Cerebral Glucose Metabolism during Pharmacologic Studies: Test-Retest under Placebo Conditions

Mark E. Schmidt, Monique Ernst, John A. Matochik, Jose Ma. Maisog, Bai-Shen Pan, Alan J. Zametkin and William Z. Potter

Section on Clinical Pharmacology, Experimental Therapeutics Branch, Section on Clinical Brain Imaging, Laboratory of Cerebral Metabolism and Section on Functional Brain Imaging, Laboratory of Psychology and Psychopathology, National Institute of Mental Health, Bethesda, Maryland

The reliability of serial [^{18}F]fluorodeoxyglucose (FDG) PET scans for psychopharmacologic studies was tested by using placebo infusions. **Methods:** FDG scans were obtained before and after a 30 min placebo infusion (n = 10; Group 1) or after each of two bolus infusions with placebo (n = 8; Group 2). Subjects performed a continuous performance task (CPT) during each scan. Cardiovascular measures and ratings of anxiety were obtained in all subjects. Samples for determination of plasma norepinephrine (NE) were taken at multiple time points in Group 1. **Results:** A slight increase in apparent global metabolism occurred between scans in both Groups 1 and 2. A few regions significantly increased in both groups. While an apparent increase in sympathetic activity occurred during the placebo infusion, neither NE levels, anxiety ratings nor cardiovascular measures correlated with global or regional FDG uptake. **Conclusion:** Test-retest differences of global and regional glucose metabolism were highly consistent across two experimental designs. While increases in cerebral glucose metabolism appeared to occur during the second scan, differences between scans were small. This method may offer advantages for selected psychopharmacologic studies.

Key Words: validation; placebo; FDG-PET

J Nucl Med 1996; 37:1142–1149

PET imaging of the brain using glucose metabolic tracers provides a potentially powerful and direct method for examining effects of pharmacologic compounds on neuronal activity. Reliable measurement of these effects, however, is uncertain because of the variability of brain activity both within and between subjects as well as error due to technical factors (*1,2*).

An important determinant of variability in brain activity is the behavioral state of the subject (*3*). Variation in general attributes of behavior such as arousal, habituation, and anxiety have been reported to diffusely influence measurements of brain blood flow and metabolism, presumably through widely distributed effects on cerebral activity (*3–7*). Given the nature of these attributes, it is not surprising that global cerebral glucose metabolic rate (CMRglu) has been found to account for most of the intersubject and intrasubject variance in FDG-PET studies. Indeed, normalization of regional activity to global CMRglu, as is commonly employed, reduces interscan variance. When specific regional changes in brain activity are anticipated, global differences assumed to be due to differences in behavior state in fact may be considered noise (*2,8,9*).

In psychopharmacological studies, however, this "noise" may represent targets of drug action. Many compounds are of interest because of their effects on anxiety and arousal. Such compounds may do so by affecting systems with distribution throughout the brain, so transformation of data by "correcting" for the global CMRglu factors out a potential effect signal.

Serial FDG scans have been advanced as a method for minimizing potential differences in behavioral state between measurements of cerebral metabolism (*10,11*). Consecutive measurements of glucose metabolism with FDG are made by subtracting the residual activity from the first scan from that of the second scan. Methodologic variance from sources such as head repositioning during repeated PET scans and processing of arterial samples is also likely to be reduced.

This method has previously been validated under resting conditions and with cognitive tasks (*10,11*). Subjects' expectations regarding the effects of drugs may result in additional behavioral variability during psychopharmacologic studies. To determine the reliability of this method for psychopharmacologic studies, global and regional estimates of CMRglu from serial FDG scans obtained before and after placebo infusions were compared.

MATERIALS AND METHODS

Subjects

Subjects were entered after a diagnostic interview and clinical screening. Exclusion criteria included any metabolic, neurologic or psychiatric disorders except as described for Group 2 subjects. All subjects provided written consent after an explanation of the purpose and risks, in accordance with standards established by the National Institutes of Health Radiation Safety Committee and the National Institute of Mental Health Institutional Review Board.

Group 1. Baseline versus Placebo Infusion. Ten healthy volunteers (5 men, 5 women; age 22–52 yr, mean age 29.5 yr) were studied using a method utilized in an investigation of idazoxan, an alpha-2 antagonist (*12*). All subjects were blind to compound identity. Four subjects had previously undergone the same PET protocol during which they had received the active drug. Three additional subjects were familiar with PET scan procedures by having undergone PET studies under other protocols.

Group 2. Placebo Bolus versus Placebo Bolus. Eight subjects (5 men, 3 women; age 21–52 yr; mean age 36.1 yr) with attention deficit/hyperactivity disorder (DSM III-R) were studied after remaining medication-free for a minimum of 3 wk, using a protocol designed to examine the acute effects of dextroamphetamine (*13*). All of these subjects were naive to PET scan procedures and were blind to compound identity.

Imaging Methods

A 20-gauge catheter was inserted into the right radial artery under local anesthesia (1% lidocaine) for blood sampling and a 22-gauge catheter was inserted into a left brachial vein for infusion of placebo and tracer approximately 1 hr before the scan procedure. Physical activity was minimized in an effort to achieve a stable resting state prior to the scans. Subjects were fitted with a thermoplastic mask and the PET gantry was aligned with the

Received May 7, 1995; revision accepted Oct. 18, 1995.

For correspondence or reprints contact: Mark E. Schmidt, MD, Section on Clinical Pharmacology, National Institute of Mental Health, Room 2D46, Building 10, 9000 Rockville Pike, Bethesda, MD 20892.

canthomeatal (CM) line. All studies were performed with a seven-slice PET camera with an in-plane resolution of 5.2 mm FWHM, interslice interval of 13.5 mm and resolution in the z axis of 10–13 mm FWHM. Ten-minute emission acquisitions were obtained serially at the CM line, 4.5 mm and 9 mm above the CM line. Data were used for reconstruction of sets of seven image planes at each position. Estimates of CMRglu were calculated for each scan individually, based on its acquisition time with respect to injection. The image sets were later interleaved to create a single volume of 21 planes. Attenuation correction was calculated from transmission scans at each position using a rotating germanium (^{68}Ge) pin, obtained before the first emission scan and before and after the second emission scan (transmission plus emission).

Group 1. FDG (3 mCi) was injected over 1 min followed by a 30-min uptake period. Emission scans were then acquired. On completion of the scans, the subjects were infused with placebo (normal saline) over 30 min (200 cc/hr). On completing the infusion, a 5-mCi bolus of FDG was given, and a second set of emission scans were obtained 30–35 min later. To reduce variance in arousal subjects performed an auditory continuous performance task (CPT) during each tracer uptake period (*2,14*). This task has been described previously (*12*). The subjects' eyes were patched during the tracer uptake phase of each scan. Blood pressure and heart rates were measured via a pressure transducer on the arterial line and were recorded at regular intervals beginning with tracer injection for the first scan. Blood samples for plasma norepinephrine were collected every 10 min for 30 min prior to the placebo infusion and then for 1 hr after starting the infusion. Samples were collected into glass tubes with EDTA, kept on ice until centrifugation to separate the plasma and were stored at −70°C until assayed. At the end of the imaging session, the subjects rated their level of anxiety during the entire experiment using the Modified Spielberger State Anxiety Scale (*15*).

Group 2. Placebo (normal saline) was injected over 3 min before the 3- and 5-mCi FDG injections for the first and second scans, respectively. Before imaging, the subjects were trained to perform a visual CPT, which has been previously described (*13*). Subjects performed the visual CPT during each tracer uptake period. After each uptake period, subjects verbally completed the Modified Spielberger State Anxiety Scale; emission scans were then obtained. Heart rate and blood pressure were monitored throughout the study by a pressure transducer on the arterial line.

Image Analysis

Raw pixel values were converted to glucose metabolic rates in milligrams of glucose per 100 g of tissue per minute using a modification of the Sokoloff operational equation and a lumped constant of 0.418 (*16–18*). The second emission scan was corrected for residual activity from the first scan (*10*). Five transaxial planes were selected from each set of 21 planes to match a standard set previously selected as containing neuroanatomic areas of interest as identified by the atlas of Matsui and Hirano (*12,14,19*). Templates of rectangular regions of interest (ROIs) were then applied to the selected planes and adjusted for individual differences in neuroanatomy. ROIs were placed over the right and left mesial occipital cortex on the image plane immediately below the C plane in Group 1, to permit comparison with the previous active drug study (*12*). The interrater reliability of this analysis method was determined previously (*12*). The intraclass correlation coefficient (ICC) was 0.88 (*20*).

Global metabolism was defined as an average of the mean CMRglu from all cortical regions sampled across all available planes (A–E). Regional metabolism was normalized within subjects by dividing the absolute regional values by the global CMRglu. One subject in Group 2 lacked a suitable match for the A plane, so the average across the B through E planes was used in that group. Interscan percent difference in metabolic rate was calculated as:

$$100 \times (ROI_{test} - ROI_{retest})/[(ROI_{test} + ROI_{retest})/2].$$

Plasma aliquots were assayed for norepinephrine (NE) using high-performance liquid chromatography and electrochemical detection (*21*).

Statistical Analysis

Regional data were evaluated by paired Student t-tests with $p < 0.05$ set as the level for significance. All tests were two-tailed unless otherwise noted, uncorrected for multiple comparisons. Chi square distribution was used to test the probability of regions differing by chance, assuming an equal likelihood of an increase or decrease in metabolic rate. Two-way ANOVA was used to test previous PET experience as a factor affecting CMRglu (CMRglu as a repeated measure). Plasma NE levels were log transformed prior to analyses. NE levels and cardiovascular parameters were analyzed by repeated measures ANOVA. Mean baseline and postinfusion values were calculated from all cardiovascular and NE measurements collected prior to the placebo infusion and during the uptake phase for the second scan. Means and differences (scan 2 − scan 1) in metabolic rate were used to calculate Pearson's correlation coefficients with peripheral measures and with anxiety ratings. Correlations were calculated for only those image regions that significantly changed.

RESULTS

Table 1 lists the mean global and absolute regional metabolic values from both Groups. Table 2 lists the global and regional percent differences for absolute and normalized values from each group.

Global Metabolism

Group 1. The global metabolic rate was slightly higher during the second scan. Global CMRglu during the second scan was significantly correlated with the global metabolic rate of the first scan ($r = 0.77$, $p < 0.01$, $n = 10$).

Global metabolism during the first scan was significantly greater in subjects naive to PET scans compared to experienced subjects (mean 12.46 ± 0.74, $n = 3$, versus 10.30 ± 1.00, $n = 7$; $F = 10.96$, $p < 0.01$), however, there was no interaction between previous experience and change in global CMRglu ($F = 1.53$, $p > 0.25$).

Group 2. Global CMRglu was slightly higher during the second scan. There was a trend for a correlation between the global metabolic rate during the second scan with the global metabolic rate of the first scan ($r = 0.66$, $p < 0.10$, $n = 8$).

Regional Metabolism

Group 1. Regional absolute metabolic values either increased or showed no change (Tables 1, 2). Six regions were significantly higher in the second scan, more than expected by chance ($\chi^2 = 14.47$, $p < 0.001$, $df = 2$). In contrast, the differences in normalized regional values were bidirectional. Two normalized regions were significantly different of which one (the middle mesial cortex region, A plane) had significantly differed in absolute metabolic rate. This was no different than expected by chance ($\chi^2 = 0.41$, $df = 2$).

Significant interactions ($p < 0.05$) between test-retest differences and previous PET experience occurred in four regions, both absolute and normalized values, all in the D plane: anterior medial frontal, left posterior frontal, left middle temporal and left posterior temporal. Examination of the simple effects revealed the metabolic rate to be higher (absolute and relative) in naive subjects during the initial scan. In the second scan,

TABLE 1
Glucose Metabolic Rates during Test and Retest Scans

	Group 1		Group 2	
	Scan 1	Scan 2	Scan 1	Scan 2
Global metabolic rate				
	10.95 (1.37)	11.39 (1.13)	11.16 (1.16)	11.68 (1.25)
Region				
A plane				
Anterior medial frontal	11.26 (2.19)	11.73 (1.69)	11.70 (1.35)	11.42 (1.51)
Middle medial cortex	10.71 (1.22)	11.78 (1.61)*†	11.82 (1.61)	12.40 (2.02)
Posterior medial cortex	11.78 (1.01)	12.19 (1.72)	12.75 (1.87)	13.38 (2.29)
Left anterior frontal	11.58 (1.49)	12.00 (1.53)	12.00 (1.12)	11.67 (1.12)†
Right anterior frontal	11.84 (2.06)	12.11 (1.69)	11.61 (1.29)	11.49 (1.54)
Left posterior frontal	11.34 (1.51)	11.67 (1.43)	12.34 (1.07)	12.40 (1.03)
Right posterior frontal	11.85 (2.13)	12.30 (1.84)	12.01 (1.04)	12.19 (0.89)
Left parietal	10.59 (1.30)	11.17 (1.06)	11.37 (1.04)	11.55 (1.02)
Right parietal	11.48 (1.41)	11.42 (1.14)†	11.36 (1.20)	11.82 (0.97)
B plane				
Anterior medial frontal	10.83 (1.52)	11.15 (1.71)	10.84 (1.01)	11.22 (1.47)
Superior occipital	10.43 (1.29)	11.08 (1.36)	11.69 (2.19)	12.67 (1.34)
Left anterior frontal	11.54 (1.70)	12.00 (1.61)	11.56 (1.23)	12.05 (1.83)
Right anterior frontal	11.42 (2.20)	12.18 (1.68)	11.41 (0.93)	11.56 (1.44)
Left posterior frontal	11.61 (1.90)	12.65 (1.59)	12.27 (1.40)	12.63 (1.32)
Right posterior frontal	12.13 (2.58)	12.39 (2.21)	12.45 (1.73)	12.95 (1.82)
Left rolandic	9.97 (1.26)	10.24 (1.19)	10.44 (1.55)	11.26 (1.48)
Right rolandic	10.00 (1.37)	10.27 (1.55)	10.41 (1.62)	10.96 (1.56)
Left parietal	10.33 (1.26)	11.17 (1.20)*	11.22 (1.16)	11.89 (1.47)
Right parietal	10.72 (1.25)	10.99 (2.16)	11.09 (1.73)	11.44 (1.31)
Middle cingulate	10.25 (1.33)	10.99 (2.16)	10.44 (1.02)	11.76 (1.69)*†
C plane				
Anterior medial frontal	10.94 (1.96)	11.38 (1.30)	10.80 (1.34)	11.26 (1.53)
Occipital	10.96 (1.62)	11.35 (1.32)	12.68 (2.39)	13.95 (2.36)
Left anterior frontal	11.11 (1.53)	11.68 (1.38)	12.02 (1.09)	12.35 (1.68)
Right anterior frontal	11.76 (2.13)	12.03 (1.57)	11.91 (0.87)	12.13 (1.13)
Left posterior frontal	11.26 (1.21)	11.70 (1.42)	11.94 (1.27)	12.39 (1.51)
Right posterior frontal	12.15 (1.97)	12.69 (1.60)	12.17 (1.38)	12.54 (1.15)
Left sylvian	10.49 (1.98)	11.07 (1.52)	10.83 (1.55)	10.97 (1.76)†
Right sylvian	10.68 (1.51)	11.12 (1.15)	10.89 (1.36)	11.49 (1.48)
Left parietal	10.44 (1.67)	10.97 (1.60)	10.76 (1.03)	11.47 (1.52)
Right parietal	10.47 (1.45)	10.75 (1.03)	10.81 (1.49)	11.42 (1.33)
Left parietal/occipital	8.97 (1.04)	9.38 (0.82)*	9.56 (1.51)	10.02 (0.77)
Right parietal/occipital	9.39 (1.08)	10.00 (1.00)	9.84 (1.42)	10.61 (1.44)
C minus 1 plane				
Left primary visual	10.60 (1.29)	10.68 (1.35)		
Right primary visual	10.60 (1.14)	11.55 (1.34)		

*$p \leq 0.05$, paired Student's t-tests, absolute rCMRglc.
†$p \leq 0.05$, paired Student's t-tests, normalized rCMRglc.
Mean (s.d.) metabolic rates in: mg glucose · 100 g tissue^{-1} · min^{-1}.

metabolism increased (absolute and relative) in these regions in experienced subjects and decreased in naive subjects.

Group 2. Three regions were significantly higher in the second scan (Tables 1, 2). This was no more than would be expected by chance ($\chi^2 = 3.0$, df = 2). After normalization, three regions were significantly different, of which the middle cingulate had also shown a significant increase in absolute metabolic rate. The number and direction of regional differences in normalized metabolism were also no different than expected on the basis of chance ($\chi^2 = 0.33$, df = 2).

Cardiovascular Response

There were no significant changes in blood pressure or heart rate in either group. No significant correlations between any of the cardiovascular parameters at the time of each injection of tracer and the subsequent measured global metabolic rate were present. Additionally, there were no significant changes in the global metabolic rate or correlations with any behavioral measure in either of the groups.

Norepinephrine Response

In two subjects, the assay variation exceeded 15%. Therefore, the data were analyzed from eight subjects in Group 1 (Fig. 1). Plasma NE concentrations during the placebo infusion were significantly larger than baseline values (main effect for time: $F = 2.72$, $p < 0.01$, df = 7, 9). While blood pressure did not significantly change for the group as a whole during the procedure, change in plasma NE was significantly correlated with change in mean arterial pressure ($r = 0.84$, $p < 0.01$ $n = 8$) and with change in diastolic blood pressure ($r = 0.88$, $p < 0.01$ $n = 8$) following the infusion. No measure of NE (baseline, postinfusion or change) significantly correlated with global or regional brain metabolism during either scan nor did the change in plasma NE correlate with global or regional

TABLE 1
Continued

	Group 1		Group 2	
	Scan 1	Scan 2	Scan 1	Scan 2
D plane				
Anterior medial frontal	10.89 (1.88)	11.47 (1.18)	10.55 (1.15)	11.23 (1.20)
Left anterior frontal	11.29 (1.79)	11.72 (1.24)	11.73 (0.83)	12.10 (1.31)
Right anterior frontal	11.98 (2.07)	12.26 (1.85)	12.09 (1.67)	12.17 (1.45)
Left posterior frontal	10.80 (1.69)	11.59 (1.57)*	11.17 (1.57)	12.07 (1.90)
Right posterior frontal	11.45 (1.88)	12.03 (1.59)	11.25 (1.55)	11.94 (1.86)
Left anterior temporal	10.65 (1.41)	11.15 (1.58)	10.76 (1.24)	11.05 (1.34)
Right anterior temporal	11.06 (1.43)	11.55 (1.50)	10.99 (1.42)	11.41 (1.47)
Left middle temporal	10.59 (1.51)	11.33 (1.12)*	11.22 (1.32)	11.84 (1.28)
Right middle temporal	10.85 (1.47)	11.25 (1.12)	11.18 (1.51)	11.97 (1.54)*
Left posterior temporal	9.72 (1.04)	10.40 (0.91)	10.49 (1.10)	11.13 (1.07)
Right posterior temporal	10.05 (1.32)	10.35 (1.31)	10.15 (1.28)	10.73 (0.92)
Left thalamus	10.20 (2.18)	10.90 (2.07)	11.72 (1.46)	12.40 (1.29)
Right thalamus	10.04 (1.87)	11.21 (2.45)*	10.78 (1.23)	12.65 (1.34)*
Basal ganglia				
Left caudate (head)	10.58 (1.79)	10.66 (1.86)	10.35 (1.07)	11.05 (2.02)
Right caudate (head)	10.48 (1.62)	10.69 (1.52)	10.09 (1.41)	11.03 (1.63)
Left anterior putamen	10.13 (1.51)	10.66 (1.86)	10.78 (2.01)	11.38 (2.67)
Right anterior putamen	11.08 (2.60)	10.98 (2.17)	10.87 (2.01)	11.36 (1.68)
Left posterior putamen	9.55 (1.68)	9.89 (1.56)	10.15 (1.35)	10.46 (1.46)
Right posterior putamen	9.74 (1.68)	9.83 (1.47)	10.05 (1.28)	10.84 (1.27)
E plane				
Anterior medial frontal	10.96 (1.73)	11.41 (1.56)	10.81 (1.63)	11.05 (1.26)
Left anterior frontal	11.52 (1.36)	11.56 (1.24)	11.47 (1.32)	11.97 (1.20)
Right anterior frontal	11.86 (1.88)	11.87 (1.60)	11.90 (1.21)	11.76 (1.42)
Left posterior frontal	10.87 (1.46)	11.20 (1.80)	10.98 (1.28)	11.75 (1.59)
Right posterior frontal	11.07 (2.08)	11.16 (1.69)	11.17 (1.24)	11.57 (1.55)
Left temporal	10.28 (0.96)	10.56 (1.31)	10.49 (1.37)	11.00 (1.51)
Right temporal	10.56 (1.42)	10.72 (1.52)	10.39 (1.85)	11.06 (1.50)
Left hippocampus	8.38 (0.81)	9.11 (1.05)	9.11 (1.08)	9.28 (1.16)
Right hippocampus	8.33 (0.92)	8.69 (1.06)	8.82 (1.14)	9.28 (0.60)

*$p \leq 0.05$, paired Student's t-tests, absolute rCMRglc.
†$p \leq 0.05$, paired Student's t-tests, normalized rCMRglc.
Mean (s.d.) metabolic rates in: mg glucose · 100 g tissue^{-1} · min^{-1}.

change in metabolism across scans, nor were there any significant correlations with CPT performance or self rated anxiety.

Behavioral Response

Group 1. Auditory CPT data were complete from both scans for nine subjects. There were no significant differences in performance between the two scans (scan 1: 68% ± 26% correct, scan 2: 72% ± 24% correct; mean ± s.d.). Performance during the first scan was highly correlated with performance on the second scan ($r = 0.95$, $p < 0.0001$) therefore the percent CPT correct scores were averaged as a single measure of attention over both scans. Average performance on the CPT showed a trend for a positive correlation with the increase in global metabolism between the two scans ($r = 0.63$, $p < 0.10$). Two of the six regions that significantly increased in absolute metabolic rate showed a significant correlation between the increase in regional metabolism from the first to the second scan and average CPT performance (A plane: anterior medial cortex: $r = 0.75$, $p < 0.05$; D plane: right posterior frontal: $r = 0.72$, $p < 0.05$,) and two of the six regions showed a trend for a positive correlation with average performance (A plane: right parietal: $r = 0.63$, $p < 0.10$; D plane: right thalamus: $r = 0.63$, $p < 0.10$). The mean state anxiety score and range from the Modified Spielberger State Anxiety Scale was 30.8 (23-to-47). The mean ± s.d. state anxiety score from a reference group of healthy young adults is 36.5 ± 10.2 (*15*). There were no significant correlations between the Spielberger rating and any regions that significantly differed or with global brain metabolism.

Group 2. Visual CPT data were complete for seven subjects. There were no significant differences in performance between the two scans (scan 1: 85% ± 17 correct, scan 2: 87% ± 15 correct; mean ± s.d.). Performance during the first scan was not correlated with performance on the second scan ($r = 0.37$, $p > 0.10$) therefore the performance scores were compared only to the global and regional metabolic rate during the time the CPT data were acquired. There were no significant correlations between CPT performance and concurrently measured global metabolism or regional metabolism in regions that significantly differed between the two scans. There were no differences in scores on the Spielberger rating during scan 1 (mean and range: 34.7, 22 to 41) versus scan 2 (mean and range: 35.0, 22 to 57). There were no significant correlations between the Spielberger rating and any cardiovascular parameter or global brain metabolism. The anxiety rating during scan 1 did correlate with performance on the CPT during scan 1 ($r = 0.84$, $p < 0.01$).

DISCUSSION

There have been a limited number of reports of test-retest variability of brain glucose metabolism. Several have been summarized in Table 3, along with the results from the present

TABLE 2
Percent Difference between Test and Retest Scans

	Group 1		Group 2	
	Absolute	Normalized	Absolute	Normalized
Global metabolic rate	4.2 (8.1)		4.5 (8.9)	
Region				
A plane				
Anterior medial frontal	4.7 (8.1)	0.4 (7.0)	−3.7 (11.2)	−7.4 (3.3)
Middle medial cortex	9.3 (11.9)	5.0 (6.8)	2.1 (11.8)	−1.6 (5.5)
Posterior medial cortex	2.8 (8.0)	−1.5 (8.8)	3.6 (11.9)	−0.1 (5.4)
Left anterior frontal	3.6 (10.6)	−0.6 (4.5)	−2.9 (8.4)	−6.7 (4.2)
Right anterior frontal	2.6 (9.2)	−1.6 (5.4)	−1.7 (11.1)	−5.4 (8.9)
Left posterior frontal	3.0 (13.1)	−1.2 (5.4)	0.3 (7.3)	−3.4 (5.5)
Right posterior frontal	4.2 (10.6)	0.0 (7.2)	1.4 (11.0)	−2.3 (6.5)
Left parietal	5.6 (10.6)	1.4 (6.6)	0.3 (10.2)	−3.4 (5.4)
Right parietal	0.2 (11.9)	−4.5 (5.8)	2.6 (7.9)	−1.1 (6.1)
B plane				
Anterior medial frontal	2.7 (10.5)	−1.5 (8.9)	3.0 (8.8)	0.6 (5.1)
Superior occipital	6.1 (12.0)	1.9 (10.8)	9.0 (14.7)	2.6 (6.8)
Left anterior frontal	4.1 (12.7)	0.1 (6.3)	3.5 (14.5)	−0.5 (9.6)
Right anterior frontal	7.2 (14.7)	3.0 (8.8)	0.8 (14.5)	−4.6 (7.0)
Left posterior frontal	9.0 (18.3)	4.8 (11.8)	2.9 (5.7)	−1.5 (9.2)
Right posterior frontal	2.6 (10.2)	−1.6 (6.4)	3.9 (13.2)	1.0 (7.2)
Left rolandic	2.8 (8.3)	−1.5 (8.0)	7.6 (17.6)	1.1 (11.3)
Right rolandic	2.5 (11.0)	−1.7 (7.5)	5.3 (14.0)	2.3 (7.6)
Left parietal	8.0 (10.5)	3.7 (5.9)	5.6 (13.6)	0.7 (7.0)
Right parietal	3.3 (9.6)	−0.9 (5.1)	3.6 (9.0)	0.7 (6.7)
Middle cingulate	5.9 (13.0)	1.7 (10.1)	11.3 (13.3)	7.1 (7.3)
C plane				
Anterior medial frontal	4.7 (10.2)	0.5 (6.0)	4.0 (10.6)	0.6 (5.1)
Occipital	3.8 (14.8)	−0.4 (12.7)	9.6 (14.3)	5.1 (10.0)
Left anterior frontal	5.2 (9.0)	0.9 (4.0)	2.2 (13.0)	−0.3 (6.8)
Right anterior frontal	2.9 (7.9)	−1.3 (4.9)	1.7 (10.5)	−0.5 (5.8)
Left posterior frontal	3.7 (10.6)	−0.6 (6.3)	3.5 (11.6)	−1.6 (7.6)
Right posterior frontal	4.7 (9.0)	0.5 (5.3)	3.2 (11.3)	−0.0 (6.9)
Left sylvian	6.0 (15.0)	1.8 (8.7)	1.1 (9.5)	−2.7 (4.2)
Right sylvian	4.4 (8.7)	0.2 (6.4)	5.2 (11.9)	2.2 (7.5)
Left parietal	5.2 (11.9)	0.9 (8.2)	6.0 (12.9)	1.6 (11.4)
Right parietal	3.1 (8.8)	−1.1 (5.3)	5.7 (11.0)	4.1 (4.6)
Left parietal/occipital	4.8 (4.9)	0.5 (5.7)	5.5 (14.0)	0.1 (12.7)
Right parietal/occipital	6.5 (12.2)	2.3 (12.7)	7.7 (11.4)	5.0 (7.9)
C minus 1 plane				
Left primary visual	0.70 (9.7)	−3.5 (9.3)		
Right primary visual	8.4 (12.6)	4.2 (6.5)		

Values are given as mean (s.d.).

study. While differences in the conditions, image resolution, data analysis and tracer used in those studies render direct comparisons difficult, some rough contrasts can be made.

The consistency in the test-retest differences in global CMRglu across the two groups in the present study and with a previous report on this method suggests that these data provide an accurate estimate of the variability of CMRglu measured by this method (*10*). The estimated ranges of mean global and regional CMRglu differences we observed are also similar to those calculated for other same day measurements of CMRglu (*11,24,26*). They were substantially less than the ranges calculated for almost all studies comparing CMRglu on different days (*22,23,25*), the exception being the study by Duara et al. (*2*) who used a visual preference task, specifically to reduce interscan differences.

The average global metabolic rate was higher during the second scan by nearly the same amount in both groups. Furthermore, mean CMRglu was modestly higher in every region in Group 1 and in all but four regions in Group 2 during the second scan, and more regions were significantly higher in Group 1 than were expected by chance. The potential sources of such an apparent systematic increase are of interest.

A source of variance specific to the paired FDG method is its dependence on k_4, the dephosphorylation rate constant, when correcting for residual activity from the first scan. Due to the slow rate of dephosphorylation, k_4 can be eliminated from the metabolic rate model in single injection studies without compromising accuracy (*27*). In sequential FDG studies, sufficient time elapses that measurable dephosphorylation can occur and result in clearance of tracer from the first injection during the second scan. If the mean k_4 values of both samples was substantially lower than the constant value used, then part of the apparent global increase during the second scan could be explained by an undercorrection for residual activity. The k_4 in gray matter calculated for a small sample of diabetic patients studied with the same camera used in the present study was

TABLE 2
Continued

	Group 1		Group 2	
	Absolute	Normalized	Absolute	Normalized
D plane				
Anterior medial frontal	6.0 (11.7)	1.8 (6.7)	6.3 (10.8)	3.8 (7.0)
Left anterior frontal	4.3 (14.1)	0.1 (8.8)	2.8 (14.1)	−1.6 (8.3)
Right anterior frontal	2.6 (10.4)	−1.6 (5.8)	0.8 (9.6)	−1.1 (5.4)
Left posterior frontal	7.2 (11.9)	3.0 (9.7)	7.4 (16.0)	2.4 (11.8)
Right posterior frontal	5.3 (7.1)	1.1 (7.0)	5.6 (10.7)	2.2 (5.4)
Left anterior temporal	4.4 (12.6)	0.2 (5.7)	2.6 (9.9)	−0.9 (6.1)
Right anterior temporal	4.3 (12.3)	0.1 (6.7)	3.7 (6.5)	−0.3 (7.5)
Left middle temporal	7.2 (10.3)	2.9 (5.8)	5.4 (11.7)	1.1 (4.6)
Right middle temporal	4.0 (8.3)	−0.2 (4.9)	6.9 (8.3)	5.2 (9.0)
Left posterior temporal	6.9 (10.3)	2.6 (4.9)	6.0 (9.5)	3.1 (7.9)
Right posterior temporal	3.1 (11.2)	−1.1 (10.3)	5.9 (11.1)	2.6 (6.8)
Left thalamus	7.1 (14.9)	2.9 (12.6)	5.8 (9.8)	1.7 (5.0)
Right thalamus	10.5 (13.1)	6.3 (10.2)	15.7 (18.1)	14.9 (17.2)
Basal ganglia				
Left caudate (head)	0.6 (16.0)	−3.5 (12.2)	8.7 (16.4)	5.6 (15.4)
Right caudate (head)	2.1 (15.7)	−2.1 (9.3)	6.0 (16.2)	3.8 (15.8)
Left anterior putamen	4.6 (14.2)	0.4 (15.9)	5.1 (22.9)	0.2 (25.9)
Right anterior putamen	0.3 (16.0)	−4.5 (13.9)	5.4 (22.8)	3.3 (22.8)
Left posterior putamen	3.8 (17.3)	−0.4 (10.8)	2.9 (13.2)	1.7 (11.6)
Right posterior putamen	1.2 (14.6)	−3.0 (10.7)	7.6 (13.7)	5.5 (7.3)
E plane				
Anterior medial frontal	4.2 (9.0)	0.0 (8.3)	2.7 (10.7)	−0.6 (5.4)
Left anterior frontal	0.5 (8.5)	−3.8 (5.6)	4.4 (7.4)	0.9 (5.2)
Right anterior frontal	0.3 (10.8)	−3.9 (6.8)	−1.5 (11.3)	−4.8 (8.3)
Left posterior frontal	2.6 (13.4)	−1.6 (9.2)	6.5 (12.9)	2.1 (6.5)
Right posterior frontal	1.3 (10.3)	−2.9 (6.5)	3.3 (11.5)	0.5 (5.2)
Left temporal	2.4 (10.8)	−1.8 (6.2)	4.5 (10.1)	−0.5 (5.0)
Right temporal	1.4 (9.7)	−2.8 (8.1)	7.0 (13.3)	4.2 (14.1)
Left hippocampus	8.1 (15.0)	4.8 (13.2)	1.8 (14.8)	1.9 (8.2)
Right hippocampus	4.1 (8.9)	−0.1 (7.9)	5.7 (9.3)	2.7 (8.4)

Values are given as mean (s.d.).

0.0064 ± 0.0011 (*29*), which is in close agreement with the k_4 value used for both of our samples (0.0068 ± 0.0014, from Phelps and others (*17,29*). Extrapolating from previous error analyses of this method, if 0.0064 was a better k_4 estimate for the subjects in the present study the error in the calculated retest CMRglu would be less than 1% (*10,28*). Ultimately, calculation of k_4 over the time period required for serial FDG-PET scans and with the dose ratio used in these studies would be necessary to accurately determine the variance contributed by the k_4 estimate to the second CMRglu measurement.

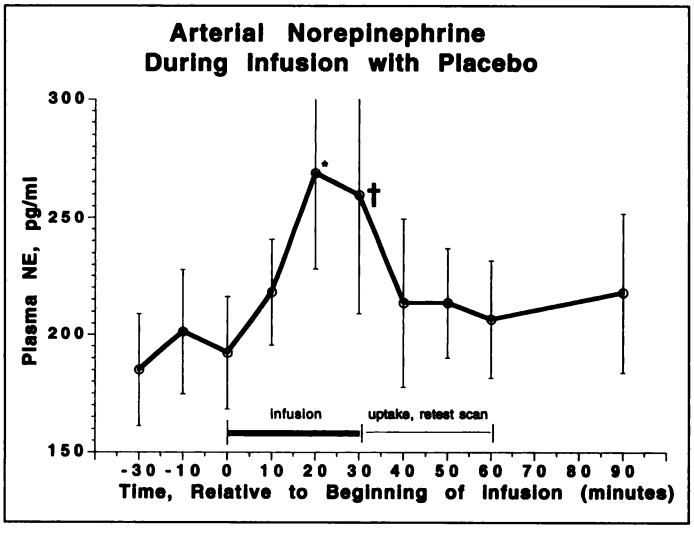

FIGURE 1. Group 1 mean plasma norepinephrine (NE) concentrations ± s.e.m. (n = 8). *Significant difference ($p < 0.05$) from pre-infusion levels. † Trend ($p < 0.10$) in a difference from pre-infusion levels.

Biological factors that have been hypothesized to influence variability of CMRglu include habituation, anxiety, diurnal variation and arousal. Of these factors, habituation has been suggested to account for a reduction in cortical activity observed between repeated blood flow measurements (*4*). Indeed, glucose metabolism was lower in subjects in Group 1 who had undergone previous PET procedures, and has been reported to be lower during repeat measurements in nearly all studies measuring CMRGlu on different days (*1,2,22,23,25*). In contrast, mean CMRglu has been reported to be higher during the second scan in two prior studies comparing same day measurements, in addition to the present study (*10,24*). While habituation must occur between same day measurements, it may not influence variability in brain glucose metabolism to the global extent suggested by studies on different days.

Significant regional interactions did occurr between test-retest differences and previous experience with PET procedures in Group 1. The interactions in each of the four regions was due to decreases in regional metabolism in naive subjects, while experienced subjects had increases. Decreases would be consistent with habituation, although the small number of naive subjects in Group 1 render any interpretation highly speculative. None of the four regions decreased in Group 2, all of whom

TABLE 3
Test-Retest Differences in Cerebral Glucose Metabolism

Study	No. of subjects	Scan interval	Condition	Mean difference (% ± s.d.)	95% Confidence interval
			Global CMRglu		
Gur et al. (22)†	8	7–23 wk	Resting	−1.0 ± 22.5	−54.2–52.2
Maquet et al. (23)	9	1–12 wk	Resting	−7.9 ± 15.5	−43.6–27.7
Duara et al. (2)†	9	1–6 wk	Resting	−24.4 ± 17.5	−64.8–15.9
	7	1–6 wk	VPT	−7.8 ± 5.1	−20.2–4.6
Bartlett et al. (24)*	12	3 hr ('am/pm')	Resting	7.0 ± 9.0	−5.8–19.8
	10	21 hr	Resting	0.6 ± 8.0	−17.5–18.7
Brooks et al. (10)	4	1–1.5 hr	Resting	2.3 ± 6.8	−19.4–23.6
This study (Group 1)	10	1.5 hr	Auditory CPT	4.3 ± 8.1	−14.1–22.6
This study (Group 2)	8	1.5 hr	Visual CPT	4.5 ± 8.9	−16.5–25.4
			Regional CMRglu		
Tyler et al. (25)	5	48 hr	Resting	−6.5 ± 4.4 (right)	−18.8–5.7
				−7.0 ± 3.0 (left)	−15.3–−1.3
Reivich et al. (26)*	8	2 hr	Resting	−0.8 ± 2.5	−6.7–5.0
Chang et al. (11)	5	1 hr	PPT	−0.7 ± 1.8	−5.6–4.3
	4	1 hr	WFT	−1.4 ± 1.8	−4.8–2.5
This study (Group 1)	10	1.5 hr	Auditory CPT	4.3 ± 2.4	−1.1–9.7
This study (Group 2)	8	1.5 hr	Visual CPT	4.3 ± 3.3	−3.5–12.1

*[^{11}C]deoxyglucose study.
†Normal control subjects only.
PPT = picture preference task; WFT = word fluency task. 95% confidence intervals were calculated from published data to facilitate comparison across different sample sizes.

were naive to PET. Nonetheless, the possibility of regional effects of previous PET experience on test-retest differences cannot be excluded.

Variation in anxiety level among normal subjects has been associated with increases, decreases, and no differences in CMRglu (*5,24,30,31*). We found no correlations between reported anxiety level, cardiovascular measures, or plasma NE levels with global or selected regional changes in CMRglu. If anxiety does influence CMRglu in normal subjects, then there are several possible reasons why we did not find a relationship. Anxiety ratings were obtained after completion of both (Group 1) or each (Group 2) scan. Transient anxiety related to the initiation of the scans may not have been reflected in our post scan ratings. Most of the subjects in Group 1 were very familiar with the procedures and only one of the subjects in Group 2 reported experiencing significant anxiety during the scan (2 s.d. above the mean rating for the age matched control group), so the range of anxiety experienced by subjects may not have been sufficient to demonstrate a relationship (*15*).

While subjects in Group 1 did not report significant anxiety, plasma NE modestly increased during the placebo infusion, which was consistent with a centrally driven sympathetic response. Plasma catecholamines have been reported to not change in response to PET scan procedures involving passive sensory stimulation (*32*). This suggests that pharmacologic studies may bring an additional potential source of variance to PET studies. The increases were transient and were not significantly correlated with CMRglu during the subsequent scan. Nonetheless, a change in anxiety or arousal set in motion by subjective responses to the infusion remains a possible explanation for the changes in metabolic rate during the second scan.

Arousal has been suggested to be a predominant influence on the variability of cerebral metabolism (*7*). Behavioral correlates of arousal vary through the day and have been hypothesized to parallel the circadian variation in CMRglu seen in preclinical models (*33*). To the extent that vigilance reflects level of wakefulness, the correlations we observed in Group 1 between CPT performance and changes in global and regional CMRglu also are consistent with arousal influencing metabolic variability. Intuitively, high levels of arousal would have effects on brain activity inverse to habituation, so the higher baseline CMRglu in Group 1 subjects naive to PET scans could also be interpreted as due to increased arousal in naive subjects.

One of the reasons CPT tasks were included was to reduce variance by maintaining a consistent, minimum level of wakefulness. The confidence intervals for mean global or regional changes in the present studies do not substantially differ from those calculated for other studies comparing same day measurements, under resting conditions (*10,24,26*). This suggests that vigilance tasks do not appreciably reduce variance between same day studies, as has reported between studies performed on different days (*2*). Inclusion of tasks like the CPT may be warranted for other reasons, such as providing a measure of drug response, or to maintain wakefulness during studies of highly sedating compounds. Unnecessary inclusion of such a task, however, may render interpretation of effects more difficult, due to potential confounds between drug and task effects.

Interestingly, the only regional finding common to both studies was a significant increase in absolute metabolic rate in the right thalamus. High variability in the regional metabolic rate in thalamus has been observed in several comparisons of repeated measurements (*1,23–25*). This may be due to functional and neuroanatomical heterogeneity of the thalamus or that activity in thalamic nucleii is uniquely sensitive to behavioral state. Indeed, activity in thalamic regions appears to be highly sensitive to level of arousal. Regional CMRglu has been reported to be greatly decreased in thalamus during all sleep stages, falls significantly following infusion with a sedating dose of benzodiazepine and is negatively correlated with the level of sleepiness so generated (*34–36*). Caution is therefore warranted in the evaluation of metabolic change in thalamic regions.

CONCLUSION

We found modest regional and global increases in the absolute CMRglu between serial FDG studies. The range of mean global and regional variability was remarkably consistent across two independent samples, and two different protocols. This suggests that variability in CMRglu generated by changes in behavioral state is reduced by this method, relative to measurements obtained on different days. Moreover, if the study undertaken does not involve strong hypotheses of global effects, or if highly regional effects are anticipated, the present results confirmed that normalization to global CMRglu may "correct" for this systematic effect and potentially reduce the regional differences due to nonspecific effects (*37*). Ultimately, any findings from a pharmacologic study using this method are best confirmed through replication, and cautious use of corroborative and preclinical data.

ACKNOWLEDGMENTS

We thank Laura Kwako and Phyllis Storch from the Walter Johnson High School National Institutes of Health internship program for assisting in data management; Roseanne Leakan, RN, for assisting during the imaging studies; and the PET technologists in the Department of Nuclear Medicine, Clinical Center, National Institutes of Health, under the direction of Peter Herscovitch, MD.

REFERENCES

1. Camargo EE, Szabo Z, Links JM, Sostre S, Dannals RF, Wagner HN. The influence of biological and technical factors on the variability of global and regional brain metabolism of 2-[^{18}F] fluoro-2-deoxy-D-glucose. *J Cereb Blood Flow Metab* 1992; 12:281–290.
2. Duara R, Gross-Glenn K, Barker WW, et al. Behavioral activation and the variability of cerebral glucose metabolic measurements. *J Cereb Blood Flow Metab* 1987;7:266–271.
3. Mazziota JC, Phelps ME, Carson RE, Kuhl DE. Tomographic mapping of human cerebral metabolism: sensory deprivation. *Ann Neurol* 1982;12:435–444.
4. Risberg J, Maximilian AV, Prohovnik I. Changes of cortical activity patterns during habituation to a reasoning test. *Neuropsychologia* 1977;15:793–798.
5. Gur RC, Gur RE, Resnick SM, Skolnick BE, Alavi A, Reivich M. The effect of anxiety on cortical cerebral blood flow and metabolism. *J Cereb Blood Flow Metab* 1987;7:173–177.
6. Warach S, Gur RC, Gur RE, Skolnick BE, Obrist WD, Reivich M. Decreases in frontal and parietal lobe regional cerebral blood flow related to habituation. *J Cereb Blood Flow Metab* 1992;12:546–553.
7. Yoshii F, Barker WW, Chang JY, et al. Sensitivity of cerebral glucose metabolism to age, gender, brain volume, brain atrophy and cerebrovascular risk factors. *J Cereb Blood Flow Metab* 1988;8:654–661.
8. Clark C, Carson R, Kessler R, et al. Alternative statistical models for the examination of clinical positron emission tomography/fluorodeoxyglucose data. *J Cereb Blood Flow Metab* 1985;5:142–150.
9. Friston KJ, Frith CD, Liddle PF, Dolan RJ, Lammertsma AA, Frackowiak RSJ. The relationship between global and local changes in PET scans. *J Cereb Blood Flow Metab* 1990;10:458–466.
10. Brooks RA, DiChiro G, Zukerberg BW, Bairamian D, Larson SM. Test-retest studies of cerebral glucose metabolism using fluorine-18 deoxyglucose: validation of method. *J Nucl Med* 1987;28:53–59.
11. Chang JY, Duara R, Barker W, Apicella A, Finn R. Two behavioral states studied in a single PET/FDG procedure: theory, method and preliminary results. *J Nucl Med* 1987;28:852–860.
12. Schmidt ME, Matochik JA, Risinger RC, et al. Regional glucose metabolism following acute alpha-2 blockade by idazoxan. *Clin Pharm Therap* 1995;57:684–695.
13. Ernst M, Zametkin AJ, Matochik JA, Liebenauer L, Fitzgerald GA, Cohen RM. Effects of intravenous dextroamphetamine on brain metabolism in adults with attention-deficit hyperactivity disorder (ADHD): preliminary findings. *Psychopharmacol Bull* 1994; 30:219–225.
14. Cohen RM, Semple WE, Gross M, Holcomb HH, Dowling MS, Nordahl TE. Functional localization of sustained attention: comparison to sensory stimulation in the absence of instruction. *Neuropsych Neuropsychol Behav Neurol* 1988;1:3–20.
15. Spielberger CD, Gorsuch RL, Lushene RE. *Manual for the state-trait anxiety inventory*. Palo Alto, CA: Consulting Psychologists Press; 1983.
16. Brooks RA. Alternative formula for glucose utilization using labeled deoxyglucose. *J Nucl Med* 1982;2:538–539.
17. Phelps ME, Huang SC, Hoffman EJ, Selin C, Sokoloff L, Kuhl DE. Tomographic measurement of local cerebral glucose metabolic rate of humans with [^{18}F]1-fluoro-2-deoxy-D-glucose: validation of method. *Ann Neurol* 1979;6:371–388.
18. Huang SC, Phelps ME, Hoffman EJ, Sideris K, Selin CJ, Kuhl DE. Noninvasive determination of local cerebral metabolic rate of glucose in man. *Am J Physiol* 1980;238:E69–E82.
19. Matsui T, Hirano A. *An atlas of the human brain for computerized tomography*. New York: Igaku-Shoin Medical; 1978.
20. Bartko J. The intraclass correlation coefficient as a measure of reliability. *Psych Rep* 1966;19:3–11.
21. Mefford I, Caliguri EJ, Grady RK, Capella P, Durkin TA, Chevalier P. Microbore HPLC of biogenic amines in small biological samples. *Meth Enzymol* 1986;124:402–412.
22. Gur RE, Resnick SM, Gur RC, et al. Regional brain function in schizophrenia. II. Repeated evaluation with positron emission tomography. *Arch Gen Psychiatry* 1987;44:126–129.
23. Maquet P, Dive D, Salmon E, von Frenckel R, Franck G. Reproducibility of cerebral glucose utilization measured by PET and the [^{18}F]-2-fluoro-2-deoxy-d-glucose method in resting, healthy human subjects. *Eur J Nucl Med* 1990;16:267–273.
24. Bartlett EJ, Brodie JD, Wolf AP, Christman DR, Laska E, Meissner M. Reproducibility of cerebral glucose metabolic measurements in resting human subjects. *J Cereb Blood Flow Metab* 1988;8:502–512.
25. Tyler JL, Strother SC, Zatorre RJ, et al. Stability of regional cerebral glucose metabolism in the normal brain measured by positron emission tomography. *J Nucl Med* 1988;29:631–642.
26. Reivich M, Alavi A, Wolf A, et al. Use of 2-deoxy-D-[1-^{11}C]glucose for the determination of local cerebral glucose metabolism in humans: variation within and between subjects. *J Cereb Blood Flow Metab* 1982;2:307–319.
27. Kuwabara H, Gjedde A. Measurements of glucose phosphorylation with FDG and PET are not reduced by dephosphorylation of FDG-6-phosphate. *J Nucl Med* 1991;32:692–698.
28. Chang JY, Duara R, Barker W, et al. Two behavioral states studied in a single PET/FDG procedure: error analysis. *J Nucl Med* 1989;30:93–105.
29. Eastman RC, Carson RE, Gordon MR, et al. Brain glucose metabolism in noninsulin dependent diabetes mellitus: a study in Pima Indians using positron emission tomography during hyperinsulinemia with euglycemic glucose clamp. *J Clin Endocrinol Metab* 1990;71:1602–1610.
30. Giordani B, Boivin MJ, Berent S, et al. Anxiety and cerebral cortical metabolism in normal persons. *Psychiatry Res Neuroimaging* 1990;35:49–60.
31. Reivich M, Alavi A, Gur RC. Positron emission tomographic studies of perceptual tasks. *Ann Neurol* 1984;15(suppl):S61–S65.
32. Cameron OG, Modell JG, Hichwa RD, Agranoff BW, Koeppe RA. Changes in sensory-cognitive input: effects on cerebral blood flow. *J Cereb Blood Flow Metab* 1990;10:38–42.
33. Room P, Tielemans AJPC. Circadian variations in local cerebral glucose utilization in freely moving rats. *Brain Res* 1989;505:321–325.
34. Maquet P, Dive D, Salmon E, et al. Cerebral glucose utilization during sleep-wake cycle in man determined by positron emission tomography and [^{18}F]2-fluoro-2-deoxy-D-glucose method. *Brain Res* 1990;513:136–143.
35. Buchsbaum MS, Gillin JC, Wu J, et al. Regional cerebral glucose metabolic rate in human sleep assessed by positron emission tomography. *Life Sci* 1989;45:1349–1356.
36. Volkow ND, Wang GJ, Hitzemann, et al. Depression of thalamic metabolism by lorazepam is associated with sleepiness. *Neuropsychopharmacology* 1995;12:123–132.
37. Friston KJ, Grasby PM, Bench CJ, et al. Measuring the neuromodulatory effects of drugs in man with positron emission tomography. *Neurosci Lett* 1992;141:106–110.