

# Dose-Dependent Decrease of Activation in Bilateral Amygdala and Insula by Lorazepam During Emotion Processing

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**Background:** Functional neuroimaging may elucidate the pathophysiologic features of anxiety disorders and the site of action of anxiolytic drugs. A large body of evidence suggests that the amygdala and associated limbic structures play a critical role in the expression of anxiety and may be treatment targets for anxiolytic drugs.

**Objective:** To determine whether lorazepam dose-dependently attenuates blood oxygenation level-dependent functional magnetic resonance imaging (BOLD fMRI) activation in the amygdala and associated limbic structures during an emotion face assessment task.

**Participants and Design:** Fifteen healthy volunteers participated in a double-blind, placebo-controlled, randomized dose-response study. Subjects underwent imaging 3 times (at least a week apart) and were given either a single-dose placebo or 0.25 mg or 1.0 mg of lorazepam 1 hour prior to an MRI session. During fMRI, subjects completed an emotion face assessment task, which has been shown to elicit amygdala activation.

**Main Outcome Measures:** The BOLD-fMRI activation in amygdala, insula, and medial prefrontal cortex during the emotion face assessment task.

**Results:** Lorazepam significantly attenuated the BOLD-fMRI signal in a dose-dependent manner in bilateral amygdala and insula but not in the medial prefrontal cortex. Lorazepam did not affect the BOLD-fMRI signal in the primary visual cortex.

**Conclusions:** The current finding provides the first neuroimaging evidence of a dose-dependent change induced by an established therapeutic agent in brain regions known to be critical for the mediation of anxiety. This investigation may help to support the use of BOLD-fMRI with pharmacological probes to investigate the neural circuits underlying anxiety and the use of fMRI as a tool in the development of new anxiolytic agents.

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**A**NXIETY DISORDERS ARE among the most common psychiatric disorders but comprise a heterogeneous group of conditions. Delineating the basic neurocircuitry underlying normal and pathologic anxiety<sup>1</sup> may help to better define anxiety disorders. The amygdala plays a critical role in normal fear conditioning and is implicated in the pathophysiologic mechanism of anxiety disorders.<sup>2,3</sup> However, this structure is also important for other emotional information processing and behavior.<sup>4</sup> Functional neuroimaging studies have shown amygdala activation in fear conditioning,<sup>5</sup> reward-related processing,<sup>6</sup> encoding of emotionally salient information,<sup>7</sup> risk taking,<sup>8</sup> processing positively valenced stimuli,<sup>9</sup> and appetitive or aversive olfactory learning.<sup>10</sup>

In addition to the amygdala, a network of structures that includes the in-

sula, anterior cingulate gyrus, and medial prefrontal cortex is important to identify the emotional significance of a stimulus, generate an affective response, and regulate the affective state.<sup>11</sup> The insula has afferent and efferent connections to the medial and orbitofrontal cortex, anterior cingulate gyrus, and several nuclei of the amygdala.<sup>12</sup> Although insula activation has frequently been associated with disgust,<sup>13</sup> there is increasing evidence of a broader role for this brain structure in emotion processing.<sup>14</sup> Insula activation is thought to be involved in differential positive vs negative emotion processing,<sup>5</sup> in particular fearful face processing,<sup>15</sup> pain perception,<sup>16,17</sup> and when individuals are asked to make judgments about emotions.<sup>18</sup>

Benzodiazepine derivatives are the most commonly prescribed antianxiety agents in clinical practice. Lorazepam, a 3-hydroxy-1,4-benzodiazepine, is rapidly and

readily absorbed, reaching peak concentrations in the blood proportional to the dose approximately 1 to 2 hours after oral administration.<sup>19</sup> Previous neuroimaging investigation using 1 mg of intravenous lorazepam during a memory task found significant decreases in activation within the hippocampal, fusiform, and inferior prefrontal regions but no significant alterations in activation in the striate cortex.<sup>20</sup>

The aim of this study was to use blood oxygenation level-dependent functional magnetic resonance imaging (BOLD fMRI) to test the hypothesis that lorazepam dose-dependently attenuates activation during an emotion face paradigm in the amygdala, insula, and medial prefrontal cortex. A modified emotion face assessment task was used to determine whether this attenuation would occur with negative emotion faces (angry and fear) but not with positive emotion faces (happy). Support for this hypothesis would provide a link between the clinical efficacy of lorazepam as an anxiolytic and the biological basis of  $\gamma$ -aminobutyric acid (GABA)-ergic modulation of the amygdala and limbic structures as key targets for anxiety circuitry.

Pharmacological fMRI is an emerging discipline with the potential to address a variety of neural systems and drug development questions.<sup>21</sup> One important consideration with pharmacological fMRI is the differentiation between the drug's effect on neural tissue or on hemodynamic modulation. Some investigators have argued that pharmacological stimulation results in heterogeneous fMRI effects as opposed to global, homogeneous changes in BOLD signal. This argument is consistent with an action on specific neuronal receptor-based mechanisms.<sup>22</sup> Moreover, focusing on a priori hypothesized areas may prevent false-positive findings.<sup>23</sup> Thus, the current study used primarily a region-of-interest approach.

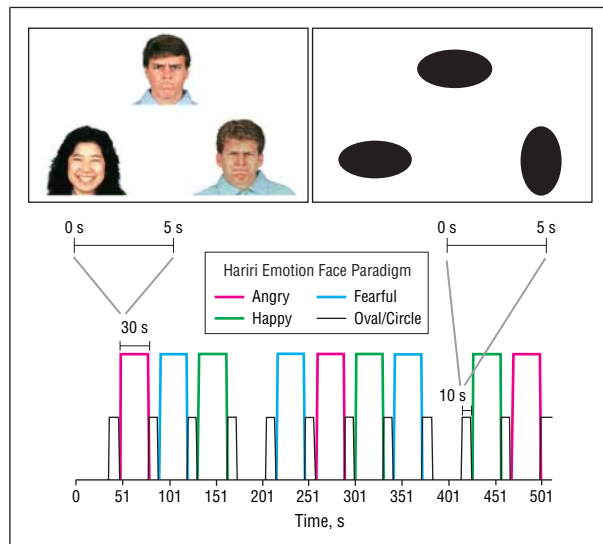
## METHODS

### SUBJECTS

The University of California, San Diego, institutional review board approved the study procedures. All participants provided written informed consent and were paid for their participation. We studied 15 healthy, nonsmoking individuals (6 women and 9 men) aged 18 to 39 years (mean  $\pm$  SD, 27.6  $\pm$  1.4 years) with 12 to 18 years of education (mean  $\pm$  SD, 15.6  $\pm$  0.3 years). Participants did not have medical or psychiatric disorders as determined by medical history and diagnoses according to the Structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders, Revised Fourth Edition*. Each subject was required to consume less than 150 mg/d of caffeine and less than 14 caffeinated beverages per week. Subjects had no history of drug or alcohol abuse and did not report a prior use of benzodiazepines. All participants passed a urine drug screen. Subjects were instructed to maintain their regular bedtimes and wake times for 1 week before and throughout the study period. Individuals presented to the MRI facility between 8 AM and 4 PM with at least 1 week between studies.

### STUDY DESIGN

The study was performed in a double-blind manner. Participants underwent each of 3 conditions in randomized order between 1 and 3 weeks apart. Lorazepam was chosen as the anxiolytic because of its well-documented behavioral effects and because it has a short metabolic half-life, which peaks at about 60 to 120 minutes after ingestion,<sup>24</sup> and no active metabolite, which eliminates the possible confounding effects of drug accumulation.<sup>25</sup> We selected doses that minimally disrupt performance of behavioral tasks and memory ( $\leq 1.0$  mg) but are used to treat anxiety. Subjects arrived at the MRI facility 60 to 90 minutes prior to the MRI study. At arrival, subjects' vital signs were assessed and subjects orally received either placebo or 0.25 mg or 1.0 mg of lorazepam suspension mixed in diet decaffeinated cola.



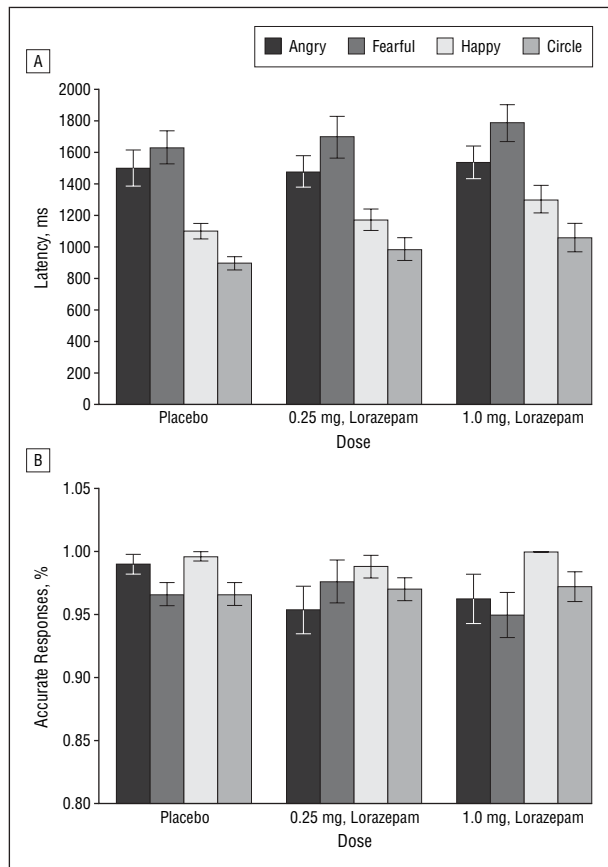
**Figure 1.** Emotion face assessment task.<sup>27</sup> The target and 2 probe faces or ovals and circles were presented for 5 seconds. Blocks of each target emotion consisted of six 5-second angry, fearful, or happy face trials; blocks of ovals or circles consisted of two 5-second trials. Blocks of fixation crosses with varying time length were interspersed between the face and oval/circle blocks.

## ASSESSMENTS AND fMRI PARADIGM

Subjects completed the Spielberger State-Trait Anxiety Inventory<sup>26</sup> (STAI) and the visual analog scales for anxiety, tension, alertness, and trembling before and after the MRI study. During fMRI, each subject was tested using a slightly modified version of the emotion face assessment task.<sup>27</sup> During each 5-second trial, a subject was presented with a target face (on the top of the computer screen) and 2 probe faces (on the bottom of the screen) and was instructed to match the probe with the same emotional expression to the target by pressing the left or right key on a button box (**Figure 1**). A block consisted of 6 consecutive trials in which the target face was either angry, fearful, or happy.<sup>28</sup> During the sensorimotor control task, subjects were presented with 5-second trials of ovals or circles in an analogous configuration and were instructed to match the shape of the probe to the target. Each block of faces and of the sensorimotor control task was presented 3 times in a pseudorandomized order. A fixation cross was interspersed between each block. For each trial, response accuracy and reaction time data were obtained.

## IMAGE ACQUISITION

During the task, one BOLD-fMRI run was collected for each subject using a 1.5-T scanner (Siemens, Erlangen, Germany) (T2-weighted echoplanar imaging, time to repeat = 2000 milliseconds, echo time = 40 milliseconds, 64  $\times$  64 matrix, twenty 4-mm axial slices, 256 repetitions). During the same experimental session, a T1-weighted image (magnetization-



**Figure 2.** Behavioral effects of placebo and 0.25 mg and 1.0 mg of oral lorazepam. Lorazepam had no effect on response latency (A), the fraction of correct responses, or accuracy (B). Error bars indicate SE.

prepared rapid acquisition gradient echo, time to repeat = 11.4 milliseconds, echo time = 4.4 milliseconds, flip angle = 10°, field of view = 256 × 256, 1-mm<sup>3</sup> voxels) was obtained for anatomical reference. For preprocessing, voxel time series were interpolated to correct for nonsimultaneous slice acquisition within each volume and were corrected for 3-dimensional motion.

## IMAGE PROCESSING

All structural and functional image processing were done with the Analysis of Functional Neuroimages software package.<sup>29</sup> Echo-planar images were coregistered to the 128th image using a 3-dimensional coregistration algorithm. The time series of motion parameters was used to obtain a mean for these 6 parameters for each subject. Three motion parameters (d-roll, d-pitch, and d-yaw, indicating change in roll, pitch, and yaw directions) were used as nuisance regressors to account for motion artifacts. The 4 orthogonal regressors of interest were (1) happy, (2) angry, (3) fearful, and (4) circle/oval sensorimotor condition. These regressors were convolved with a modified gamma variate function to account for the delay and the dispersion brain response of the BOLD-fMRI signal due to hemodynamics.<sup>30</sup> Additional regressors were used to model residual motion in the roll, pitch, and yaw directions as well as baseline and linear trends. The Analysis of Functional Neuroimages program 3dDeconvolve<sup>29</sup> was used to calculate the estimated voxelwise response amplitude. A gaussian filter with 6 mm full width at half maximum was applied to the voxelwise percent signal change data to account for individual variations of the anatomical landmarks.

Data for each subject were normalized to Talairach coordinates. A priori regions of interest (defined by the Talairach Dae-

mon atlas<sup>31</sup>) in the bilateral amygdala, medial prefrontal cortex, primary visual cortex, and insula were used as masks. Based on these areas of interest, it was determined via simulations that a voxelwise a priori probability of .05 would result in a corrected clusterwise activation probability of .05 if a minimum volume of 128  $\mu$ L and 2 connected voxels (in the amygdala) or 512  $\mu$ L and 8 connected voxels (in all other regions of interest) was considered. The areas of interest were superimposed on each individual's voxelwise percent signal change brain image. Only activations within the areas of interest that also satisfied the volume and voxel connection criteria were extracted and used for further analysis. No clusters of voxels survived the cluster threshold parameters in the medial prefrontal cortex. Thus, planned comparisons using contrasts for repeated-measures designs were performed for only bilateral amygdala and insula when significant omnibus F values were found. The voxelwise percent signal change data were entered into a mixed-model analysis of variance with task contrast (face type vs circle/oval comparison condition) and dose (placebo or 0.25 or 1.0 mg of lorazepam) as fixed factors and subjects as a random factor. In addition, a whole-brain analysis was carried out to determine whether lorazepam affected nonhypothesized areas. Activations clusters were extracted and corrected for multiple comparisons using an a priori  $P < .05$  threshold and a volume mask of greater than 1000  $\mu$ L.

## STATISTICAL ANALYSIS

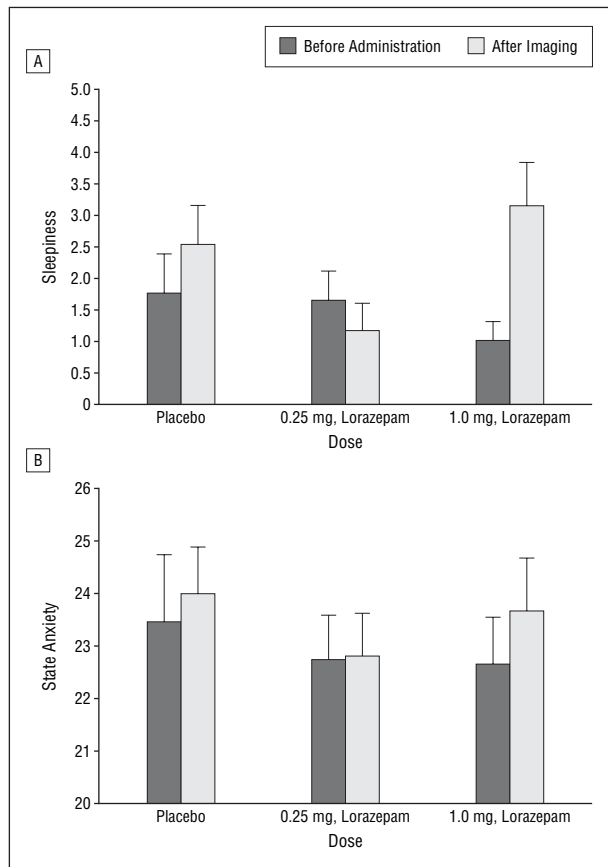
All behavioral analyses were carried out with SPSS version 10.0.<sup>32</sup> A repeated-measures multivariate analysis of variance, with dose (placebo or 0.25 or 1.0 mg of lorazepam) as the within-subjects factor, was used to analyze the behavioral measures and neural activation patterns. Behavioral measures are reported as an interaction between dose and task type. Self-rating measures are reported as interactions between the drug and ratings before and after the MRI study. Correlational analyses were conducted for placebo administration by examining the relationship between self-rating scales or performance measures and activation in the bilateral insula and amygdala during viewing of angry, fearful, and happy faces.

## RESULTS

### BEHAVIORAL RESULTS

Subjects matched the probe face to the target face with nearly perfect accuracy (mean  $\pm$  SD, 97.0%  $\pm$  0.7%), which was not affected by either the 0.25-mg or 1.0-mg dose of lorazepam ( $F_{2,28} = 0.40$ ,  $P = .60$ ,  $\eta^2 = 0.03$ ). As shown in **Figure 2**, although latency was affected by face type (ie, longer for matching angry or fearful faces when compared with happy faces and circles or squares [ $F_{3,12} = 48.8$ ,  $P < .001$ ,  $\eta^2 = 0.92$ ]), neither dose of lorazepam affected response latency ( $F_{2,28} = 1.47$ ,  $P = .25$ ,  $\eta^2 = 0.09$ ).

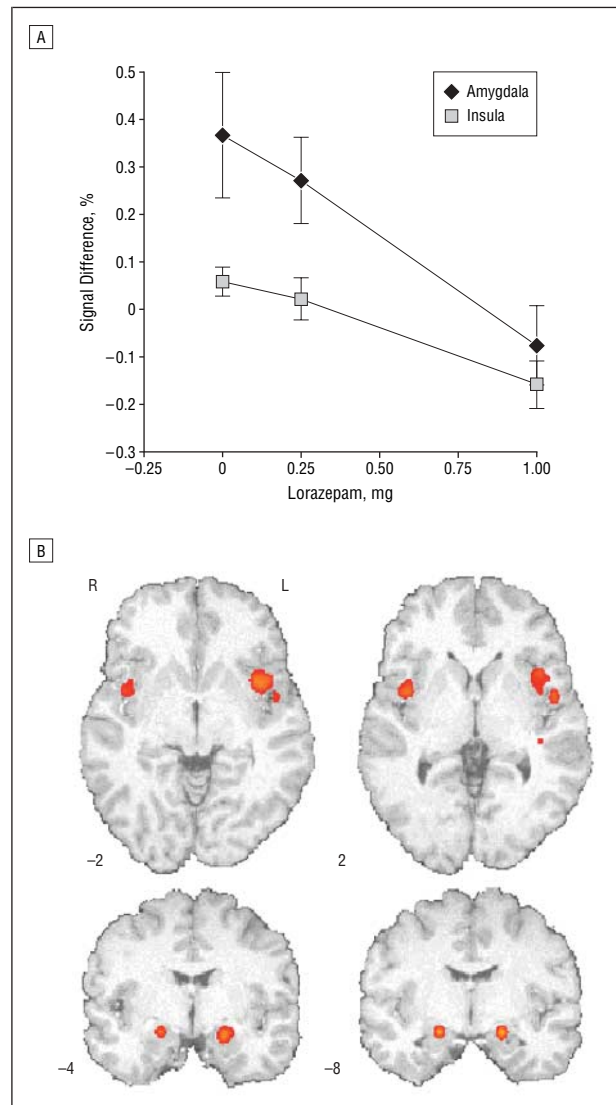
As shown in **Figure 3**, lorazepam did not affect the level of anxiety, either measured by visual analog scales ( $F_{2,22} = 1.71$ ,  $P = .19$ ,  $\eta^2 = 0.14$ ) or by the STAI ( $F_{2,28} = 0.31$ ,  $P = .73$ ,  $\eta^2 = 0.02$ ). Moreover, visual analog ratings of tension ( $F_{2,22} = 0.57$ ,  $P = .57$ ,  $\eta^2 = 0.05$ ) or trembling ( $F_{2,22} = 1.82$ ,  $P = .18$ ,  $\eta^2 = 0.14$ ) did not significantly change across drug administration. However, individuals reported increased sleepiness after 1.0 mg of lorazepam, as evidenced by a dose by pre-post interaction ( $F_{2,22} = 7.2$ ,  $P = .004$ ,  $\eta^2 = 0.40$ ) (Figure 2).



**Figure 3.** A, A high dose of lorazepam increased sleepiness (0=not at all; 10=extremely) at the 1.0-mg dose. B, Neither a low nor a high dose of lorazepam had a significant effect on state anxiety.

### FUNCTIONAL NEUROIMAGING RESULTS

Irrespective of face type, subjects showed bilateral activation of the amygdala and anterior insula during the emotion face assessment task relative to the sensorimotor control task (see **Figure 4** and the **Table** for statistics). Lorazepam attenuated the activation in a dose-dependent fashion in the amygdala and insula (Table, **Figure 5A**). Specifically, activation in bilateral amygdala after administration of 1.0 mg of lorazepam was significantly lower than after 0.25 mg ( $t_{14}=4.83, P<.001$ ) and significantly lower than after placebo administration ( $t_{14}=2.85, P=.01$ ). There was no significant difference between placebo and 0.25 mg of lorazepam ( $t_{14}=0.62, P=.54$ ). Similarly, activation in the bilateral insula after 1.0 mg of lorazepam was significantly lower than after 0.25 mg ( $t_{14}=3.41, P=.004$ ) and significantly lower than after placebo administration ( $t_{14}=3.63, P=.003$ ). There was no significant difference between placebo and 0.25 mg of lorazepam ( $t_{14}=0.70, P=.50$ ). There was no significant effect of lorazepam on activation in the bilateral visual cortex for placebo or the 2 doses of lorazepam (Table, Figure 5B). A whole-brain analysis revealed significant effects of lorazepam in the right inferior temporal gyrus (coordinates: 43,-63,0), left insula (-27,7,-6), amygdala (-9,-11,-10), and fusiform gyrus (-29,-93,-13). In all areas, there was a significant attenuation of the signal in the 1.0-mg lorazepam condition relative to both placebo and 0.25 mg of lorazepam.



**Figure 4.** A, The blood oxygenation level-dependent functional magnetic resonance imaging effects (mean and SE) of placebo and 0.25 mg and 1.0 mg of oral lorazepam. B, Lorazepam dose-dependently attenuated the signal difference between the face-matching task and the sensorimotor control task in bilateral amygdala and insula. Numbers indicate Talairach z coordinates.

### CORRELATIONS BETWEEN BEHAVIORAL AND FUNCTIONAL NEUROIMAGING RESULTS

There were no significant correlations between visual analog scales before administration or after imaging with the degree of activation in the bilateral insula or amygdala during task performance. Moreover, behavioral measures such as latency of response or accuracy of response during the emotion face assessment task did not correlate with the degree of activation in the bilateral insula or amygdala.

### CONCLUSIONS

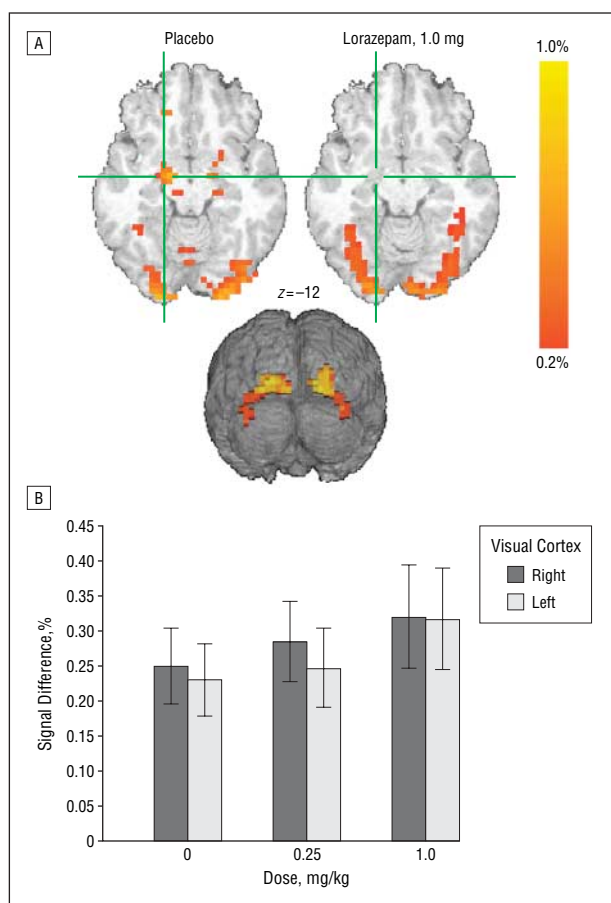
The results of this investigation show for the first time that a known anxiolytic, the benzodiazepine lorazepam, dose-dependently attenuates task-induced activation in bilateral amygdala and insula but has no effect

**Table. Statistical Analyses of Regions of Interest**

Effect	Amygdala			Insula			Visual Cortex		
	F Value	P Value	Partial Effect Size ( $\eta^2$ )	F Value	P Value	Partial Effect Size ( $\eta^2$ )	F Value	P Value	Partial Effect Size ( $\eta^2$ )
Laterality	1.65	.22	0.10	2.27	.15	0.14	0.25	.62	0.02
Dose	6.22	.006*	0.31	8.88	.001*	0.39	0.56	.58	0.04
Face†	1.39	.26	0.09	0.37	.69	0.03	2.62	.09	0.16
Side × dose	0.57	.57	0.04	3.12	.06	0.18	0.26	.77	0.02
Side × face	0.25	.78	0.02	0.10	.91	0.01	1.97	.16	0.12
Dose × face	0.52	.72	0.04	0.68	.61	0.05	0.33	.86	0.02
Side × dose × face	1.18	.33	0.08	0.35	.84	0.02	1.00	.42	0.07

\*Significant at  $P < .05$ .

†Target face: happy, fearful, or angry.



**Figure 5.** A, Percentage of signal activation during the face matching vs the sensorimotor comparison task at  $z = -12$  during administration of placebo and 1.0 mg of oral lorazepam. Cross-hairs indicate the approximate center of mass of the amygdala as defined by the Talairach Daemon atlas.<sup>31</sup> The bar indicates percent signal change from 0.2% to 1.0%. B, Blood oxygenation level-dependent functional magnetic resonance imaging percent signal difference of placebo and 0.25 mg and 1.0 mg of oral lorazepam in the visual cortex.

on the visual cortex. These effects were observed at doses of lorazepam that did not significantly affect behavior on this task and did not change levels of anxiety in these healthy (nonanxious) volunteers. In animals, a large body of evidence suggests that benzodiazepine agonists attenuate brain activity in the amygdala consistent with their

anxiolytic effects.<sup>33</sup> In humans, however, evidence of benzodiazepine effects on reducing amygdala activity had been missing.

A major focus in the field of anxiety research is the delineation of the basic neurocircuitry underlying normal and pathologic anxiety.<sup>1</sup> Amygdala activation occurs during fear conditioning, and altered amygdala activation has been implicated in the pathophysiologic mechanism of anxiety disorders.<sup>2,3</sup> For example, individuals with social anxiety disorder<sup>34</sup> or posttraumatic stress disorder<sup>35</sup> show amygdala hyperresponsivity to fearful or angry faces. Moreover, patients with panic disorder have decreased benzodiazepine receptor binding in the left hippocampus and precuneus<sup>36</sup> as well as in the right orbitofrontal cortex and right insula.<sup>37</sup> The insular cortex appears to be important for subjective feeling states and interoceptive awareness.<sup>38,39</sup> To our knowledge, this is the first study to show that an anxiolytic drug can dose-dependently reduce activation in the amygdala and insula, areas that are important for emotional processing in general and anxiety in particular. Thus, pharmacological fMRI with specific behavioral probes may prove especially useful for delineating how these brain structures are altered in anxiety disorders.

Lorazepam also attenuated activation in the fusiform gyrus. Although primarily recognized as a face-processing area,<sup>40</sup> this structure's activation patterns are related to the degree of conscious perception,<sup>41</sup> anticipation,<sup>42</sup> and identification of emotionally important visual clues.<sup>43</sup> Moreover, activation in the fusiform gyrus is altered in individuals with anxiety disorders,<sup>44</sup> and increased activation in the fusiform gyrus to fearful faces has been observed after cholinergic enhancement.<sup>45</sup> The amygdala can modulate early visual processing of emotional faces in the extrastriate cortex.<sup>15</sup> Thus, lorazepam-induced attenuation of fusiform activity may be indirectly elicited by its down-regulation of the amygdala.

The behavioral effects of benzodiazepines in healthy, nonanxious volunteers have been mixed; for example, diazepam had no effect on fear-potentiated startle response,<sup>46</sup> whereas it impaired the recognition of anger and fear but not other emotional expressions.<sup>47</sup> Single administration vs repeated administration of lorazepam can induce loss of appetite, dizziness, and physical and mental

tiredness.<sup>48,49</sup> Benzodiazepines dose-dependently disrupt learning and performance on acute vigilance tasks<sup>50</sup> and on a variety of memory tasks, presumably by reducing the normally facilitative effect of emotion on memory.<sup>51,52</sup> Thus, behavioral effects may not provide robust indicators of potential anxiolytic efficacy in healthy nonanxious subjects, whereas changes in neural activation patterns induced by these substances may be informative. A low level of state anxiety may account for the lack of correlation between attenuation of the amygdala by lorazepam and changes in anxiety levels in our study. Future studies may include individuals with high trait anxiety (and ultimately anxiety disorders) to elucidate the relationship between anxiolytic effect and amygdala attenuation.

New drugs with novel anxiolytic mechanisms have been developed, including GABA A subreceptors,<sup>53</sup> metabotropic glutamate receptor agents,<sup>54</sup> and neuro-peptide modulators.<sup>55</sup> The current results provide a strong rationale for using BOLD fMRI imaging and amygdala- and/or insula-sensitive behavioral paradigms to determine whether these substances exert effects similar to those seen with standard anxiolytics. Furthermore, evaluation of the effects of other known classes of anxiolytics, such as long-term administration of selective serotonin reuptake inhibitors, should be undertaken to further validate this paradigm. The effect sizes ( $\eta^2$ ) observed in this study provide proof in principle that small-scale (ie, sample sizes in the range of 15 to 30 subjects) BOLD fMRI studies can provide reasonably robust data to determine the site of action of putative anxiolytics. In summary, the current finding provides the first evidence of a dose-dependent change induced by an established therapeutic agent in brain regions known to be critical for the mediation of anxiety.

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