior temporal gyrus (BA 21)	−56	−10	−16	3.65

BA = Brodmann area.
Z scores correspond to voxels of peak activity whose locations are given at stereotactic coordinates referring to atlas of Tailarach and Tournoux (*23*) (contrast: patients [2-back minus reference condition] minus control subjects [2-back minus reference condition]).

condition) between healthy control subjects and schizophrenic patients. There were no significantly different fTCD changes under activation between the 2 groups. However, the mean cluster volume for significant activations in the supply area of the ACA in PET was significantly higher in schizophrenic patients ($P < 0.05$). The same holds true when the volume differences (volumes of significant activations minus volumes of significant deactivations) were compared ($P < 0.01$). Interestingly, the height of significant rCBF increase under activation was significantly lower in schizophrenic patients than that in healthy control subjects ($P < 0.005$). Therefore, when comparing the volume-weighted perfusion changes between the 2 groups, no significant difference was found because schizophrenic patients showed a significantly larger activation volume, but with a significantly lower rCBF increase, than healthy control subjects under working memory stimulation. Because the height of the fTCD signal changes ($CBFV_{activation}$ minus $CBFV_{reference\ condition}$) in the ACA depends on both the size of the activated or deactivated clusters and the averaged height of rCBF change within these clusters (multiple linear regression of fTCD signal changes with size of clusters and height of perfusion changes as predictors showed that both predictors contribute significantly to the fTCD signal: $P < 0.0005$; $r = 0.92$; adjusted $r^2 = 0.83$), the nonsignificance of the comparison of volume-weighted perfusion changes becomes plausible when taking into account the nonsignificantly different fTCD changes.

In the regression analysis of mean fTCD changes ($CBFV_{activation}$ minus $CBFV_{reference\ condition}$) and PET volume differences for all subjects ($n = 21$), only moderate correlation coefficients were obtained ($r = 0.67$; $P < 0.005$). However, the separate regression analyses for healthy control subjects ($n = 10$) as well as for schizophrenic patients ($n = 11$) showed strong and highly significant correlations ($r = 0.94$ for control subjects; $r = 0.91$ for patients; all $P < 0.0005$). As shown in Figure 6, the regression slope in

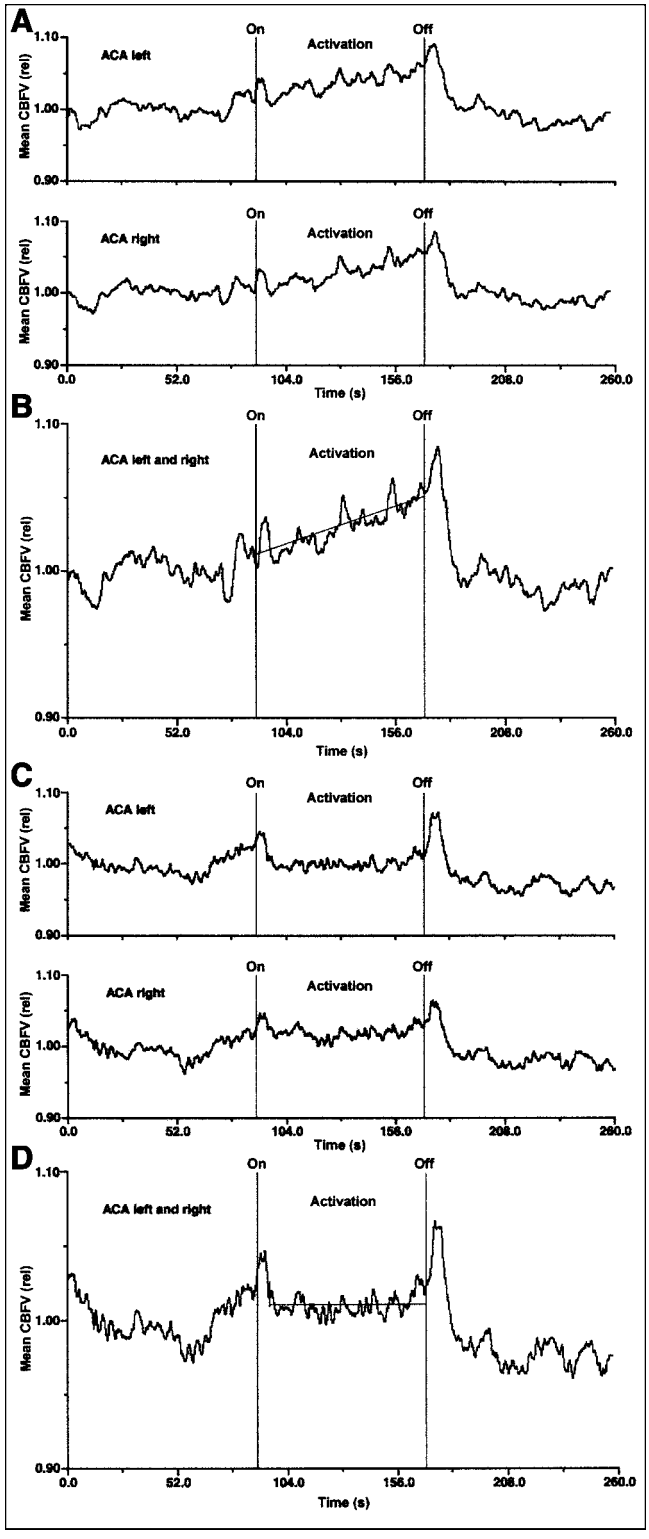

FIGURE 5. Averaged cerebral blood flow velocity (CBFV) changes of left and right ACA and mean CBFV for both sides during activation in all control subjects and schizophrenics. (A and B) Averaged CBFV changes of left (top) and right (bottom) ACA during cognitive activation (2-back) from 90 s (On) to 170 s (Off) in all healthy control subjects (A) and bilateral (averaged, ACA left and right) CBFV change (B). *x*-Axis, time (s); *y*-axis, CBVF (resting CBFV normalized to 1). During activation initial peak, then continuously increasing CBFV, followed by over- and undershoot right after activation (170 s). Regression slope of fitted regression curve of bilateral fTCD signals from 90 to 170 s (from after initial peak to over- and undershoot) was positive: CBFV = 0.9667 + 0.0005 × time. (C and D) Averaged CBFV changes of left (top) and right (bottom) ACA during cognitive activation (2-back) from 90 s (On) to 170 s (Off) in all schizophrenics (C) and bilateral (averaged, ACA left and right) CBFV changes (D). *x*-Axis, time (s); *y*-axis, CBVF (resting CBFV normalized to 1). During activation initial peak, then no continuously increasing CBFV but almost slightly decreasing, followed by over- and undershoot right after activation (170 s). Regression slope of fitted bilateral fTCD signals from 95.5 to 170 s was slightly negative: CBFV = 1.0109 − 0.00001 × time. Therefore, temporal behavior of schizophrenic patients under cognitive activation is different from that of control subjects because they show slightly decreasing CBFV during time course of activation, whereas control subjects show significant CBFV increase after initial peak under activation. rel = relative.

TABLE 2
Comparison of PET and fTCD Parameters in Supply Area of ACA Under Working Memory Stimulation Between Healthy Control Subjects and Schizophrenic Patients

Parameters of working memory stimulation imaging	Healthy control subjects (n = 10)	Schizophrenic patients (n = 11)
Activation volumes (mm^3)	1,285.8 ± 630.6*	2,442.1 ± 1,458.5*
Volume differences (mm^3)	66.6 ± 363.3†	1,769.9 ± 1,661.9†
rCBF increases under activation (mL/100 g/min)	11.3 ± 4.7†	4.8 ± 2.3†
$rCBF_{activation}$ minus $rCBF_{deactivation}$ (mL/100 g/min)	5.1 ± 7.2‡	0.09 ± 5.1‡
Volume-weighted perfusion changes (mm^3 × mL/100 g/min)	5,678.9 ± 8,894.9	8,963.6 ± 10,248.5
fTCD changes (%)	0.53 ± 2.97	1.08 ± 2.62

*$P < 0.05$.
†$P < 0.01$.
‡$0.05 < P < 0.1$.
All values are mean ± SD.
Activation volumes = mean cluster volume for significant activations; volume differences = volumes of significant activations minus volumes of significant deactivations; rCBF increases under activation = calculated by projecting significant activation clusters onto averaged PET images of each task and computing mean rCBF increase in significant activation clusters ($rCBF_{activation}$ minus $rCBF_{reference\ condition}$); $rCBF_{activation}$ minus $rCBF_{deactivation}$ = rCBF change of activated clusters minus rCBF change of deactivated clusters; volume-weighted perfusion changes = volume-weighted perfusion change of significant activation clusters minus volume-weighted perfusion change of significant deactivation clusters; fTCD changes = fTCD signal differences under working memory stimulation ($CBFV_{activation}$ minus $CBFV_{reference\ condition}$).

schizophrenic patients is significantly lower than that in control subjects ($P < 0.05$). This means that schizophrenic patients show significantly larger PET volumes of activation minus deactivation for the same height of fTCD changes than control subjects. Therefore, it becomes clear why the correlation coefficient for all subjects together is considerably lower.

Figure 7 shows that in the regression analyses of mean fTCD changes versus PET volume-weighted rCBF changes in control subjects and patients there are strong and highly significant correlations ($r = 0.95$ for control subjects; $r = 0.94$ for patients; all $P < 0.0005$) and that regression slopes are not significantly different between patients and control subjects. Therefore, the regression analysis for all subjects together ($n = 21$) also shows a strong correlation ($r = 0.94$; $P < 0.0005$). It becomes evident that the height of the fTCD changes depends not only on volume but on both volume of the clusters and the averaged height of rCBF change within these clusters. Therefore, for both groups during cognitive stimulation of the working memory, there is a very good correlation between the CBFV changes measured with fTCD and the volume-weighted rCBF changes measured with PET in the supply area of the ACA.

Correlation Analyses of Neuropsychologic Data to fTCD and PET Data

Finally, correlations of neuropsychologic performance during the 2-back task with the fTCD changes, PET volume differences, PET rCBF changes, and PET volume-weighted rCBF changes were performed for control subjects as well as patients. Interestingly, there were only significant correlations between neuropsychologic performance (reaction times) and neuroimaging parameters for healthy control subjects (fTCD changes: $r = -0.65$, $P < 0.05$; PET volume differences: $r = -0.83$, $P < 0.005$; PET rCBF changes: $r = -0.64$, $P < 0.05$; PET volume-weighted rCBF changes: $r = -0.86$, $P < 0.005$; the negative correlations indicating shorter reaction times [better neuropsychologic performance] correlate well with fTCD–PET parameters), whereas there were no significant correlations found in schizophrenic patients (all $r < 0.3$; all $P > 0.4$). This means that despite the fact that there were no significant differences in neuropsychologic performance between the 2 groups, schizophrenic patients revealed no significant correlations of cognitive performance under activation to blood flow–dependent imaging parameters. Therefore, this indicates a qualitative difference in cognitive processing between the 2 groups.

DISCUSSION

It is well known that CBFV shows good correlation with rCBF changes in intracranial physiology (*4,18,24,25*). CBFV of the MCA has been shown to correlate well with nonsimultaneous blood oxygen level–dependent signal of fMRI in a verbal fluency task (*26*).

Using truly simultaneous fTCD–PET acquisition, to our knowledge, it has not been demonstrated previously that CBFV changes measured with fTCD under working memory activation in the supply area of the ACA show a very good correlation with volume-weighted rCBF changes measured with PET in schizophrenic patients as well as in healthy control subjects (Fig. 7). It should be emphasized that the height of the CBFV changes depends not only on volume but on both the volume of the significant activation or deactivation clusters and the averaged height of rCBF

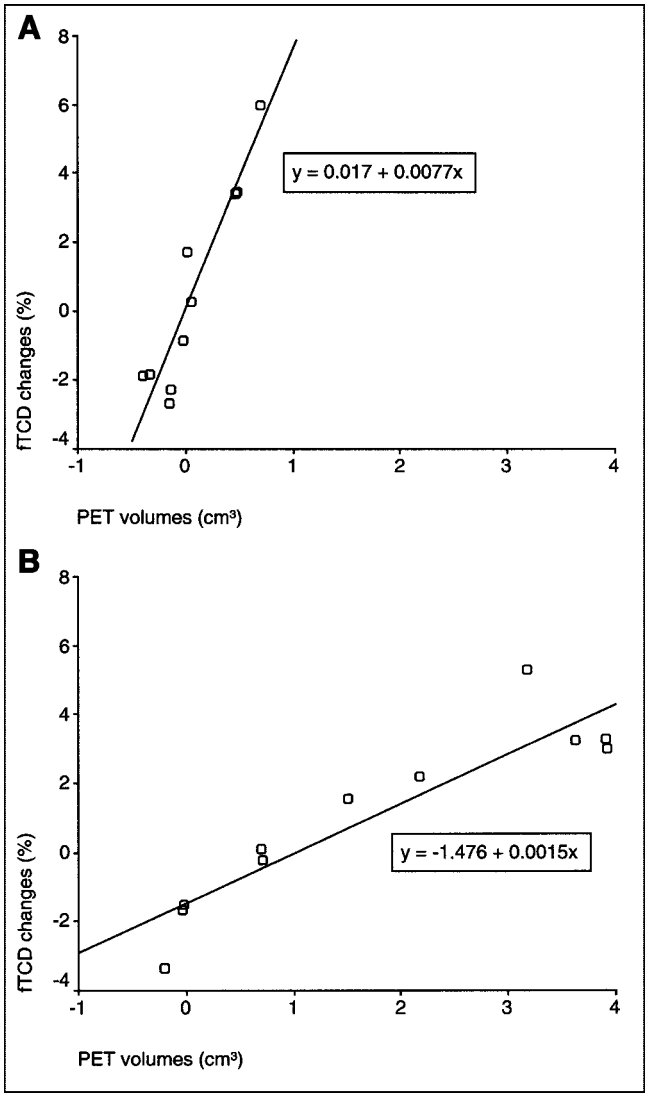

FIGURE 6. Regression analysis of mean fTCD changes ($CBFV_{activation}$ minus $CBFV_{reference\ condition}$) of ACA vs. PET volume differences (volume of activated clusters minus volume of deactivated clusters) in healthy control subjects and schizophrenic patients. (A) There is strong and highly significant correlation between mean fTCD changes and PET volume differences in supply area of ACA in healthy control subjects: $y = 0.017 + 0.0077x$; $r = 0.94$; $P < 0.0005$. (B) There is also strong and highly significant correlation between mean fTCD changes and PET volume differences in supply area of ACA in schizophrenic patients: $y = -1.476 + 0.0015x$; $r = 0.91$; $P < 0.0005$. Note that regression slope in schizophrenic patients is significantly lower than that in control subjects: 0.0015 (95% confidence interval, 0.0009–0.0019) vs. 0.0077 (95% confidence interval, 0.0054–0.0099); $P < 0.05$. This means that schizophrenic patients show significantly larger PET volumes of activation minus deactivation for same height of fTCD changes.

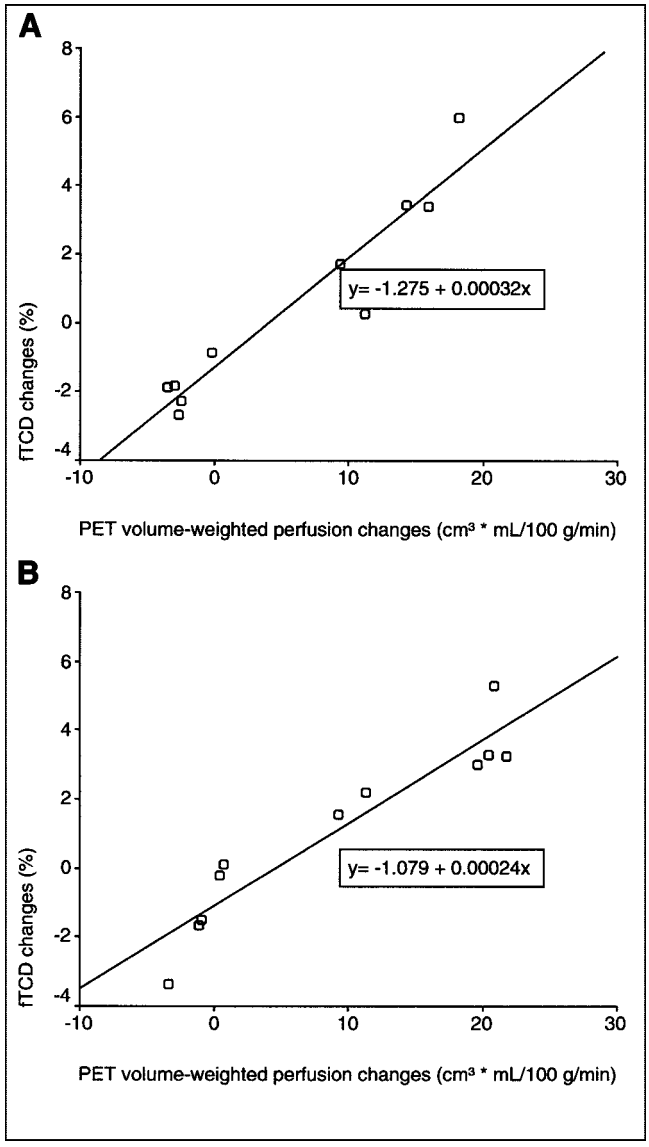

FIGURE 7. Regression analysis of mean fTCD changes ($CBFV_{activation}$ minus $CBFV_{reference\ condition}$) of ACA vs. PET volume-weighted rCBF changes (volume-weighted perfusion change of significant activation clusters minus volume-weighted perfusion change of significant deactivation clusters) in healthy control subjects and schizophrenic patients. (A) There is strong and highly significant correlation between mean fTCD changes and PET volume-weighted rCBF changes in supply area of ACA in healthy control subjects: $y = -1.275 + 0.00032x$; $r = 0.95$; $P < 0.0005$. (B) There is also strong and highly significant correlation between mean fTCD changes and PET volume-weighted rCBF changes in supply area of ACA in schizophrenic patients: $y = -1.079 + 0.00024x$; $r = 0.94$; $P < 0.0005$. Note that regression slope in schizophrenic patients is not significantly different from that in control subjects: 0.00024 (95% confidence interval, 0.00018–0.00037) vs. 0.00032 (95% confidence interval: 0.00024–0.00040); $P > 0.2$. This means that for both groups during cognitive stimulation of working memory, there is strong and highly significant correlation between CBFV changes measured with fTCD and volume-weighted rCBF changes measured with PET in supply area of ACA.

change within these clusters, as shown by multiple linear regression. Schmidt et al. (*24*) determined cognitive hemispheric lateralization during a cognitive visuospatial task by fTCD of the MCA and cross-validated fTCD with nonsimultaneous fMRI in 14 healthy control subjects. They

found a significant, but only moderate, correlation between fTCD (CBFV) changes and fMRI volume of activated clusters ($r = 0.54$; $P = 0.02$). However, they did not take into account the influence of the volume of significant deactivations during this task on the CBFV changes. Furthermore, the height of the fMRI signal change should have also been considered, as we showed for PET in our study. Therefore, our correlation of fTCD with PET was much higher ($r = 0.95$, $P < 0.0005$ for healthy control subjects; $r = 0.94$, $P < 0.0005$ for schizophrenic patients).

Regarding working memory activation, Cupini et al. (*10*) studied 22 healthy volunteers with fTCD of the MCA during visuospatial and verbal working memory tasks. They suggested that the high temporal resolution of this technique would be promising for further application in CBFV change monitoring during neuropsychologic studies. Furthermore, it could be very well comparable with results from PET and fMRI because all of these methods determine blood flow–dependent imaging parameters as surrogate parameters of neuronal activity. However, to our knowledge, there is no report in the literature of the role of fTCD of the ACA in working memory and of the role of fTCD of the ACA in schizophrenia. Clearly, the reason for this is that during monitoring, the MCA is technically much easier to insonate continuously over several minutes than the ACA (*27*).

Owen et al. (*28*) demonstrated that working memory processes within the human middorsolateral and midventrolateral frontal regions are organized according to the type of processing required rather than according to the nature (i.e., spatial or nonspatial) of the information being processed, as has been widely assumed. Up to this time, PET and MRI studies on the verbal and nonverbal working memory in healthy subjects usually showed broad frontal and parietal activations even when the stimuli were held constant and only the memory load changed (*29–31*). Thus, it seems that MCA-supplied structures are the main ones affected, but activations have also been reported for the medial cortical ACA-supplied structures, such as the cingulate gyrus or the medial portions of the superior frontal gyrus (*30,32–37*), which would indicate that these are also part of a disseminated neuronal system for mediating working memory processes. Postmortem studies of the brains of schizophrenic patients have also shown abnormalities of cortical areas within the working memory network, including the dorsolateral prefrontal cortex, cingulate, and temporal cortices (*5*).

However, in our study we demonstrated that schizophrenic patients compared with healthy control subjects showed significantly different activations, especially in the supply area of the ACA and the left temporal areas (Fig. 4; Table 1). Artiges et al. (*11*) investigated brain regions involved in working memory control processes in patients with schizophrenia. The results suggested a cinguloparietal dysfunction underlying the impairment of working memory control processes during a random number generation task in patients with schizophrenia (*11*). Bertolino et al. (*5*) researched 13 schizophrenic patients with proton magnetic resonance spectroscopic imaging (to measure *N*-acetylaspartate as a marker of neuronal pathology) and with PET during performance of an N-back task. Patients showed worse task performance compared with control subjects. A traditional criticism of functional neuroimaging studies assessing differences in activation by working memory tasks between schizophrenic patients and healthy control subjects has been that patients usually perform worse on these tests, thus making the comparison unfair (*1,5*). Carter et al. (*13*) studied 8 patients with schizophrenia and 8 matched comparison subjects using PET and the N-back task. The rCBF response to increased working memory load was significantly reduced in the patients' right dorsolateral prefrontal cortex, but the patients' task performance was significantly worse ($P < 0.0001$). We therefore studied 11 clinically stable chronic schizophrenic patients with no significant difference in performance of a 2-back task to detect differences that cannot be challenged as artifacts of poor performance. Because we recently showed that different positive symptoms in schizophrenics correlate exclusively with either hyper- or hypoperfusion of the cerebral cortex (*2,3*), we avoided an artificial influence of strongly pronounced positive symptoms on rCBF measurements under the 2-back task by using special patient selection criteria. Furthermore, control subjects were matched individually for age, sex, education, and IQ because several studies indicate age differences in the frontal lateralization of verbal and spatial working memory revealed by PET (*38–40*). The fact that our schizophrenic patients showed significantly more activations in the supply area of the ACA and left temporal areas (with a task performance comparable with that of control subjects) could be interpreted as recruitment to compensate for partial neural dysfunction, as is speculated for age-related neural declines and more extended activations in control subjects (*40*). However, the fact that only healthy control subjects show a good correlation between task performance and blood flow–dependent imaging parameters indicates a qualitative difference in cognitive processing between control subjects and schizophrenic patients. Artiges et al. (*11*) also found no correlation between activity in the anterior cingulate (supply area of the ACA) and task performance in schizophrenics.

Using truly simultaneous fTCD–PET acquisition, to our knowledge, it has not been shown previously that schizophrenic patients show a significant and qualitative difference in the temporal (Fig. 5) and spatial resolution (Fig. 4; Table 1) of cognitive processing compared with healthy control subjects. Under working memory stimulation, they activate a significantly larger cortical volume, perhaps to ensure adequate task performance but with a significantly lower increase of rCBF in this volume, than control subjects. The healthy subjects activated a certain number of neurons in the ACA-supplied area (qualitative strategy, Fig. 3). After an initial peak at the beginning of the task, which is physiologically quite normal in activation studies using fTCD, they showed a slight reactive rise in CBFV that

increased steadily throughout the task (Fig. 5). This would suggest that, when executing a cognitive task, control subjects continuously increased neuronal activity. This increase in activity caused an increase in the neuronal metabolism, which in turn caused an increase in rCBF and a continuous rise in CBFV (Fig. 5). Thus, during a working memory task, healthy subjects were capable of adapting their neuronal activity, and hence rCBF, to the current need. In contrast, schizophrenic patients showed a completely different strategy. They activated a significantly larger cortical volume (i.e., much greater number of neurons) (Figs. 3 and 4; Table 2). After the initial peak and a likewise slight reactive rise in CBFV, their CBFV remained essentially constant throughout the task and even decreased slightly (Fig. 5). This would suggest that patients activate a larger number of specific and unspecific neurons already at the beginning of the test (quantitative strategy). The patients evidently then could not further increase neuronal activity during the time course of cognitive activation, as shown by the patients' essentially unchanged reactive CBFV (Fig. 5).

The temporal weight of flow information in bolus-injected $H_2{}^{15}O$ PET scans is not uniform throughout the scan duration. In fact, the CBF-sensitive part of the scan is mainly restricted to a relatively short time window of 15- to 20-s length after the arrival of the radiotracer in the brain (*41*). This CBF-sensitive window may be too short for complex activation paradigms, particularly those of longer duration (e.g., the go-'n-get-me-a-beer-task (*41*)). Regarding the N-back task, the bolus injection method with PET scans of 60-s duration starting 20 s after injection seems to be well suited as shown by other groups (*5,13*). However, one might suggest, that the wider PET activation clusters in our patient group could be due to the relatively higher flow velocity during the very initial part of the activation task (peaks) in the patient group compared with healthy control subjects as shown in the fTCD–CBFV diagram (Fig. 5). It is therefore important to emphasize that the PET scans were started 20 s after the injection of the radiotracer, whereas the activation task and the fTCD acquisition were started immediately upon injection of the radiotracer. Thus, the PET scans were securely started after the initial flow peak in the fTCD–CBFV diagrams.

CONCLUSION

We showed that schizophrenic patients exhibit qualitative differences in the spatial and temporal resolution of cognitive processing. All facts could be interpreted as a sign of alternative, less-efficient problem-solving strategies in schizophrenia, less efficient than those used by healthy control subjects, which lead to the working memory deficits observed during the further course of this disease. A truly simultaneous combination of fTCD and $H_2{}^{15}O$ PET can be used in neuroscience to add fundamental new information on both spatial and temporal cognitive activation behavior to understand the true physiologic nature of the disease-specific differences of mental illnesses that are seen as disorders of the mind arising in the brain (*42*).

ACKNOWLEDGMENTS

The authors thank Keyvan Setani for patient management, Alejandro Rodón for language editing, and Klaus Willmes for guidance and supervision during the statistical analysis. This study was supported by a grant from the Interdisciplinary Center for Clinical Research-Central Nervous System (IZKF-ZNS) of the Aachen University of Technology.

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