Unilateral Voluntary Hand Movement and Regional Cerebral Uptake of Technetium-99m-Exametazime in Human Control Subjects

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The study examines the sensitivity of a region of interest approach to detect functional changes in brain metabolism with SPECT and split-dose ^{99m}Tc-exametazime by replicating a simple hand movement experiment previously carried out with PET. Regional uptake of ^{99m}Tc-exametazime was determined in 12 healthy controls before and during a thumb-digit opposition task. Analysis of regional uptake was carried out blind to the hand used in the opposition task and showed a significant unilateral activation effect in a pericentral region of interest with opposite results in left- and right-handed activation. The maximum contralateral increase in tracer uptake was 16% before and 26% after correction for back diffusion. This is in good agreement with previous results employing absolute cerebral blood flow determination with PET and confirms the usefulness of ^{99m}Tc-exametazime SPECT for the examination of functional metabolic changes.

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Technetium-99m-exametazime was introduced on the basis that it gives an estimate of regional cerebral blood flow (rCBF) (*1*). Its quantitative properties in the estimation of rCBF at rest have been determined against several other methods, such as ^{133}Xe (*2*), ^{15}O-labeled CO_2 positron emission tomography (PET) (*3–5*), ^{18}F-labeled fluoromethane PET (*6*) and radiolabeled microspheres (*7*). These studies have shown a monotonic, but non-linear, relationship between uptake of ^{99m}Tc-exametazime and rCBF.

In addition to the measurement of resting rCBF, single-photon emission tomography with ^{99m}Tc-exametazime can be employed to examine changes in rCBF during physiological, psychological or pharmacological activation (*8–10*). We have recently reported a split-dose technique for the study of such activation effects, using a repeat injection of 250 MBq ^{99m}Tc-exametazime applied in the same imaging session, which yielded good reproducibility of results in repeat baseline studies (*11*). The purpose of the present study was to employ a specific physiological activation paradigm that had been examined previously using PET and to determine whether the predicted local activation effect could be detected and whether the magnitude of activation was comparable to the effect observed with PET. For this purpose, we used a finger-thumb opposition task, which results in a metabolic activation of the pre- and post-central gyrus of the contralateral cerebral cortex (*12–16*).

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MATERIALS AND METHODS

Subjects

Twelve control subjects were recruited from relatives and friends associated with the local branch of the Alzheimer's disease society. They included four men and eight women with a mean age of 63 yr (s.d. 3.5, range 59–68 yr). Patients were examined using the Annett Handedness Scale, scored according to Briggs & Nebes (*17*). They were assessed neuropsychologically using the National Adult Reading Test (*18*), the block design and digit symbol tests of the Wechsler Adult Intelligence Scale R (*19*) and the Wechsler Memory Scale-R (*20*) to ascertain that no subject showed evidence of cognitive or psychomotor impairment. All subjects gave informed written consent according to a procedure approved by the local ethics committee.

Imaging Procedure

Subjects were imaged with a single slice multi-detector dedicated head scanner (Multi-X 810, Strichman Medical Equipment Inc., Boston, MA) after two repeated injections of 250 MBq of ^{99m}Tc-exametazime, as described in detail elsewhere (*11*). With the use of the intermediate 572 whole collimators, the in-slice resolution of the scanner is given as 7.5 mm (FWHM) by 15 mm slice thickness. The sensitivity of the instrument was measured as 520 cps/1 kBq/ml (*11*). An indwelling intravenous catheter was inserted in an arm vein 15–30 min before the injection. For the injection, subjects were positioned comfortably on the imaging table with eyes patched and ears unplugged. A metronome was calibrated to 90 bpm and activated for 20 beats before and 5 min after start of the injection (bolus injected over 5 sec). During this time, the subject was instructed not to move in rhythm to the beat, and ambient nose was kept to a minimum. The subject's head was then placed in a moulded headrest, positioned with the help of two crossed light beams, and fixed with two pressure pads over the zygomatic arches. Five slices were acquired parallel to the orbito-meatal line at +55, +65, +71, +77 and +83 mm. The

subjects were then instructed to perform a "finger opposition task," touching each of four fingers successively with their thumb (*12*). They had to perform this movement in rhythm with the metronome, one half movement, i.e., touch or extend, per beat and moving briskly from one extreme position to the other. Subjects were alternatively allocated to right- and left-hand movement conditions. Two short training sessions were performed about 10 min after the first injection and again, after the first scan, immediately before the second injection. Once the task was performed accurately, the subject was positioned comfortably, all muscle groups not involved in the movement task were relaxed, and the second injection of ^{99m}Tc-exametazime was given. Movements were started after a signal given by the experimenter lightly touching the subject's forearm 10 beats after the metronome was started. The second intravenous bolus of tracer (250 MBq in 5 sec) was given after an additional 10 beats. Movements were stopped by a second touch signal from the experimenter 3 min after the injection. For the remaining 2 min, conditions were identical to baseline. Subjects were repositioned and the same slices were acquired as for the baseline condition. The acquisition time for the second scan was 2.5 min per slice, half the time required for the baseline scan. We routinely use two half-doses instead of a larger dose for the second (activation) injection, because the potential gain in signal-to-noise ratio afforded by the latter is offset in psychiatric populations by increased movement artifacts during the longer acquisition time of the first (lower dose) scan. The design of the activation and imaging procedure is illustrated in Figure 1. Lipophilicity of the tracer after reconstitution was measured in six subjects at baseline as 85% (s.d., 6.1%) and for the activation scans as 87% (s.d., 3.2%). The mean difference in lipophilicity between scans was 2% with a 95% confidence interval from −5% to 10%. The radiation dose for each subject was approximately 8 mSv.

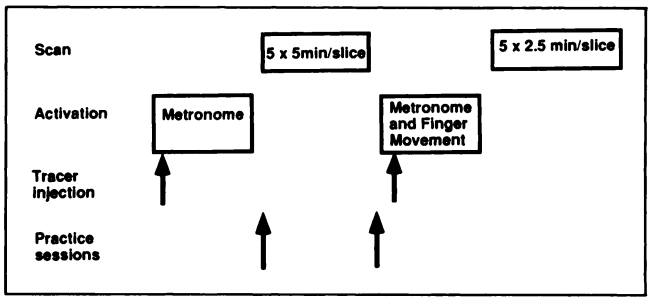

FIGURE 1. Protocol for tracer injection and activation procedures.

Data Analysis

A template was drawn outlining the slice hemispheres and a pericentral region of interest (ROI) in the shape of parallelogram. The whole template was fitted to the outline of the hemispheres defined after removing the lower 40% of the intensity spectrum. The parallelogram had its anterior boundary approximately 30% and its posterior boundary 60% of the distance from the anterior to the posterior pole of the brain. It was chosen to include the pre- and post-central gyrus in a standard brain atlas (Fig. 2). When applied to the three slices at 71, 77 and 83 mm above the orbito-meatal (OM) line, the mean area of the pericentral ROI was 13 cm^2 (s.d. 2.1) left and 13.4 cm^2 (s.d. 2.1) right. The inter-rater reliability of the measurement of mean regional count densities in this ROI was determined by normalizing the regional count densities to the whole slice, calculating the difference of measures between two raters (NJD and KPE) and expressing these differences as a percentage of the mean of the two raters. The inter-rater error, defined as twice the s.d. of these percentage difference values, amounted to 5% for the pericentral ROI. There was no systematic difference between the two raters, so that 95% of all inter-rater differences were situated between −5% and +5%. This means that in a comparison of two groups of six subjects, differences in group means of more than 3.2% can be detected with confidence. Whole brain activity was calculated from hemislice activities of the three slices at 71, 77 and 83 mm. The ROI-to-whole brain ratio (R) was calculated for ROIs in all three slices. R values were corrected for flow-dependent backdiffusion using the Lassen algorithm as suggested by Inugami et al. (*4*) and Woods et al. (*8*):

$$R_{corr} = \alpha R/(1 + \alpha - R),$$

where $\alpha = 2$. The net uptake during the activation procedure (second injection) was calculated by subtracting time-corrected counts measured during the first scan from the total counts measured during the second scan, as described elsewhere (*11*).

ROI-to-whole brain ratios before and after Lassen correction were compared between the two groups of patients with left- and right-handed activation using multivariate analysis of variance with: (1) left/right side of brain, (2) three slice levels and (3) baseline/activation scans as three within-subjects factors. This method takes into account all repeated measures of variance and co-variances and represents a relatively conservative omnibus-test for the presence of an activation effect. Crucial to the detection of activation would be a significant interaction between side of activation, side of the brain and condition. This derives from the hypothesis that increases in rCBF were expected in one or more slices for the side of the brain contralateral to hand movement only during the activation condition.

Because the level of the slice showing an activation effect could not be predicted a priori, uptake ratios were averaged post hoc across three slices and a right-left index was calculated as 200 × (R−L)/(R+L) to determine an effect size. One-tailed t-tests were used to compare indices between subjects with right- and left-sided hand movement. After these tests had been conducted blind to the side of activation, a more specific examination of the locus of activation effect was carried out by selecting the slice with the

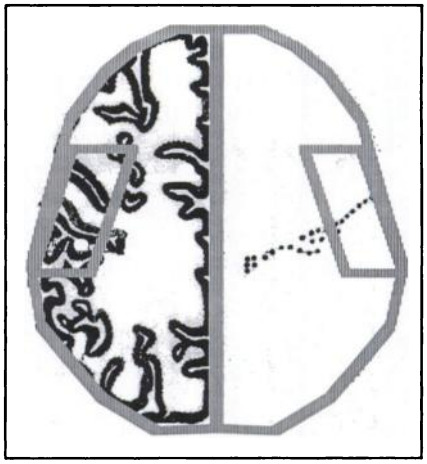

FIGURE 2. Configuration of the template with two hemislice and two pericentral ROIs (the underlying atlas-slice is reprinted with permission from Ref. 21).

TABLE 1
Descriptive and Neuropsychological Screening Variables

Test	Mean	Range
Handedness (Briggs & Nebes, 1975)	22.8	20–24
Years of education	15.9	12–21
NART-IQ (Nelson & Willison)	119.3	111–127
Block design (WAIS-R) (Wechsler, 1981)	13.3	11–17
Digit symbol (WAIS-R)	12.2	9–15
Attention/Concentration (WMS-R) (Wechsler, 1987)	114.2	71–130
General memory (WMS-R)	109.6	92–133
Delayed recall (WMS-R)	119.6	93–138

greatest increase in mean uptake in the pericentral ROI of the contralateral hemisphere and by estimating the maximum size of the activation.

RESULTS

The subjects were all right-handed; their neuropsychological test results (Table 1) indicated that none showed cognitive deterioration.

That increases in rCBF occurred in the pericentral ROIs of the hemispheres contralateral to the activation only during the activation condition was supported by the F value of the appropriate omnibus test (side of activation × side of brain × activation condition – $F_{1,10} = 7.37$, $p = 0.022$). The results were identical if uptake ratios were corrected for backdiffusion at higher flow rates ($F_{1,10} = 7.77$, $p = 0.019$).

Our conservative estimate of the lateral shift in rCBF of pericentral ROIs was based on the average uptake of tracer in pericentral ROIs across the three slices. Mean values were calculated separately for left- and right-handed activation groups. These are shown in Table 2 during baseline and activation scans with and without Lassen correction. Although at baseline both groups showed an asymmetry towards the right as predicted from the literature (*22*), there was a nonsignificant trend for the right-left index to be smaller in the left hand than the right-hand activation group. This suggests relatively greater uptake contralateral to the side of the cannulated, nonactivated side, which might have received some tactile and proprioceptive input. As predicted, left-handed activation results in an increase in uptake to the right side (i.e., a high index), whereas right-handed activation reverses the baseline right asymmetry towards the left (index < 0).

To determine the effect of size of contralateral rCBF increases, it was assumed that the pericentral ROI showing the highest contralateral increase in uptake from baseline to activation scan included the brain area with specific functional activation due to hand movements. Table 3 shows the corresponding percentage increases before and after Lassen corrections. The maximum increase in perfusion and, therefore, the putative locus of activation tended to be in the highest slice, 83 mm above the OM line, although in a few cases, maximum changes were observed up to 12 mm lower (Table 3).

The anatomy of such a peak effect in one subject (no. 2, Table 3) is illustrated in Figure 3. Slices at 71, 77, and 83 mm above and parallel to the OM line are depicted before and during left hand activation, in combination with the pixel by pixel subtraction image.

DISCUSSION

The results show that the distribution of ^{99m}Tc-exametazime can be used to illustrate dynamic changes in rCBF in neocortex which parallel changes in neuronal activation associated with physiological stimuli (*12–15, 24, 25*). As a group, subjects with right-hand movement showed increased uptake to the left pericentral cortex of slices 71, 77 and 83 mm above the OM line. Subjects with left-handed activation showed an opposite effect. While the level of slices and the location of the ROI was predetermined by reference to a previous PET study (*12*), measurements were made blind to the site of activation. In addition, the omnibus multivariate analysis of variance did not rely on the determination of areas with peak change as used in Woods et al's. (*8*) study of visual stimulation, thus further avoiding the possibility of spuriously positive results.

The magnitude of effect produced in motor and proprioceptive sensory activation was determined post-hoc and resulted in a percentage increase very similar to that reported by Colebatch et al. (*12*) who used a quantitative

TABLE 2
Right-Left Indices Calculated from Uptake to Pericentral ROIs Averaged Across Three Slices

	Right-Left index (s.e.)			
	Right-Handed activation	Left-Handed activation	t (d.f. = 10)	p (one-tailed)
No Lassen correction				
Baseline	3.2 (2.1)	1.4 (1.6)	−0.67	0.52
Activation	−2.1 (2.9)	7.4 (2.4)	2.53	0.03
Lassen correction				
Baseline	4.7 (3.1)	2.2 (2.4)	−0.63	0.54
Activation	−3.3 (4.4)	11.3 (3.6)	2.56	0.03

TABLE 3
Mean %Increase in Tracer Uptake in the ROI and Slice with the Highest Contralateral Increase

Subject no.	Side of cortical activation	Slice	%Increase	%Increase after Lassen correction
1	R	+71 mm	21	36
2	R	+71 mm	13	22
3	R	+83 mm	−1	−2
4	R	+83 mm	19	29
5	R	+83 mm	21	32
6	R	+83 mm	34	56
7	L	+77 mm	29	49
8	L	+77 mm	−2	−2
9	L	+83 mm	14	21
10	L	+83 mm	17	27
11	L	+83 mm	13	20
12	L	+83 mm	16	25
Mean (s.d.)			16 (10)	26 (17)

integral/dynamic method of CBF determination with ^{15}O-labeled CO_2 (*23*). The calculation of percentage increase in uptake using a correction for backdiffusion resulted in values slightly in excess of those reported with PET (21%) (*12*). Guenther et al. (*15*) reported a similar experiment using opening and closing of the right hand and ^{133}Xe SPECT with a tomographic scanner. These authors report an increase of rCBF in the left motor cortex of eight healthy controls of 26%.

In two subjects (nos. 3 and 8, Table 3), no contralateral activation effect could be observed. This is likely due to uncontrolled variability in task performance, e.g., an inadvertent and unobserved activation of other muscle groups which could have lead to an overall increase of tracer uptake and, therefore, a masking of any unilateral changes in regional uptake into sensory-motor cortex.

In addition to primary sensory-motor activation, Colebatch et al. (*12*) also report increased blood flow in supplementary motor cortex. An attempt to fit a small ROI over this area in our scans did not yield a significant activation effect. This may be because the ^{99m}Tc-exametazime method does not allow resolution of such small effects, or because the effect is actually less robust than the PET report suggests. With the use of larger ROIs, any activation effect is likely to disappear due to background noise. The solution to this problem may be a pixel-by-pixel subtraction procedure to remove background activity and to identify functional hot spots. With such a method it will again be essential to identify the anatomical localization of activated areas using parallel MRI scans or a stereotactic reference. Finally, the additional use of a three-dimensional reconstruction procedure (e.g., Neuro 900, Strichman Medical Equipment Inc.) or a multiple-headed rotating gamma camera might have increased transaxial resolution and the likelihood of detecting smaller changes.

CONCLUSION

Our results suggest that the measurement of regional uptake of ^{99m}Tc-exametazime provides a sensitive method for detecting cerebral correlates of physiological activation. The order of magnitude of the effect observed compares well with the results derived from ^{15}O PET and ^{133}Xe SPECT studies. While PET studies employing ^{15}O or ^{18}F will remain restricted to few centers and relatively cooperative patients and controls, and ^{133}Xe SPECT studies have certain drawbacks due to their poorer spatial resolution, ^{99m}Tc-exametazime SPECT is easily available for the examination of metabolic abnormalities in more disturbed patient populations. First results suggest that simple physiological activation procedures might accentuate differences in tracer uptake between patients and controls (*15*). Such methods, extended to neuropsychological activation (*26*) offer a potential means for identifying the functional abnormalities in psychiatric disorders and the action of drugs for their treatment.

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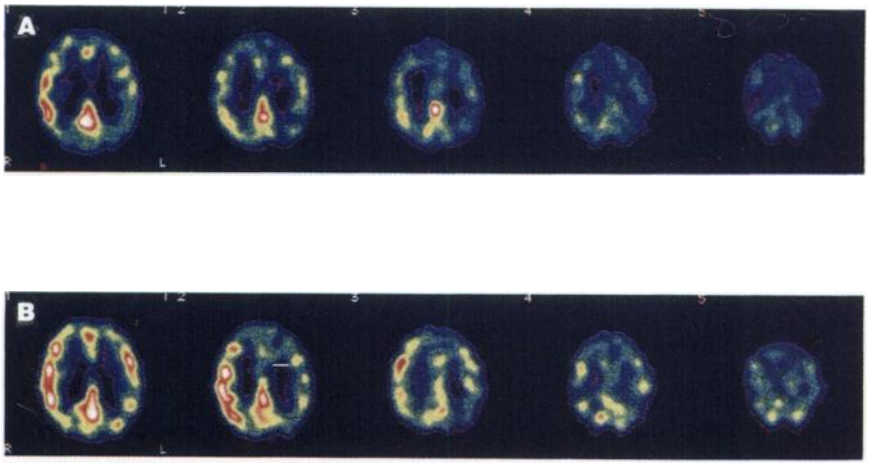

FIGURE 3. Demonstration of pre- and post-central gyrus activation during the finger opposition task. (A) Resting baseline scan. (B) Scan during left-handed activation, transaxial slices from 71 mm above and parallel to the OM line at 6 mm intervals for subject 2 (Table 3).

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