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Midazolam Changes Cerebral Blood Flow in Discrete Brain Regions

An $H_2{}^{15}O$ Positron Emission Tomography Study

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Background: **Changes in regional cerebral blood flow (rCBF) determined with $H_2{}^{15}O$ positron emission tomographic imaging can identify neural circuits affected by centrally acting drugs.**

Methods: **Fourteen volunteers received one of two midazolam infusions adjusted according to electroencephalographic response. Low or high midazolam effects were identified using *post-hoc* spectral analysis of the electroencephalographic response obtained during positron emission tomographic imaging based on the absence or presence of 14-Hz spindle activity. The absolute change in global CBF was calculated, and relative changes in rCBF were determined using statistical parametric mapping with localization to standard stereotactic coordinates.**

Results: **The low-effect group received 7.5 ± 1.7 mg midazolam (serum concentrations, 74 ± 24 ng/ml), and the high-effect group received 9.7 ± 1.3 mg midazolam (serum concentrations, 129 ± 48 ng/ml). Midazolam decreased global CBF by 12% from 39.2 ± 4.1 to 34.4 ± 6.1 ml · 100 g^{-1} · min^{-1} ($P < 0.01$ at a partial pressure of carbon dioxide of 40 mmHg). The rCBF changes in the low-effect group were a subset of the high-effect group. Decreased rCBF ($P < 0.001$) occurred in the insula, the cingulate gyrus, multiple areas in the prefrontal cortex, the thalamus, and parietal and temporal association areas. Asymmetric changes occurred, particularly in the low-effect group, and were more significant in the left frontal cortex and thalamus and the right insula. Relative rCBF was increased in the occipital areas.**

Conclusion: **Midazolam causes dose-related changes in rCBF in brain regions associated with the normal functioning of arousal, attention, and memory. (Key words: Benzodiazepines: midazolam. Cerebral blood flow. Electroencephalography. Radionuclide imaging: $H_2{}^{15}O$. Sedation. Sleep: drug effects. Tomography: emission computed.)**

MIDAZOLAM is used most commonly in the clinical setting to produce temporary drug-induced amnesia. Although serum concentrations required for amnesia have been determined,[1] and it is clear that midazolam, like other benzodiazepines, has an agonist action at the gamma-aminobutyric acid (GABA-A) receptor complex, it is not known how the drug acts on the central nervous system to produce specific effects such as sedation and amnesia. If specific neural structures are affected by midazolam and these could be identified, then a better understanding of the mechanisms of sedation and amnesia might be possible. Neural structures affected by drug administration can be identified using positron emission tomography (PET) using ^{15}oxygen (called "O15-PET").[2-6] If neural structures affected by midazolam correspond to neural networks previously shown to be important in memory, attention, and sedation, then it would be reasonable to assume that the clinical effects of midazolam are expressions of midazolam's effects on these specific structures. This hypothesis

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could be tested with other drugs that have similar clinical properties.

Benzodiazepines have been shown to decrease cerebral blood flow (CBF) globally,[7-11] with some studies demonstrating regional changes in flow.[7] Many of these studies used techniques that yielded minimal data on regional effects. These localized effects may be important in understanding the mechanism of action of drugs in the brain.

In this study, O15-PET was used to identify changes in regional CBF (rCBF) after normalization for global CBF changes during midazolam sedation. We hypothesized that midazolam would not only decrease global CBF but also result in dose-related changes in rCBF. To minimize interindividual variability in pharmacokinetics, volunteers received an infusion of midazolam adjusted to produce one of two clearly distinguishable electroencephalographic (EEG) effects associated with greater and lesser degrees of sedation.

Materials and Methods

This investigation was approved by the local Institutional Review Board and Radiation Safety Committee before accrual of subjects took place.

Participants

Fourteen healthy men (ages 21-44 yr; mean ± SD: 28.1 ± 5.8 yr) participated in this study after giving informed consent. All but one were right-handed, and none had any history or physical evidence of neurologic or psychiatric illness. All volunteers participated in a familiarization session before the study day in which all tasks and procedures were practiced, including placing the volunteers in the PET scanner for 45-60 min. On the day of the study, two intravenous catheters were placed, one for D5$^1/_2$NS infusion at 150 ml/h and midazolam administration, and the other for $H_2{}^{15}O$ administration. A radial arterial catheter was placed in the opposite extremity (left hand) for blood sampling.

Study Design

All the volunteers were scanned in three conditions on a single day: control (scans 1, 3, and 5), an attention task (scans 2, 4, and 6), and during midazolam infusion (scans 7, 8, and 9). The results of the attention task have been reported separately.[12] This report concerns data collected from scans 1, 3, and 5 and 7, 8, and 9. After scan 6, midazolam was infused using a computer-assisted continuous infusion device (CACI)§ during simultaneous EEG monitoring.

EEG Recording

EEG recordings were obtained simultaneously with each PET scan. Nineteen channels of EEG were recorded from the standard International 10-20 System montage referenced to linked mastoids at a sampling rate of 100 Hz and bandwidth of 0.3-40 Hz, using the NeuroScan programmable SynAmps and software (Neuro Scan, Herndon, VA). The vertical and horizontal electro-oculogram and chin electromyogram were recorded for later use in visual editing for artifact rejection. Spectral analysis was performed using epochs of 10.24 seconds, a fast Fourier transform, and a Hann window for its anti-aliasing effect. Broad-band beta power was calculated in the bandwidth of 12-20 Hz. Within this range, spectral peaks were identified and their signal-to-noise ratios were calculated. The signal-to-noise ratio is defined as the ratio of the area of the spectral peak itself to the area of "pedestal" under the peak.[14] Two-dimensional brain maps (fig. 1) of the topographical distribution of beta power over the head were constructed by plotting power in the beta band at each measurement electrode and interpolating for points between electrodes.

Drug Infusion to EEG Effect

Volunteers were randomly assigned to receive midazolam infusion by computer-assisted continuous infusion to produce either a low or high midazolam effect on the EEG. Initial effect site target concentrations were 70 ng/ml for the low-effect group and 100 ng/ml for the high-effect group. The low effect resulted in high-frequency, low-amplitude EEG activity in the beta frequency range (13-20 Hz), whereas the high effect was obtained when "sleep" spindles at approximately 14 Hz were present. If spindles were not present after 5 min, when predicted equilibration between serum and effect site concentrations is 80-90% complete, the initial target concentration was increased by 10 ng/ml increments until this EEG response was evident. To confirm the visual inspection of the analog EEG recording, a 5-min sample of EEG was analyzed using power spectral analysis to confirm that a spectral peak centered at approximately 14 Hz was present (fig. 1C). After predicted equilibration between serum and effect site concentra-

§ Glass PSA and Jacobs J, Duke University Medical Center, Durham, NC. See Veselis *et al.*[13] for details on the use of this device.

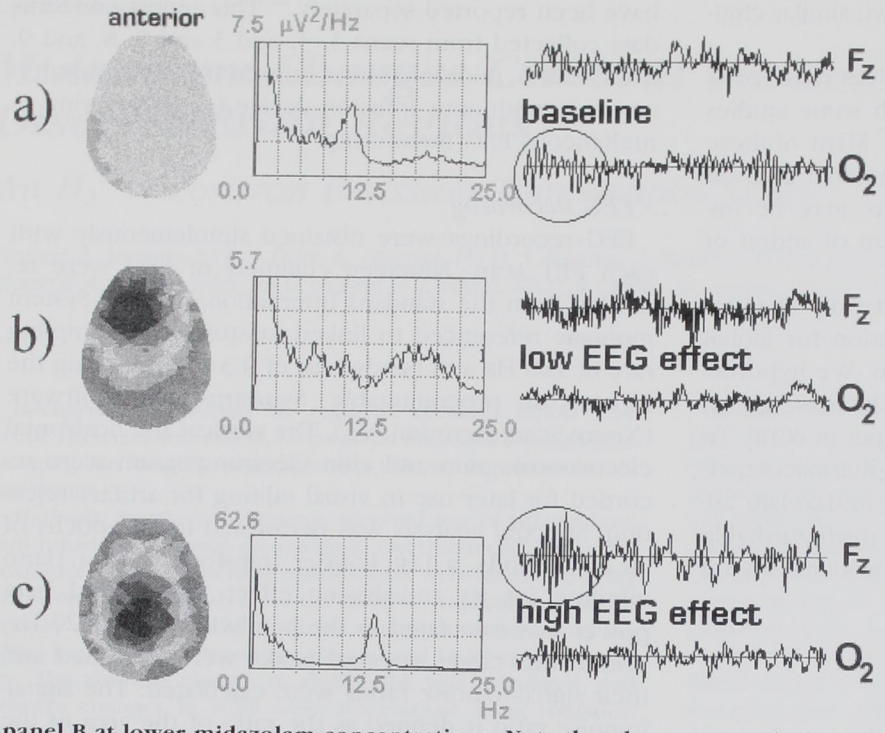

Fig. 1. Representative electroencephalographic (EEG) recordings from the participants in different conditions. From left to right: brain map of the distribution of beta power (12–20 Hz) over the head (anterior is at the top), power spectrum of EEG, and analog EEG recording from two electrodes. (*A*) The baseline condition, in which little beta power is present. The predominant EEG rhythm is a posterior alpha rhythm (circled) at approximately 10 Hz. (*B*) The low-effect group (LOW; midazolam concentration approximately 75 ng/ml), which demonstrates a broad-band increase in beta power but no identifiable rhythmical activity. The analog EEG shows diffuse frontal high-frequency EEG activity, with the distribution of the beta power being anterior. The alpha rhythm present at baseline is largely absent. (*C*) The high-effect group (HIGH; midazolam concentration approximately 130 ng/ml). The presence of rhythmic 14 Hz "spindles" (circled), which are apparent as a peak in the power spectrum. This "spindle" activity has a more central distribution than the increase in beta power seen in panel B at lower midazolam concentrations. Note that the power scale (y axis) in this figure is different, indicating that this rhythmic activity is of very high power compared with baseline alpha power.

tions at the desired target concentration, 15 more minutes were allowed to elapse before any additional scans were performed. Arterial blood samples were obtained immediately after each scan for arterial blood gas and midazolam determinations, and separate values were averaged for each participant. Midazolam concentrations were determined by high-performance liquid chromatography.[15]

Neuropsychological State during Positron Emission Tomographic Scanning

Every attempt was made to make the neuropsychological state similar between control and midazolam conditions. To complete the study in a single day, the midazolam condition always followed the control condition. In both control and midazolam conditions, participants listened to binaural auditory tones at 1000 Hz, 80 dB, with an interstimulus interval of 1.1 second, presented beginning 30 seconds before isotope injection and continuing throughout the scanning procedure. Presentation of auditory tones was done so that the control condition could be used in comparisons with both the auditory attention task[12] and midazolam infusion conditions. During control and midazolam conditions, participants were instructed to pay no attention to the tones ("just relax and let the tones just float by"). Participants received auditory stimulation through a pair of miniature earphones inserted into their ears. The volunteers were blindfolded, and their heads were held in a constant position throughout the study using a thermosetting face mask. A condom catheter was placed so the participants would not need to leave the scanner to urinate during the lengthy scanning procedure. During scanning, the room was dimly lit and background noise was minimal.

Study Groups

Because the EEG response *during* PET scanning could be different than the EEG effect seen just before PET scanning, the participants were classified into low- and high-effect groups based solely on the EEGs obtained during PET scanning. After accrual of all participants, the EEG spectra obtained during scanning were reviewed for determination of study groups for data analysis. Three experienced encephalographers (R.V., R.R., V.F.) examined EEG spectra from four derivations across the scalp (F_z, C_z, P_z, and O_2). Because three scans were obtained in each condition, a total of 12 power

Table 1. EEG Parameters for Low and High Effect Groups

	Total β Power (μV^2; 12–20 Hz)		Signal–Noise Ratio (SNR) of Spectral Peak at 13–15 Hz†	
	Low Effect	High Effect	Low Effect	High Effect
Baseline condition	64.3 ± 32.1	78.3 ± 37.3	NA	NA
Midazolam condition	166.5 ± 95.0*	325.7 ± 286.1*	0.69 ± 0.16	2.51 ± 0.80‡

NA = not applicable.

* $P < 0.05$ *versus* baseline (paired *t* test).

† The size of the spectral peak (area under the curve) is directly related to the SNR.

‡ $P < 0.05$ between low and high effect (Mann-Whitney U test).

spectra in both the baseline and midazolam conditions were examined for each participant (four electrodes × three scans). Spectra were classified without knowledge of which participant or study group they were associated with. Participants with a clearly visible peak in the power spectrum centered at 14 Hz during the drug condition (fig. 1C) were included in the high midazolam effect group. Generally a spectral peak is not clearly visible until the signal-to-noise ratio of the peak is greater than 1.0. Participants without a clearly visible spectral peak were assigned to the low midazolam effect group (fig. 1B). After this classification, the high- and low-effect groups consisted of seven participants each (table 1). This classification was congruent with the initial randomization to low- or high-effect groups, except for three participants. Two had been randomized to the high-effect group, but the spectral peak signal-to-noise ratio was less than one. One had been randomized to the low-effect group, but power spectral analysis revealed a spectral peak with a signal-to-noise ratio of approximately 2.

Memory Test

Participants were asked to memorize a list of 16 words presented auditorily (a modified Rey Auditory-Verbal Learning Task). Two tests were administered: "baseline," which occurred between scans 2 and 3 before midazolam infusion; and "drug," which occurred between scans 7 and 8 during midazolam infusion. The degree of memory loss was determined by the number of presented words recognized at the end of the study day. We must emphasize that this word memory test was administered between PET scans. There was no memory or attention task administered during PET scans.

Positron Emission Tomographic Scanning

For each scan, 20 mCi $H_2{}^{15}O$ in 10 ml were infused during 20 seconds into the antecubital vein using a constant-rate infusion pump. The interval between successive $H_2{}^{15}O$ administrations was approximately 15 min to allow for isotope washout between scans. Three scans were obtained in each study condition to increase the activation signal relative to background noise in the images.

Positron emission tomographic scans were obtained with a PC4600 NeuroPet scanner (Cyclotron Corp., Berkeley, CA; no longer in business), with a 10 cm axial field of view and a 1.2 cm in-plane resolution. Participants were positioned so that the center of the field of view was approximately 3 cm above the orbitomeatal line. As a result, some of the superior cortex was not in the field of view, because the scanner could only image up to 8 cm above the orbitomeatal line. To construct an approximation of the arterial input function to the brain, arterial blood was sampled through a sodium iodide well counter at 5 ml/min for 4 min starting 30 seconds before $H_2{}^{15}O$ administration and then discarded. A single 10-min transmission scan using a rotating rod of $^{68}Ge/^{68}Ga$ was performed before scanning commenced to correct for attenuation of signal in its passage through bone and cerebral tissue.

Data Analysis

Univariate Statistical Analysis. Comparisons between low- and high-effect groups for demographic variables, midazolam dosage, memory scores, and EEG parameters were performed using *t* tests or the Mann Whitney U test if the data were not normally distributed (SAS version 6.01; SAS Institute, Cary, NC). Unless otherwise stated, all results are presented as means ± SD.

Cerebral Blood Flow. Absolute global flow values were computed from the arterial input function and the activity counts of the PET scanner ("quantitative image analysis"). Relative changes in rCBF between baseline and midazolam infusion were evaluated using SPM95 software‖ that normalizes for differences in global flow between comparison images ("qualitative image analysis"). SPM95 allows localization of group changes in rCBF to specific cerebral locations and also provides information on percentage change of relative rCBF between conditions at a normalized mean global CBF (semiquantitative analysis).

Quantitative Image Analysis. Values derived for absolute CBF are specific for each participant based on their particular anatomy and therefore are unsuitable for intersubject comparisons. However, global CBF values can be used for paired comparisons of the sedated to the baseline condition in each participant.[16] The slope of the partial pressure of carbon dioxide (P_{CO_2}) *versus* CBF data was determined for each participant and then averaged to obtain a correction factor for changes in P_{CO_2}, which was 0.83 $ml \cdot min^{-1} \cdot 100\ g^{-1} \cdot mmHg^{-1}$. This correction factor was applied to each participant's quantitative CBF data using analysis of covariance.

Qualitative Image Analysis. Image analysis was performed on a Hewlett-Packard (Palo Alto, CA) Apollo model 735 workstation using SPM95 implemented in PRO MATLAB (Mathworks, Natick, MA). Images were realigned to the first scan and transformed into the standard stereotactic space used in the human brain atlas of Talairach and Tournoux.[17] As a final preprocessing step, the images were smoothed using an isotropic Gaussian kernel (15-mm FWHM) to accommodate normal variability in functional and gyral anatomy for group analysis. The three scans obtained for a particular volunteer in each condition (*i.e.*, baseline, midazolam) were identified as replicated measurements for that volunteer in the SPM95 analysis.

After specifying the appropriate design matrix for each group separately (multiple subject with two conditions and three replications), the condition and participant effects were estimated according to the general linear model at each and every voxel. A subject-specific analysis of covariance model was used that accounts for the fact that the relation between global CBF and rCBF can be different among individuals. Mean global CBF was normalized by SPM95 to 50 $ml \cdot 100\ g^{-1} \cdot min^{-1}$. This analysis generated a mean pixel value with associated error variance for each condition. Differences in the adjusted condition means were assessed for the group as a whole using the t and Z statistics. Pixel-by-pixel comparison between conditions was then made by SPM95, and mean pixel values of control and experimental condition scans were compared for significant differences by *t* test.

Comparisons were performed between control and low-effect conditions and between control and high-effect conditions. Foci of significant decreases or increases of activity were identified and expressed as Z scores. SPM images were compared for significant change within each group, with a threshold Z value of >3.09, which corresponds to $P < 0.001$. Significant maxima were mapped using the Talairach atlas to determine the anatomic location and corresponding Brodmann's area, where applicable. Another criterion was also applied to assess the significance of identified regions. This is the probability that an effect of significance $Z > 3.09$, occurring by chance alone in the area of the size of the volume analyzed, would be less than $P = 0.05$ (P(Zmax > u) in the SPM analysis).

Foci of significant rCBF changes were considered to be present in both the low- and high-effect groups if the coordinates of foci occurred within 15 mm of each other in a three-dimensional space in both comparisons (control–low effect and control–high effect). This value of 15 mm was chosen based on the PET camera resolution and image smoothing. Foci were considered to be significant effects only if they met the P(Zmax > u) criterion in the high-effect group. The same focus in the low-effect group, although meeting the criterion of $Z > 3.09$, may not necessarily meet the P(Zmax > u) criterion (indicating a small area of rCBF change). However, because these same foci appeared in the high-effect group, these foci were considered to be significant effects in the low-effect group. The regional corrected blood flows (adjusted so that mean global CBF was 50 $ml \cdot 100\ g^{-1} \cdot min^{-1}$) were determined at each of these foci and the percentage change compared with baseline was calculated.

Results

Study Groups

No differences in demographics were observed between the high and low EEG effect groups (for all parti-

‖ Available from the Wellcome Department of Cognitive Neurology and MRC Cyclotron Unit, London, UK. SPM96 is now available at the following internet site: http://www/fil.ion.bpmf.ac.uk/spm/spm96.html

Table 2. Relative rCBF Decreases Related to Midazolam Effect

Control–Low Effect Comparison				Control–High Effect Comparison					
Significance by Z Score	x (mm)	y (mm)	z (mm)	Significance by Z Score	x (mm)	y (mm)	z (mm)	BA	Location
4.91*	46	10	4	4.89*	44	12	0		Right insula
4.43*	−10	34	24	4.13*	−2	42	20	9–32 s	Left medial frontal gyrus
									Left anterior cingulate gyrus
4.19*	−20	50	24	5.14*	−22	56	12	10	Left superior frontal gyrus
4.01*	−36	40	24	6.08*	−42	40	12	46	Left middle frontal gyrus
3.94*	20	28	−16	5.15*	24	28	−16	11–47 s	Right inferior/middle frontal gyrus
3.75	54	−28	0	4.77*	62	−32	−4	21	Right middle temporal gyrus
3.34	26	56	4	4.64*	16	58	4	10	Right superior frontal gyrus
3.26	−4	−20	0	4.6*	−4	−16	4		Left thalamus, dorsomedial n.
3.23	−30	6	−4	3.88*	−30	6	0		Left claustrum
3.19	−56	−38	20	5.08*	−54	−46	24	40 s	Left inferior parietal lobule
3.11	−26	26	−12	5.14*	−18	28	−16	11–47 s	Left inferior/middle frontal gyrus

Z score = the magnitude of statistical significance: a Z score of ≥3.09 corresponds to $P < 0.001$; BA = Brodmann's areas (if focus is in a border between two areas, both are indicated); "s" = focus was deep in a sulcus.

x, y, z coordinates are given according to the atlas of Talairach and Tournoux[17] for significant maxima that are within 15 mm of each other in both the low and high effect groups.

* The probability of an area this size or larger occurring by chance alone in the volume of pixels analyzed is <0.05.

cipants combined: weight, 79.5 ± 9.7 kg; body mass index, 26 ± 3.4; weight above ideal, 10.3 ± 14.6%; sleep before the study day, 5.9 ± 1 h). Significant differences were present in the total dose of midazolam administered (7.5 ± 1.7 mg *vs.* 9.7 ± 1.3 mg; $P = 0.02$ by *t* test) and average serum concentrations during scanning (74 ± 24 ng/ml *vs.* 129 ± 48 ng/ml; $P = 0.02$ by *t* test). There were no significant hemodynamic changes. No quantitative data regarding degree of sedation were obtained. However, review of the taped notes of one author's (R.V.) observations of the participants' state indicated that those in the low-effect group were sedated to varying degrees. Participants in this group were noted to be "not sleepy at all," "readily responsive to verbal command," "able to complete the word test without difficulty," and "a little woozy." One participant was "almost asleep . . . requiring repeated verbal stimulation to respond." Participants in the high-effect group were heavily sedated and usually required physical stimulation to respond. Some volunteers in this group were noted to be "snoring" and "not responding during word the test." EEG analysis confirmed the expected effects (table 1). Participants in both groups suffered memory loss during midazolam administration. From a 16-word list administered during midazolam infusion, the number of words recognized at the end of the study day were 5 ± 4.5 ($P < 0.002$) and 1.6 ± 1.7 ($P < 0.001$) words for the low- and high-effect groups, respectively (compared with baseline scores by paired *t* test). The significance of the difference between recognition memory scores between high- and low-effect groups during midazolam sedation was $P = 0.10$ by *t* test.

Quantitative Blood Flow Analysis

The arterial P_{CO_2} increased after drug administration in both the low- and high-effect groups, the increase being 3.5 ± 3.1 mmHg ($P = 0.04$) in the low-effect and 5.1 ± 4.1 mmHg ($P = 0.03$) in the high-effect group. After correction for these P_{CO_2} changes, there is approximately a 12% decrease in global CBF from 39.2 ± 4.1 $ml \cdot 100\ g^{-1} \cdot min^{-1}$ in the baseline condition to 34.4 ± 6.1 $ml \cdot 100\ g^{-1} \cdot min^{-1}$ in the midazolam condition ($P < 0.02$ by *t* test; P_{CO_2} normalized to 40 mmHg).

Qualitative Image Analysis

For relative decreases in rCBF, 11 significant foci were found in both the low- and high-effect groups (table 2; figs. 2A, 2B). Similarly for relative increases in rCBF, five foci were present in both the low- and high-effect groups (table 3; figs. 2C, 2D). In general, the significance of the change in rCBF at each focus was larger for the high-effect group than for the low-effect group. Figure 3 shows the percentage changes of the semiquantitative blood flows derived from SPM analysis between baseline and low-effect or high-effect groups.

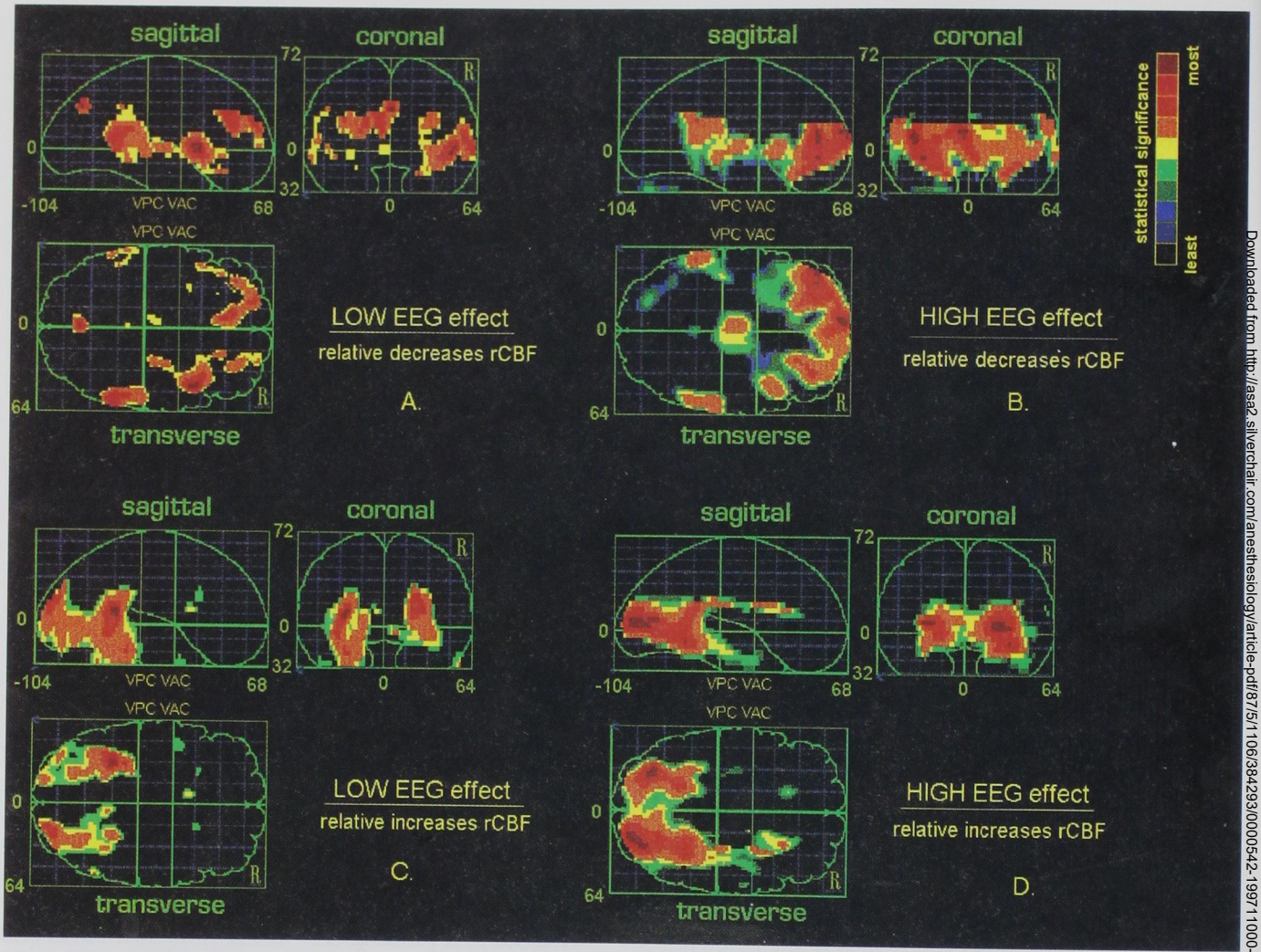

Fig. 2. SPM projection images indicating regions of statistically significant changes in relative regional cerebral blood flow (rCBF). These images represent changes in the entire volume of imaged brain collapsed onto either the sagittal, coronal, or transverse planes. The field of view did not include the topmost portion of cortex. The changes seen in the low-effect group (*A* and *C*) are a subset of the changes in the high-effect group (*B* and *D*). Midazolam significantly decreases rCBF in the right insula, bilateral prefrontal cortices, thalamus, and temporal auditory association cortices. The relative increases probably represent areas that are less affected by midazolam and occur in the occipital areas. VAC, vertical anterior commissure line; VPC, vertical posterior commissure line. Numeric labels indicate distance in millimeters. From the respective reference lines: sagittal view: VAC, anterior commissure–posterior commissure (AC-PC) line, labeled as O; coronal view: midline, AC-PC line; transverse view: midline, VAC.

Discussion

The results of this study indicate that midazolam sedation not only causes global decreases in CBF but also produces significant regional changes that likely represent the neural structures affected by midazolam. These structures represent neural networks involved in arousal and memory.[18–23] A large decrease of rCBF occurred in the dorsomedial nucleus of the thalamus, a critical location for maintaining vigilance and effortful information processing.[23,24] Regional CBF also decreased in the superior, middle, and medial frontal gyri (BA 10, 46, 9), which are areas of "working" memory and executive control functions and receive input from the dorsomedial thalamic nucleus.[19,22,23,25–29] The cingulate gyrus (BA 32), which is activated in tasks involving anticipation or attention to competing inputs[30] was also affected by midazolam. Midazolam impairs memory by interfering with acquisition or encoding of material and by decreasing the capacity of "working" memory.[31,32]

Table 3. Relative rCBF Increases Related to Midazolam Effect

Control–Low Effect Comparison				Control–High Effect Comparison					
Significance by Z Score	x (mm)	y (mm)	z (mm)	Significance by Z Score	x (mm)	y (mm)	z (mm)	BA	Location
4.85*	22	−92	16	5.72*	20	−90	4	18	Right middle occipital gyrus
4.13*	26	−44	16	5.08*	28	−48	12		Right tapetum
3.99*	28	−68	−4	5.62*	28	−80	4	19–18	Right lingual gyrus
3.90*	36	−50	−8	5.54*	30	−52	−8	19	Right fusiform gyrus
3.82*	−32	−84	16	5.91*	−28	−74	8	19	Left middle occipital gyrus

See Table 2 footnotes.

The left prefrontal cortex is preferentially activated during the encoding of information, and the right prefrontal cortex is activated during retrieval of information,[20,21,33,34] a process that is relatively unimpaired by benzodiazepines.[31] The findings of this study correspond to this functional localization, with the low-effect group having relative decreases in rCBF in the left, but not right, prefrontal cortex.

If we assume that at these doses benzodiazepines do not uncouple CBF and metabolism, then we can also compare our results with studies of cerebral metabolism. Of ten previous studies examining the regional effects of benzodiazepines, four found significant changes in regional blood flow or cerebral metabolic rate for glucose,[7,35–38] although two of these report on the same group of patients (table 4).[36,37] Regional CBF changes in the 1985 study of Mathew et al.[7] correspond closely to those seen in our low-effect group. A later study of Mathew and Wilson[8] did not find any regional changes, although significance was borderline ($P < 0.06$), and the CBF measurements were done when diazepam concentrations were predicted to be one third of those in the 1985 study. Using lorazepam, Volkow et al.[35] found a 23% decrease in glucose metabolism in the thalamus, the largest change of any region. In the current study, the largest percentage of rCBF decrease also occurred in the thalamus, with a relative rCBF change of 9%. As the global CBF decreased 12%, the absolute

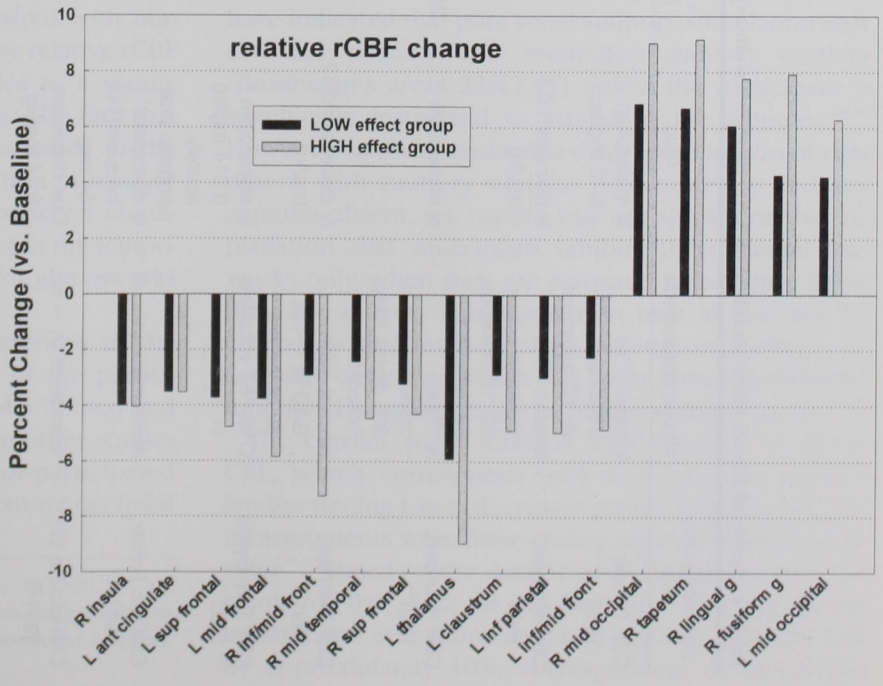

Fig. 3. Percentage change in relative regional cerebral blood flow (rCBF) at specific locations compared with baseline condition, after normalization of mean CBFs between participants and conditions. The location labels are in order from left to right to correspond with their locations in tables 2 and 3 from top to bottom (highest to lowest statistical significance in the low-effect group; L, left; R, right; g, gyrus. Because global CBF decreased 12% between baseline and drug conditions, the relative increases (group of bars on the right side of the graph) probably represent areas of brain that experienced significantly smaller decreases in rCBF than mean CBF changes, rather than representing true increases in rCBF. Note that the largest relative decreases in rCBF occurred in the thalamus and bilaterally in several regions in the prefrontal cortex. Many of these areas are involved with the maintenance of arousal, attention, and normal memory function.

Table 4. Cerebral Blood Flow/Metabolism Effects of Benzodiazepines

CBF/CMR Author, Year	Method and Subjects	Drug	= [MDZ]* (ng/ml)	Behavioral/EEG/Task	Global Decrease (%)	Regional Decreases
CBF Forster, 1982[9]	Inhaled ^{133}Xe Volunteers (n = 8), 16 detectors	iv midazolam 0.15 mg/kg	300	Lose consciousness for 10 min	34	None found
CBF Mathew, 1985[7]	Inhaled ^{133}Xe Volunteers (n = 20; 10 placebo); 32 detectors	iv diazepam 0.1 mg/kg	110	Awake, eyes open; EEG "no indication of drowsiness/sedation"	8–10	R > L frontal, temporal, inferior parietal, occipital
CBF Knudsen, 1990[44]	iv ^{133}Xe; craniotomy (n = 30); global Kety-Schmidt	iv midazolam	300/600	Fully anesthesized: fentanyl, N_2O, pancuronium	No change	Only global effects measured
CBF Mathew, 1991[8]	Inhaled ^{133}Xe GAD (n = 15); 32 detectors	iv diazepam 0.12 mg/kg	40	None sleepy during CBF measurement	3–11	None found (P = 0.06)
CBF Roy-Byrne, 1993[11]	O15 PET volunteer (n = 8)	iv alprazolam 0.014 mg/kg acutely, chronic oral	50	50% loss of word memory, visual continuous performance task	7–40 acute; disappears with chronic	None found
CMRglc Foster, 1987[43]	Alzheimer's (n = 5)	iv diazepam infusion (14–54 mg)		EEG stage 2 sleep; no blink response; arousable to tactile stimulation	30 (highly variable; NS)	None found
CMRglc De Wit, 1991[38]	Volunteers (n = 8)	Oral diazepam 0.14 mg/kg	15	No different from placebo (decreased anxiety); visual monitoring task	11	None found
CMRglc Buchsbaum, 1987;[36] Wu, 1991[37]	GAD (n = 18)	Chronic oral clorazepate		Decreased Hamilton Anxiety Score; visual vigilance task	19	R > L frontal, occipital
CMRglc Volkow, 1995[35]	Volunteers (n = 21)	iv lorazepam 30 μg/kg	90	Tired and sleepy; told to keep eyes open	13	Thalamus (23%), occipital cortex
CBF Veselis, 1997 (this study)	O15 PET Volunteers (n = 14)	iv midazolam infusion	70/130	EEG ± 14 Hz spindles; awake to asleep; auditory stimulation	12	L prefrontal cortex, L thalamus, R insula

GAD = generalized anxiety disorder; CMRglc = cerebral metabolic rate for glucose determined using PET and ^{18}FDG; NS = not significant.

* Equivalent serum concentration for midazolam: when possible STANPUMP was used to predict effect site concentration; equivalent potencies were obtained from references 48–52.

rCBF decrease should be approximately 20%, which corresponds well with Volkow and colleagues' data.[35]

The fact that other studies found no regional effects, whereas we did, may be explained by several factors. These include the insensitivity of previous methods to detect rCBF changes and failure to correct for global CBF changes with appropriate normalization procedures. For instance, using O15-PET, Roy-Byrne et al.[11] did not find any regional CBF effects of alprazolam, but they used a region-of-interest analysis, which examines relatively large areas of the brain as a unit, and subtle regional effects may not be detectable. Interindividual variability may be important in obscuring rCBF effects, because those studies that detected regional differences generally had many participants (10 to 21) compared with those that found no regional effects (5 to 8 participants). A major difference between our study and the others reviewed is that the drug infusion method we used resulted in constant serum concentrations of drug during PET imaging. Rather than using a fixed dose of drug, we administered midazolam to a defined electrophysiologic effect. Although serum concentrations of drug may have been variable among participants, the state of the brain may have been consistent.

Studies finding regional changes in cerebral metabolic rate for glucose or CBF after a benzodiazepine also found decreases in the occipital regions.[7,35-37] However, the present study found relative rCBF increases in the occipital areas. Based on the 12% decrease in global CBF, and the 9% relative increase compared with normalized baseline rCBFs, we interpret these relative rCBF increases as areas where rCBF decreases to a significantly lesser extent than global changes. The fact that these areas are represented as rCBF increases in the analysis is a by-product of the normalization procedure that equates mean global blood flows between conditions. Volkow et al.[35] found a similar effect in the temporal lobes when cerebral metabolic rate for glucose was normalized.

Another possible reason for the differences in the occipital lobe findings is the condition of the participants. In our study, participants were blindfolded and received auditory stimulation, whereas in other studies participants either kept their eyes open or participated in a visual vigilance task, which would activate occipital cortex.[7,35-37] If a drug effect is more evident in stimulated areas of the brain, then regions that are activated in both the control and drug condition may demonstrate changes. This can account for the decreases in occipital regions in studies in which visual stimuli were used and the decreases in the temporal lobe region in the present study in which auditory stimuli were used. Thus the baseline state of the participant may be an important confounding factor when auditory or visual stimulation is present in both the control and drug conditions.

The method of analysis that we used here is similar to a "subtractive" comparison technique (actually a *t* test comparison of voxel means and variances between two conditions). Thus in theory any rCBF changes due to stimulation should be present to the same extent in both conditions and result in no significant differences seen between conditions. However, if the reaction of the participant to the stimulus, as represented by a rCBF effect, is altered in any way, such as by a drug effect, this will be apparent in the comparison image. Although participants were instructed not to pay attention to the tones presented, they may have paid even less attention during the drug condition than during the baseline condition. Although an attentional task was not formally performed, the auditory stimulation may have activated some degree of automatic attentional processing in the control condition, resulting in an apparent decrease during drug administration. This is an unlikely explanation of all of the results found, as previous PET studies have indicated that pure tonal auditory stimulation only activates primary and secondary auditory cortices (Brodmann's areas 22,42,41) unless the participant is specifically instructed to attend to the stimulus.[39,40] However, in the absence of a direct comparison of conditions with auditory stimulus present *versus* auditory stimulus absent, we cannot rule out the alternative explanation that midazolam inhibits these neural networks only when they are activated rather than when they are at rest. This distinction may in fact not be crucial, as this method of examining drug effects on activated neural networks has been recommended for functional imaging studies of pharmacologic agents.[41,42]

The current study found a 12% decrease in global CBF, which corresponds well with previous studies. Studies finding larger decreases probably did so because measurements were done during high# drug concentrations.[9,43] Based on the data presented in table 4 and the results of this study, it can be stated that during the initial stages of sedation, benzodiazepines decrease CBF by approximately 10%, corresponding to midazolam

Using pharmacokinetic modeling software, STANPUMP (S. Shafer, Stanford University, Palo Alto, CA, available through the internet at http://pkpd.icon.palo-alto.med.va.gov) predicted as effect-site concentration of 300 ng/ml.

concentrations of approximately 50–100 ng/ml. Higher doses, causing loss of consciousness, decrease global CBF by approximately 30% and correspond to serum concentrations of midazolam of approximately 300 ng/ml. Based on Knudsen *et al.'s* data,[44] there seems to be a ceiling effect, with no further decrease in global CBF occurring at higher concentrations.

The absolute values we obtained for resting global blood flow are somewhat low when compared with traditionally accepted resting CBF values in the literature, but agree well with other resting global CBF values obtained using O15-PET.[11,16,45] Low global CBF values probably result from the presence of nonperfused cerebral structures in the PET images in the middle third of the brain, which represented a large portion of the data in our study. The correction factor from our P_{CO_2} *versus* CBF data of 0.83 $ml \cdot min^{-1} \cdot 100\ g^{-1} \cdot mmHg^{-1}$ translates to 2.3% mmHg at a global mean CBF of 35 $ml \cdot 100\ g^{-1} \cdot min^{-1}$ and corresponds well with data from previous literature.[46,47] The increase in P_{CO_2} of 5 mmHg in our high-effect group corresponds closely to the increase in P_{CO_2} found by Forster et al.[9]

In conclusion, the findings of our study show that midazolam is affecting distinct neural pathways relevant to arousal, memory, and attention. The ability to demonstrate regional effects of benzodiazepines may be closely related to specific methodologic considerations, particularly those that decrease interindividual variability. Regional CBF changes may be most evident at lower, sedative concentrations of benzodiazepines that produce specific cognitive effects such as amnesia and drowsiness. In addition, regional CBF changes associated with drug effects may be more evident when the affected neural networks are in an activated state. The methods described here show great potential in delineating the mechanisms of action of various drugs that affect human memory and attention processes.

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