Measuring Reproducibility of Regional Brain Metabolic Responses to Lorazepam Using Statistical Parametric Maps

Gene-Jack Wang, Nora D. Volkow, Alejandro V. Levy, Christoph A. Felder, Joanna S. Fowler, Naomi R. Pappas, Robert J. Hitzemann and Christopher T. Wong

Medical and Chemistry Departments, Brookhaven National Laboratory, Upton; Departments of Radiology and Psychiatry, State University of New York, Stony Brook; and Psychiatry Service, Veterans Affairs Medical Center, Northport, New York

Statistical parametric mapping (SPM) is a method for localizing differences in brain activation patterns without the need for anatomic predefined constraints. The purpose of this study was to assess the reproducibility of the patterns of activation obtained with SPM for baseline measures and for metabolic changes in response to lorazepam on a test-retest design. The results were compared with those we previously published using region-of-interest (ROI) methods. **Methods:** Sixteen healthy right-handed men were scanned twice with PET and [^{18}F]fluorodeoxyglucose (FDG): before placebo and before lorazepam (30 μg/kg). The same double FDG procedure was repeated 6–8 wk later to assess test-retest reproducibility. Image datasets were analyzed by using SPM95 software. Difference images between baseline and lorazepam were compared for the first and second evaluations, both for relative decreases as well as increases in metabolism. Significance level was systematically varied to $P < 0.001$, $P < 0.01$ and $P < 0.05$. **Results:** There were no differences in the baseline SPM maps obtained for the first and second evaluations. SPM showed similar, although not identical, differences in response to lorazepam between the two evaluations. Both evaluations showed significant decreases in occipital cortex (9.7% and 10%) and significant relative increases in left temporal pole (6.8% and 10.4%). However, the second evaluation showed a decrease in the left frontal cortex (areas 6 and 8), which was not present in the first evaluation. The results were very similar to those we had obtained with ROI methods, except for the activation in the left temporal pole, which we had not observed with ROI analyses. **Conclusion:** Although the overall pattern of lorazepam-induced activation depicted by SPM was reproducible in pattern and magnitude, there were some differences that included a left frontal area of deactivation during the second but not the first evaluation. Results with SPM are similar to those with the ROI method, and, because it systematically analyses the whole brain, SPM can uncover patterns not seen with the ROI method.

Key Words: benzodiazepines; cerebral glucose metabolism; fluorodeoxyglucose; PET; statistical parametric mapping

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For correspondence or reprints contact: Gene-Jack Wang, MD, Medical Department, Brookhaven National Laboratory, Upton, NY 11973.

Statistical parametric mapping (SPM) is a statistical method used for image analyses and can automatically determine statistical differences between sets of images obtained between different experimental conditions or between sets of images obtained from different groups of subjects. Although it can be used to compare images that reflect biochemical or pharmacologic parameters, its most common application is to assess significant changes in regional brain activity (*1–5*).

The method is based on the averaging of images across groups of subjects distinguished on the basis of biologic variables (*6*), disease processes (*7*) and/or activation conditions (*8–13*). This is accomplished with a series of steps, which involve: (a) a linear spatial registration (spatial realignment), (b) a nonlinear spatial registration (plastic transformation), (c) an anatomic normalization to reference space (Talairach and Tournoux *Atlas*) (*14*), (d) a linear global functional normalization and (e) the computation of statistical significance parameters p(Z) and p(k), based on the intensity (Z) and area (k) of the activation region (*15*).

The measurement of regional brain glucose metabolism with PET and [^{18}F]fluorodeoxyglucose (FDG) has been used to assess cerebral dysfunction in neuropsychiatric disorders (*16*) and disease progression (*17*) and to evaluate the effects of treatment (*18,19*). With region-of-interest (ROI) methods in which predefined anatomic areas were evaluated, studies have shown that baseline metabolic measurements (*20,21*) and regional metabolic responses to drugs are reproducible (*22*) for test-retest conditions. This is relevant in that, if one is to evaluate the effect of a treatment intervention or disease progression, it is important for the control measures to be reproducible.

This study evaluates the reproducibility of the pattern of responses obtained with SPM for the regional brain metabolic changes induced by lorazepam on two separate evaluations performed at a 6- to 8-wk interval in 16 healthy control subjects. The results were compared with those we previously reported on test-retest reproducibility for lorazepam-induced metabolic responses in these subjects using predefined ROIs (*23*).

TABLE 1
Summary of SPM Results of Significant Difference Between Baseline and Lorazepam Administration in Study 1 and Study 2

	Study 1					Study 2				
Region	Size (k)	P ($n^{max} > k$)	Z	P ($Z^{max} > u$)	Coordinates x, y, z (mm)	Size (k)	P ($n^{max} > k$)	Z	P ($Z^{max} > u$)	Coordinates x, y, z (mm)
Decreased metabolism										
Occipital	6050	0.001	5.62	0.0001	2, −80, −4	6106	0.001	5.62	0.0001	6, −74, 0
			5.05	0.0001	4, −100, −12			5.55	0.0001	4, −96, −8
			5.04	0.0001	−8, −32, 32			5.38	0.0001	−16, −88, −12
Left frontal			4.47	0.005	−26, 26, −12			4.89	0.001	−42, 12, 40
Right frontal			4.40	0.007	42, 12, 40			4.01	0.022	44, 22, 36
			3.93	0.036	28, 30, −16					
Thalamus			3.95	0.034	4, −10, 8			4.07	0.017	2, −16, 8
Increased metabolism										
Left temporal	4658	0.001	5.14	0.0001	−50, −38, −8	5681	0.001	5.02	0.0001	−42, 6, −28
			5.05	0.0001	−40, −10, −16			4.60	0.002	−38, −20, −12
			4.18	0.015	−50, 0, −12			3.61	0.076	−8, −54, −24
Right temporal	4332	0.002	4.47	0.002	34, −4, −20	3686	0.007	4.31	0.007	52, −8, −24
			4.67	0.002	46, −58, 12			4.18	0.012	38, −2, −8
			4.24	0.012	48, 4, −16			4.13	0.014	46, 0, −12
Cingulate			4.27	0.011	−8, 30, 0			3.87	0.034	−4, 22, 16

Study 1: Threshold (u) = 2.33; Volume [S] = 59130 voxels; df = 15; FWHM = [24.4 30.2 27.6] mm (i.e., 46 RESELS).
Study 2: Threshold (u) = 2.33; Volume [S] = 59130 voxels; df = 15; FWHM = [27.1 32.2 30.1] mm (i.e., 36 RESELS).
$P < 0.05$.

MATERIALS AND METHODS

Subjects

Sixteen healthy, right-handed men (mean age 38.3 ± 11.2 y, age range 23–58 y) who consumed less than five alcoholic drinks per week were selected for the study. To exclude subjects with medical or neuropsychiatric illnesses, subjects received complete physicals, neurologic and psychiatric examinations and routine laboratory tests, including urine toxicology. Subjects were instructed to refrain from drinking alcoholic beverages and to discontinue any over-the-counter medication 1 wk before the scan. Informed consent was obtained from each participant after the nature of the experiment was fully explained.

Experimental Design

Two sets of identical studies were performed in each subject 6–8 wk apart. Each set consisted of two PET scans performed with FDG on 2 separate days within 1 wk of each other. On the first day, subjects were injected with a placebo (3 mL saline solution) given 40–50 min before the FDG (baseline-FDG) scan. On the second day, subjects were injected with lorazepam (30 μg/kg) given 40–50 min before the FDG (lorazepam-FDG) scan. The subjects were unaware of the drug received. To avoid circadian variability (*21*), the four scans for a given subject were performed at the same time of day (±1 h).

PET Scanning

Subjects were asked to refrain from smoking, drinking caffeine-containing drinks and eating for at least 4 h before the study. PET scans were performed with a CTI-931 tomograph (resolution 6 × 6 × 6.5 mm full width at half maximum [FWHM], 15 slices; CTI/Siemens, Inc., Knoxville, TN). Procedures for subjects' positioning, scanning protocol, arterialized blood sampling and conditions of study were as previously described (*24*). Briefly, a 20-min emission scan was obtained beginning 35 min after injection of 145–185 MBq (4–5 mCi) FDG. Metabolic images were computed as described previously (*24*).

Statistical Parametric Maps Measurement

Metabolic images were analyzed with the software package for Statistical Parametric Mapping SPM95 (SPM95 Software; MRC Cyclotron Unit, Hammersmith Hospital, London, UK) (*15*). Four steps were involved in the automatic image analysis.

1. Spatial registration: Each subject had a total of four FDG scans, two baseline and two lorazepam scans. For each subject, the realignment of the four scans was performed with respect to the first baseline scan using the "realign" option of the SPM95 package, which minimizes the variance of the pixel intensity ratio between scans.
2. Anatomic geometric registration: For each subject, we obtained an average scan of the realigned set of four scans, and this averaged brain image was registered to the Talairach and Tournoux *Atlas* (*14*) through a PET-like template in the SPM95 software package by using linear rigid body translations, rotations and nonlinear, (quadratic) final spatial registration, plus linear rescaling to the brain dimensions of the Talairach and Tournoux *Atlas*. By using the same parameters obtained for each subject's average brain, each of the four scans were then registered to the Talairach and Tournoux *Atlas* reference space. All the operations of this step were performed with the "normalization" option of the SPM95 package. The images were then smoothed by FWHM of 16 mm to account for the anatomic variability among subjects.
3. Functional registration: The global brain activity was computed for each FDG scan and a linear transformation was used to rescale each pixel activity so that the 64 scans from all the

subjects would have the same global activity. This step was performed using the "proportional scaling" option of the SPM95 package with the default value for the global brain activity.

4. Statistical analysis: For each task-task comparison, the t values of the scans were computed on a pixel-by-pixel basis and then converted to their Z scores to obtain a normalized distribution. SPMs were displayed in coronal, transverse and sagittal views showing only those pixels that reached a statistical significance of $P < 0.001$, $P < 0.01$ and $P < 0.05$. The pixel with the highest Z score within each isolated significant region was chosen to report its coordinates in the Talairach and Tournoux *Atlas* reference space, which uses a mid-sagittal plane containing the line between anterior and posterior commissures (AC-PC) as the y-z coordinate origin and its associated orthogonal transaxial plane to contain the x-y axes with the x and y coordinate origins in the anterior commissure.

RESULTS

SPM confirmed that the baseline metabolic measurements for the first and second evaluations were not different from each other for the significance levels of $P < 0.001$ and $P < 0.01$. However, for a significance level of $P < 0.05$, SPM showed differences in baseline measurements in the right frontal cortex (x = 32, y = 16, z = 20; $P < 0.013$); the measurements for the first scan were 5.4% lower than for the second scan.

Lorazepam induced a significant decrease in metabolic activity in occipitocerebellar regions for both evaluations ($Z > 5$, $P = 0.0001$; Table 1) and a comparable volume of activation (6050 versus 6106 pixels). In the second but not the first evaluation, lorazepam decreased metabolism in the left frontal cortex (Z = 4.9) for a significance level of $P < 0.001$ (Fig. 1). For a significance level of $P < 0.01$ (Fig. 2), SPM showed additional decrements in left orbitofrontal region and right frontal cortex for the first evaluation only (Z = 4.4; Table 1). For a significance level of $P < 0.05$ (Fig. 3), SPM showed additional decrements in SPM in the thalamic region for the first (Z = 3.95) and second (Z = 4.07) evaluations (Table 1).

The largest metabolic decrement after lorazepam administration in the first evaluation was in the occipital cortex (9.7%, Z = 5.62, $P < 0.0001$), followed by thalamus (7.7%, Z = 3.95, $P < 0.03$), right frontal cortex (5.8%, Z = 4.4, $P < 0.007$) and left orbitofrontal cortex (5.6%, Z = 4.47, $P < 0.005$). In the second evaluation, it was in the occipital cortex (10%, Z = 5.62, $P < 0.0001$), followed by thalamus (7.7%, Z = 4.07, $P < 0.02$), left frontal cortex (6.1%, Z = 4.89, $P < 0.001$) and right frontal cortex (5.1%, Z = 4.01, $P < 0.02$).

The patterns for lorazepam-induced relative increases were also similar for both evaluations, and the predominant

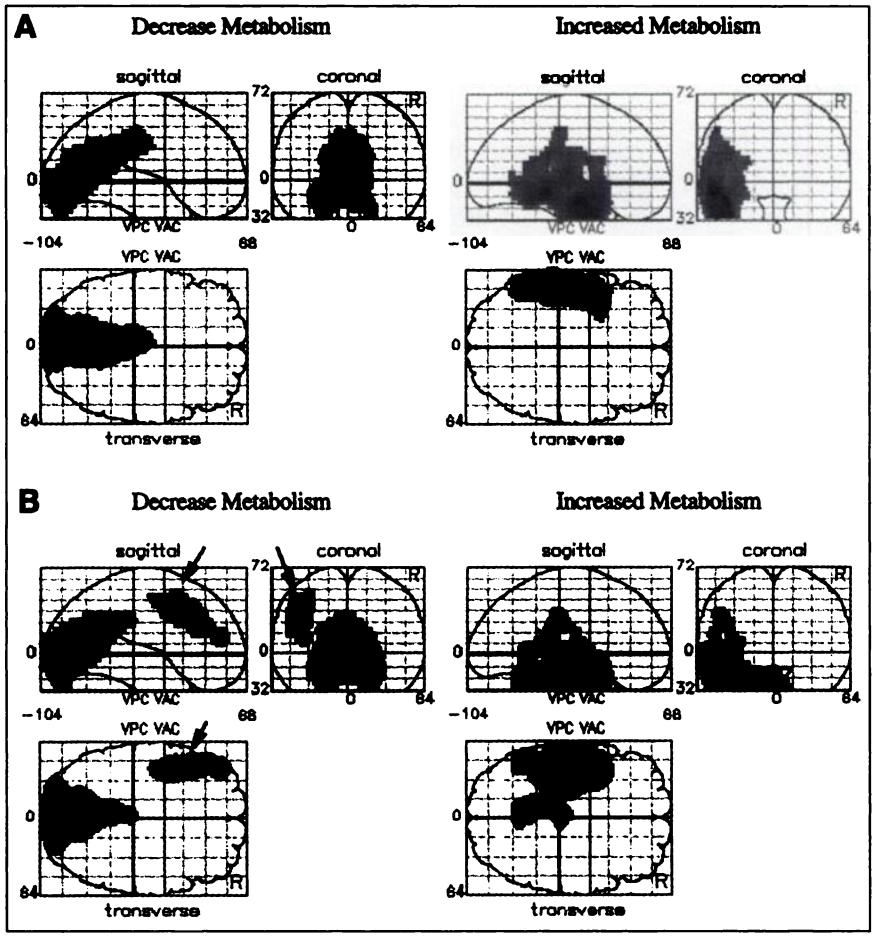

FIGURE 1. Cerebral metabolic changes after lorazepam for first evaluation (A) and for second evaluation (B) using SPM. Highlighted areas indicate regions where there were metabolic changes at significance level of $P < 0.001$. Arrows point to lorazepam-induced decreases in left frontal metabolism that were seen in second but not in first evaluation.

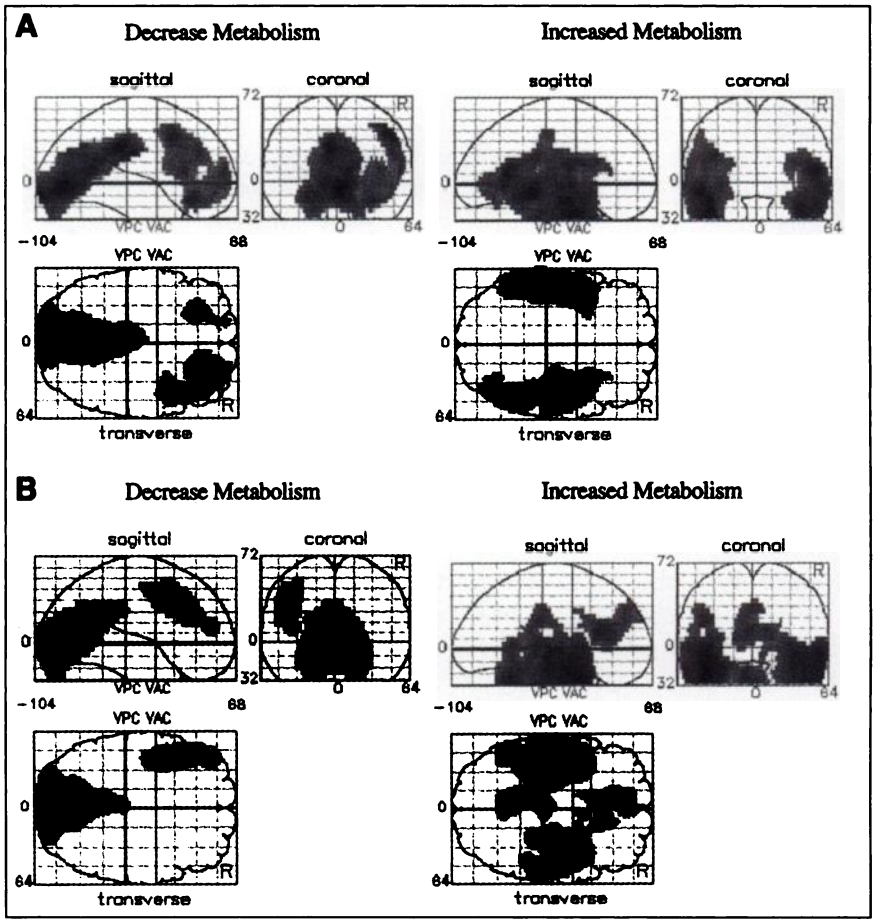

FIGURE 2. SPM of cerebral metabolic changes after lorazepam for first (A) and second (B) evaluation at significance level of $P < 0.01$. Note that additional decrements in left orbitofrontal and right frontal cortices in first evaluation as well as increments in right temporal cortex in first and second evaluation were seen from those detected with $P < 0.001$ (Fig. 1).

finding was an increase in left lower temporal cortex ($Z > 5$, $P = 0.0001$; Table 1), which included a somewhat larger area during the second evaluation than during the first (5681 versus 4658 pixels, respectively) (Fig. 1). For a significance level of $P < 0.01$ (Fig. 2), SPM showed an additional relative increase in the right lower temporal cortex for both evaluations ($Z > 4$; Table 1). The only difference in the SPM pattern of activation was seen at the significance level of $P < 0.01$, which showed that, for the first evaluation but not the second, the metabolism was increased in cingulate gyrus ($Z = 4.27$; Table 1). For a significance level of $P < 0.05$, SPM showed an additional relative increase in the cingulate gyrus for the second evaluation ($Z > 3.87$; Table 1).

The magnitude of metabolic increases after lorazepam in the first evaluation was the largest in cingulate gyrus (7.9%, $Z = 4.27$, $P < 0.01$), left lower temporal cortex (6.8%, $Z = 5.14$, $P < 0.0001$) and right lower temporal cortex (6.6%, $Z = 4.74$, $P < 0.002$). In the second evaluation, it was largest in left lower temporal cortex (10.4%, $Z = 5.02$, $P < 0.0001$) and right lower temporal cortex (7.1%, $Z = 4.31$, $P < 0.007$).

DISCUSSION

This study shows that the SPM patterns under baseline conditions are highly reproducible. In this respect, it is consistent with our previous results with ROI analysis showing that "relative" metabolic measures (region/whole brain) did not differ between evaluations but differed for the results obtained with the "absolute" metabolic measures, which showed lower brain metabolism during the second evaluation than during the first (*23*). The discrepancy is mainly because the differences in absolute values between baselines reflected a global change in metabolism, which is canceled by the SPM normalization procedure that brings all the scans to the same level of activity.

This study also shows that SPM measurements of regional brain metabolic changes induced by acute lorazepam administration are highly reproducible in a given subject when tested at a 6- to 8-wk interval. This is consistent with our previous results, using ROI analyses, showing no significant differences in lorazepam-induced changes in relative metabolism between the first and second evaluations. As for our previous results with ROI analyses, the largest changes in metabolism induced by lorazepam were in the occipital cortex and thalamus (*23,25–28*).

By using SPM, we showed a significant decrease in metabolism in the left frontal cortex during the second evaluation, which we did not document with ROI analyses. Similarly with SPM, but not with ROI analyses, we showed activation of the left temporal cortex for both evaluations.

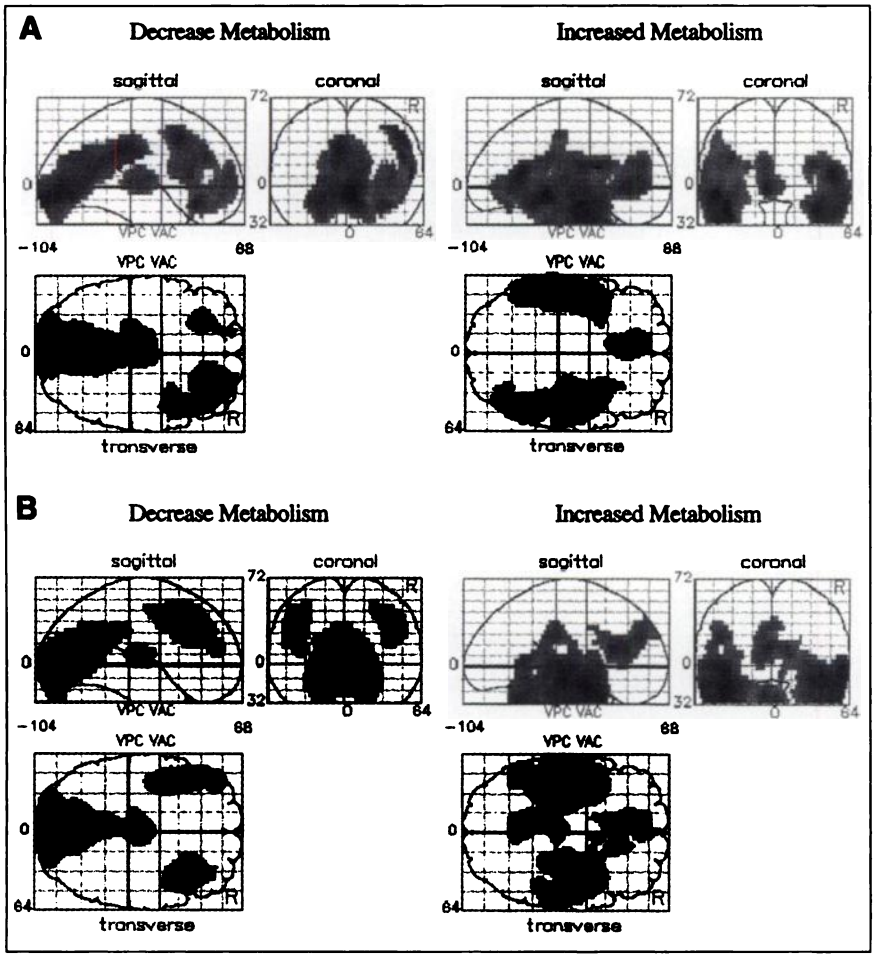

FIGURE 3. SPM of cerebral metabolic changes after lorazepam for first (A) and second (B) evaluation at significance level of $P < 0.05$. Note decrease in thalamic metabolism for both evaluations.

The left frontal cortex was more sensitive to lorazepam administration during the second evaluation than during the first. The extent to which this difference in sensitivity represents physiologic changes in response to repeated lorazepam administration and/or changes secondary to known expectations for the experiment requires further investigation.

When comparing the differences in the results obtained with the SPM and the ROI methods, we found that SPM revealed a marked difference between the hemispheres in sensitivity to the effects of lorazepam not seen with the ROI method. This is probably because, with the ROI method, one is limited to the sampling of preselected regions with arbitrary boundaries that may average out and/or miss areas where there are differences in activity between the hemispheres. In the case of lorazepam, for example, SPM showed that the drug has a marked effect in the left hemisphere, where it induces a relative decrease in the left frontal cortex and a relative increase in the left inferior temporal cortex; such a pattern is not observed in the right hemisphere. Further studies are required to assess the significance of these interhemispheric differences compared with the behavioral effects of lorazepam.

Another significant difference between the SPM and the ROI methods was that, with the ROI method, the largest changes were observed in the thalamus, whereas SPM does not detect thalamic differences as significant unless the significance level is set to $P < 0.05$. This is quite surprising, because thalamic deactivation by lorazepam is a finding that we have reported consistently in all of our studies with lorazepam, including studies in controls (*25*), alcoholics (*26*), subjects at risk for alcoholism (*27*) and cocaine users (*28*). Individual analyses for thalamic responses showed decreased metabolism in the thalamus in 13 of 16 subjects, and the magnitude of this change was in the 7.9% range. The reason for this discrepancy is unclear and may reflect the fact that the area of the thalamus that shows decreases in metabolism with lorazepam may vary in location between subjects, or it may reflect the blurring of the spatial resolution incurred as part of the SPM procedure, which cannot confidently distinguish activation foci that are less than 10–15 mm apart (*29*). Phantom studies are required to determine why SPM was unable to show the thalamic effect until the P value was lowered to $P < 0.05$.

Shifting the SPM significance level from $P < 0.001$ to $P < 0.05$ increased the variability between the baseline measures. As expected, it also increased the regions that were found to be affected by lorazepam and increased the differences between the response to lorazepam induced during the first and the second evaluations. As a result of

these comparisons, we recommend setting the SPM significance level to $P < 0.001$ for acute pharmacologic studies.

CONCLUSION

This study documents that SPM provides reproducible results for baseline as well as drug intervention studies. The pattern and magnitude of metabolic change shown by SPM was similar, although not identical, to that obtained with the ROI method. We therefore recommend that studies should report results with both methods and that caution should be exerted when using SPM to detect effects in small subcortical structures.

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