Visual Stimulation Increases Technetium-99m-HMPAO Distribution in Human Visual Cortex

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The ability of changes in the distribution of technetium-99m-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) to reflect physiologic changes in regional cerebral blood flow (rCBF) was evaluated using photic stimulation, a procedure known to increase rCBF in the striate cortex. Seven healthy subjects were injected with 740 MBq ^{99m}Tc-HMPAO on two separate days. On one day, the injection was performed following closure of the eyes and patching for 5 min. On the other day, subjects were exposed to a stroboscopic light to produce photic simulation. Images of distribution of ^{99m}Tc-HMPAO were obtained using a Strichman 810X single-photon emission computed tomogram (SPECT) brain scanner. Comparison of images obtained during light occluded versus stimulation conditions revealed a significant increase in distribution of radiopharmaceutical in visual cortex relative to whole brain (peak increase corrected for radiopharmaceutical backdiffusion 36.7% ± 6.6%). HMPAO appears to provide a useful method for detecting relative rCBF increases with SPECT.

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Technetium-99m-d,l-hexamethylpropylene amine oxime (^{99m}Tc-HMPAO) is a novel radiopharmaceutical which has been shown to distribute in the brain as a function of regional cerebral blood flow (rCBF) (*1,2*). The effects of stimuli known to produce functional changes in regional CBF on the distribution of ^{99m}Tc-HMPAO are not well established. It is particularly important to determine this relationship because of concerns that ^{99m}Tc-HMPAO may underestimate rCBF in high rCBF regions (*3,4*). The purpose of this study was to investigate the effects of visual stimulation, a stimulus known to increase rCBF in the striate or visual cortex, on the relative distribution of ^{99m}Tc-HMPAO in this area.

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MATERIALS AND METHODS

Subjects

The subjects employed in this study were seven healthy volunteers (see Table 1). No evidence of neurologic, psychiatric, or other illness was obtained by history, physical examination, and routine hematologic, serum chemistry, and urine clinical laboratory testing. All subjects gave written informed consent to participate in the study.

Acquisition

Subjects each underwent single-photon emission computed tomographic (SPECT) brain imaging examination on two separate days one week apart. On each day, subjects received an injection of approximately 740 MBq ^{99m}Tc-HMPAO per antecubital vein. Images were acquired using a Strichman 810X Brain Imager (Medfield, MA) (*5,6*), using a 576 hole medium-energy collimator. Each study consisted of 16 transaxial slices, spaced 6 mm apart with acquisition time of 2.5 min per slice (total acquisition time 40 min). The head was positioned using a laser beam so that slices were parallel to the orbitocanthal-meatal line. To facilitate repositioning and to reduce head movement, each subject wore an individualized molded plastic face mask (Precision Splint, Polymed Co., Baltimore, MD) and rested his head in an individually molded vacuum bean bag (Olympic Vac-Pac, Seattle, WA). Images were reconstructed using a two-dimensional iterative deconvolution algorithm (Strichman Medical Equipment program version 2.31, Medfield, MA). Data were normalized to a 740-MBq injection dose. Actual doses varied from 681 to 773 MBq. Data were also normalized to a 100% lipophilicity of the injectate. Actual lipophilicity varied from 79% to 100%, (see Table 1) as measured by paper chromatography within 5 min prior to injection.

Stimulus Parameters

Each subject was studied on one day under visual stimulation conditions and on the other day under visual deprivation conditions. The sequence of stimulation and deprivation was balanced. The visual stimulation condition consisted of stroboscopic white light (Grass PS22) with an intensity of 1.5×10^6 candlepower, a frequency of 10 Hz, and a flash duration of 10 msec. The subjects were asked to look directly at the light, which was held approximately 20 cm from their eyes. During visual deprivation, eyes were patched. Subjects wore a sound-attenuating headset and laid motionless for both conditions. The door to the imaging room was closed and conversation

TABLE 1
Effects of Visual Stimulation on ^{99m}Tc-HMPAO Distribution

Patient no.	Age/Sex	Visual stim.	Injectate lipophilicity	Counts per pixel*			ROI/Brain Ratio (R)				Percent increase			
							Uncorrected		Lassen-corrected‡		Uncorrected		Lassen-corrected	
				T-P† cortex	Striate cortex	Whole brain	T-P cortex	Striate cortex	T-P cortex	Striate cortex	T-P cortex	Striate cortex	T-P cortex	Striate cortex
1	23/M	S	88%	320	397	286	1.12	1.39	1.19	1.72	8.6%	17.0%	13.8%	31.6%
		D	84%	309	356	300	1.03	1.19	1.05	1.31				
2	26/M	S	91%	287	340	234	1.23	1.45	1.38	1.88	−0.2%	17.2%	−0.3%	33.4%
		D	87%	446	450	363	1.23	1.24	1.39	1.41				
3	27/M	S	99%	323	470	296	1.09	1.59	1.14	2.25	0.7%	16.8%	1.0%	35.8%
		D	92%	296	371	273	1.08	1.36	1.13	1.66				
4	25/M	S	100%	206	268	187	1.10	1.43	1.16	1.83	0.8%	24.3%	1.2%	46.4%
		D	90%	399	421	365	1.09	1.15	1.15	1.25				
5	62/M	S	92%	445	538	358	1.24	1.50	1.41	2.01	5.5%	19.2%	9.4%	38.4%
		D	91%	397	425	337	1.18	1.26	1.29	1.45				
6	25/F	S	95%	492	580	436	1.13	1.33	1.21	1.59	3.3%	16.0%	5.4%	28.7%
		D	100%	416	437	381	1.09	1.15	1.14	1.24				
7	19/F	S	100%	447	519	348	1.28	1.49	1.50	1.98	10.3%	22.3%	18.0%	44.3%
		D	79%	297	311	255	1.16	1.22	1.27	1.37				
mean											4.1%	18.8%§	6.8%	36.7%¶
s.d.											4.1%	3.1%	7.0%	6.6%

* Normalized for injectate lipophilicity and dose (see text).
† T-P, left temporoparietal cortex.
‡ Corrected using the Lassen flow-dependent backdiffusion algorithm (see text).
§ Paired $t = 8.70$, $p < 0.001$, striate vs. T-P cortex.
¶ Paired $t = 8.99$, $p < 0.001$, striate vs. T-P cortex.

and extraneous noise were minimized during tracer uptake on both days. Room light and temperature were not specifically controlled or measured but appeared to be very similar on the two study days. Stimulation or deprivation were both begun 1 min before and continued for 5 min after radiopharmaceutical injection.

Image Analysis

The image analysis method used was modeled after the method of Fox and Raichle (*7*) in order to provide a measure of comparability with their PET rCBF study of visual stimulation. In that study, the first step was to perform a direct pixel-for-pixel subtraction of deprivation from stimulation studies. Next, a 13.5 × 27 mm region of interest (ROI) was placed circumscribing the single pixel with the maximum percent change. This pixel was in the area of the striate or visual cortex in all cases.

In the current study, we were unable to perform direct pixel-for-pixel subtractions because we studied subjects on two separate days and thus had to reposition them. The following alternate method that would best approximate that of Fox and Raichle was used. Sample data illustrating the method for one subject are shown in Table 2. Five successive slices near and slightly above the level of the basal ganglia were identified as containing an area corresponding to the visual cortex by comparison to a brain atlas (*8*). The five slices were chosen from each study day by an operator initially blind to the identity of the day of photic stimulation. In order to delineate the area of peak activation, the operator then used threshold commands to identify the pixel with the highest count density in each of the five slices obtained from the visual stimulation study day. This pixel was near the midline in the posterior cortical area in all cases. Next, a rectangular 13 × 27 mm ROI was placed around this pixel in each of the five slices. The operator then placed an identical ROI in the analogous area in each of the five slices in the visual deprivation study. This allowed calculation of the mean counts per pixel in five comparable ROI pairs from the visual stimulation and deprivation days in each subject. The five transaxial slices used for evaluation of stimulation or deprivation conditions for a typical subject are shown in Figure 1. The location of the visual cortex ROI is shown in Figure 2.

In order to calculate the mean counts per pixel for the whole brain, each of the sixteen slices was then outlined with the aid of the brain atlas. If a Z-axis mispositioning error appeared to have occurred between the two test days, a slice from the top of one study and the bottom of the other were omitted from the whole brain total. This was done to minimize discrepancies in whole brain counts due to inclusion of a higher proportion of cerebellum in one of the two studies. The whole brain count density was calculated by summing the counts in each slice and the number of pixels in each slice and dividing the summed counts by the summed pixels.

The ROI/whole brain ratio (R) was calculated for each of the five slices in each study day. From the five pairs of anatomically comparable stimulation and deprivation ROIs, one pair was chosen that maximized the $R_{stimulation} - R_{deprivation}$ difference. This difference was taken to approximate the peak rCBF increase due to visual stimulation. Finally, the peak change in rCBF was defined as:

$$(R_{stimulation} - R_{deprivation})/R_{deprivation} \times 100\%.$$

As an internal control, a second ROI was placed using an identical method in the left superior temporal-inferior parietal cortex (Fig. 2).

TABLE 2
Method of Peak Change Calculation: Sample Data (Subject 3)

Stimulation Study			Deprivation Study				
Slice	Count density	ROI/WB Ratio (R_{STIM})	Slice	Count density	ROI/WB Ratio (R_{DEP})	R_{STIM}-R_{DEP} Difference	% Change
Visual Cortex Region							
9	420	1.42	9	339	1.24	0.18	14.5%
10	446	1.51	10	369	1.35	0.16	11.8%
11	470	1.59	11	371	1.36	0.23	**16.8%**
12	451	1.52	12	387	1.42	0.10	7.0%
13	419	1.42	13	345	1.26	0.16	12.7%
Temporoparietal Region							
9	278	0.94	9	289	1.06	−0.12	−11.3%
10	302	1.02	10	291	1.07	−0.05	−5.7%
11	323	1.09	11	296	1.08	0.01	**0.7%**
12	310	1.05	12	298	1.09	−0.04	−3.7%
13	296	1.00	13	302	1.11	0.11	−9.9%
Whole Brain							
WB	296	—	WB	273	—	—	—

ROI = region of interest; WB = whole brain; peak changes are in bold face.

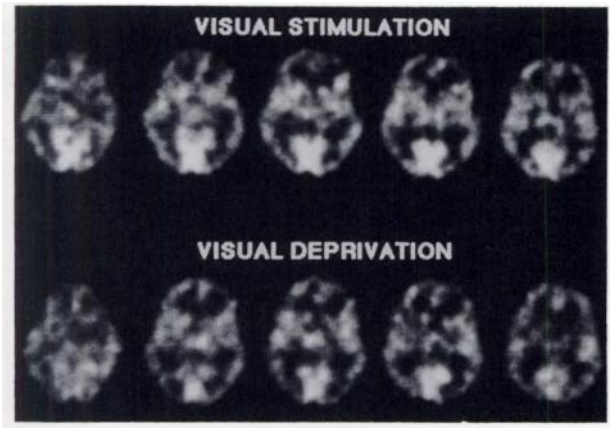

FIGURE 1
Stacks of five slices containing area of striate cortex in a typical subject (Subject 3 in Table 1). Top: visual stimulation study. Bottom: visual deprivation.

Lastly, the R values were connected for flow-dependent backdiffusion using the Lassen algorithm (*9,10*):

$$\text{corrected } R = \alpha R/(1+ \alpha - R)$$

where α=2. This algorithm has been validated versus PET (*10*).

RESULTS

The data for each subject are shown in the Table 1. The mean peak increase in rCBF was 36.7% in the Lassen-connected data (18.8% uncorrected). Every subject showed an increase, the variability across subjects was relatively small, and the difference between stimulation and deprivation was highly significant (paired t=26.7, p<0.001 uncorrected data). By contrast, the mean peak increase in rCBF in the control region was much smaller (6.8%, 4.1% uncorrected) and only just reached statistical significance (paired t=2.8, p<0.05, uncorrected). The increase in the striate region was significantly higher than the increase in the temporoparietal region (Table 1).

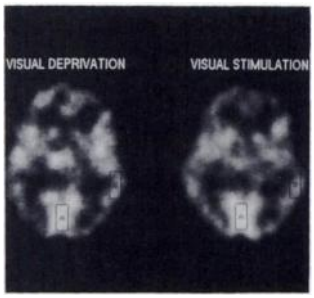

FIGURE 2
Regions of interest as placed in third slice shown in the same study as Figure 1. Region A: striate cortex region. Region B: left superior temporal/inferior parietal region.

DISCUSSION

The results suggest that the distribution of ^{99m}Tc-HMPAO is able to detect rCBF increases resulting from this physiologic stimulus. Thus, ^{99m}Tc-HMPAO may be a suitable radiopharmaceutical for further SPECT investigation of neuroanatomical correlates of rCBF changes related to other physiologic stimuli.

An important methodologic issue is involved in the method of calculating the peak change. It could be argued that the difference between the peak number in a random distribution of difference scores will tend to be positive and thus that we have biased the study toward a finding that may not exist. It is difficult to know the best way to address this argument. One method would be to choose a control region as we have done. The problems with this course are that it is not certain that the control region is unaffected by visual stimulation or that the unstimulated day-to-day variability in the control region is similar to that in visual cortex. An alternative approach would be to conduct a test-retest study to evaluate the peak change in striate cortex ROIs when no stimulus was given either day. The problem with this approach is that it would use different subjects whose visual cortex response to visual stimulation is unknown.

Using the present method, we did observe fairly large peak changes in the temporoparietal control region in two subjects. In Subject 1, the 13.4% corrected peak change was due to an isolated "cold spot" in one slice in the visual deprivation study (r=1.05). In Subject 7, there was an isolated "hot spot" (r=1.50) in a single slice in the stimulation study. These observations suggest that there is indeed some "noise" in the peak change statistic. The contribution of such noise to PET reports of peak changes in blood flow or glucose metabolism have not, to our knowledge, been reported. It is possible tht these two fairly large changes in the temporoparietal region could relate to subtle uncontrolled differences in the imaging environment. This discussion notwithstanding, the small percent peak increase in the control area relative to the striate cortex region in the current study suggests that the contribution of noise in the peak change statistic to our visual stimulation finding is small.

The percent increase in rCBF in visual cortex reported must be considered together with discussion of the stimulus parameters chosen. Our intent was to use a strong but simple stimulus, so as to maximize our chances of observing a robust effect consistently across individuals. The intensity of the light was perceived as bright enough to be slightly to moderately uncomfortable. The stimulus frequency, 10 Hz, is within the range reported to produce the maximal visual cortex evoked responses in humans (*11,12*). Visual checkerboard stimulation or complex visual stimulation (*13*) might have produced greater responses.

Direct comparisons of the present data to PET studies of visual stimulation are somewhat problematic due to differences in stimulus parameters and image acquisition and analysis techniques. For example, Fox and Raichle (*7*), using the oxygen-15-water PET rCBF technique, have reported a 31.5% ± 6.2% increase in visual cortex rCBF. While an identical sized ROI was used, Fox and Raichle's method of identifying the peak increase would tend to produce slightly higher percent increases than ours. The visual stimulus parameters were different also, in that Fox and Raichle used patterned stimulation at 7.8 Hz. Phelps et al. (*13*), using the fluorine-18-2-fluoro-2-deoxyglucose technique, have reported an approximately 38% increase in a region approximately half as large as ours in the primary visual cortex using an alternating checkboard pattern at 2 Hz.

The changes in whole brain activity between visual stimulation and deprivation days are difficult to account. Whole brain count density was fairly similar between test days in most subjects but was 50% higher on the visual stimulation day in one subject (#7). Another subject (#4) showed the opposite pattern. Presumably, these effects were unrelated to the visual stimulus. They do not appear to be due to differences in injection dose, injectate lipophilicity, or head positioning. They may possibly relate to hyperventilation, changes in radiopharmaceutical metabolism, or infiltration of the injection dose.

The ratio for the temporoparietal cortex was lower than for striate cortex in six of seven subjects during visual deprivation. Presumably, this is explained by partial volume effect, since there are two cortical thicknesses closely apposed in the striate region and only one in the temporoparietal region.

Concerns have been raised about a propensity for the nonrectilinear collimation in the Strichman 810X to result in significant inclusion within a slice of counts originating outside the slice ("cross-plane cross-talk"). If the reconstruction algorithm failed to adequately correct for this phenomenon, the increase in the striate cortex blood flow could also cause an apparent increase in flow in brain slices which did not include visual cortex. This situation would artifactually decrease the percent increase in the striate cortex/whole brain ratio related to visual stimulation. In order to evaluate this possibility, we constructed two phantoms each with a volume of 1630 cc and each containing a 6-cm diameter 128-cc compartment modeling the visual cortex. Each phantom was filled so that the visual cortex/whole phantom ratio (R) for known activity was 1.228 in the visual deprivation phantom and 1.434 in the visual stimulation phantom (16.8% "uncorrected" "increase"). SPECT images were acquired using the same protocol described for the subjects. Measured Rs were 1.250 and 1.465 (17.2% "increase"), respectively. These data suggest that the focussing collimation of the camera we employed did not distort the ROI/whole brain ratios in this study.

CONCLUSION

Overall, the data from this study permit the conclusion that a SPECT rCBF technique using ^{99m}Tc-HMPAO as the rCBF radiopharmaceutical was able to detect expected rCBF increases in visual cortex with visual stimulation. The percent increase observed in the backdiffusion-corrected data is very similar to that in previous PET rCBF and glucose metabolism reports. The results suggest that it should be possible to use SPECT and ^{99m}Tc-HMPAO in studies mapping the rCBF effects of other physiologic stimuli. In order to perform more quantitative studies of more subtle stimuli with SPECT in the future, methodologic advances will be required. It is important that algorithms correcting for flow-dependent backdiffusion causing underestimation of higher regional flows be thoroughly validated and generally accepted, as the percent increase observed without backdiffusion correction is half that previously reported with other methods. In addition, a more precise and verifiable method for head repositioning must also be developed so that direct pixel-for-pixel subtraction of stimulated and basal states can be employed.

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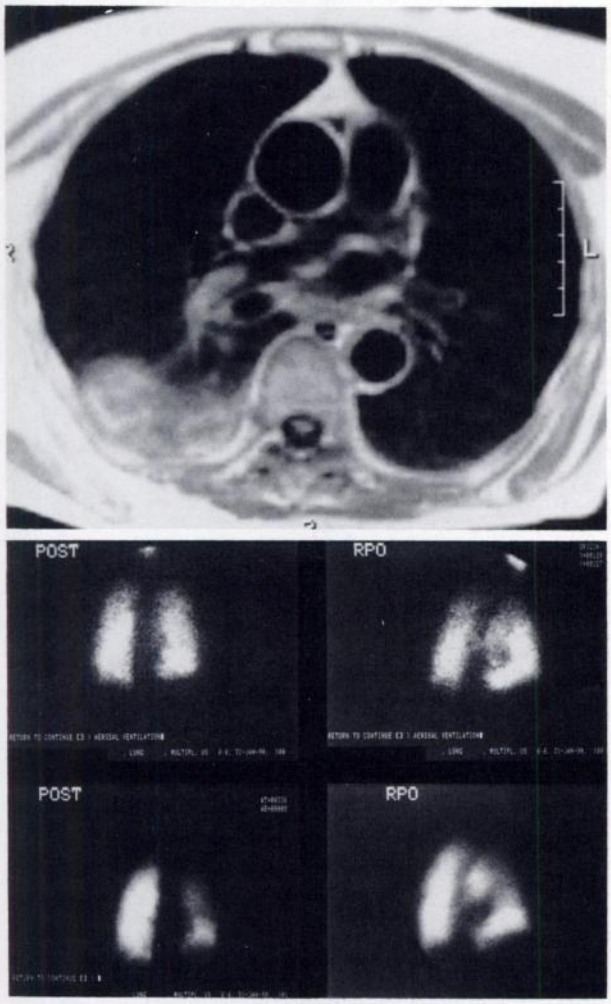

FIRST IMPRESSIONS

PURPOSE:
A 69-yr-old female presented with acute onset of right infrascapular chest pain and hemoptysis. She had fractured her left tibia in a motor vehicle accident and the cast had been removed one day prior to her admission to this hospital. The ventilation and perfusion studies showed one matched defect in the right lower lobe compatible with indeterminate probability for pulmonary embolism. The pulmonary angiogram demonstrated a large saddle embolus at the bifurcation of the right pulmonary artery. MRI of the chest was consistent with thrombus in the right pulmonary artery and consolidation in the posterior aspect of the right lower lobe.

TRACER:
^{99m}Tc-DTPA aerosol and ^{99m}Tc-MAA

ROUTE OF ADMINISTRATION:
Inhalation and intravenous injection

TIME AFTER INJECTION:
Immediate

INSTRUMENTATION:
General Electric Gamma Camera

CONTRIBUTORS:
B. Chandramouly, J. Lee, and W.M. Rumancik

INSTITUTION:
Long Island College Hospital, Brooklyn, NY