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Amygdala abnormalities in first-degree relatives of individuals with schizophrenia unmasked by benzodiazepine challenge

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Abstract

Rationale—Impaired emotion processing in schizophrenia predicts broader social dysfunction and has been related to negative symptom severity and amygdala dysfunction. Pharmacological modulation of emotion-processing deficits and related neural abnormalities may provide useful phenotypes for pathophysiological investigation.

Objectives—We used an acute benzodiazepine challenge to identify and modulate potential emotion-processing abnormalities in 20 unaffected first-degree relatives of individuals with schizophrenia, compared to 25 control subjects without a family history of psychosis.

Methods—An oral 1mg dose of the short-acting anxiolytic benzodiazepine alprazolam was administered in a balanced crossover placebo-controlled double-blind design, preceding identical 3T fMRI sessions approximately 1 week apart. Primary outcomes included fMRI activity in amygdala and related regions during two facial emotion-processing tasks: emotion identification and emotion memory.

Results—Family members exhibited abnormally strong alprazolam-induced reduction in amygdala and hippocampus activation during emotion identification, compared to equal reduction in both groups for the emotion memory task.

Conclusions—GABAergic modulation with alprazolam produced differential responses in family members vs. controls, perhaps by unmasking underlying amygdalar and/or GABAergic abnormalities. Such pharmacological fMRI paradigms could prove useful for developing drugs targeting specific neural circuits to treat or prevent schizophrenia.

Keywords

pharmacological fMRI; benzodiazepine; endophenotype; amygdala; facial emotion; GABA; schizophrenia

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Introduction

The use of functional MRI (fMRI) to elucidate the pathophysiology of psychiatric illness can be enhanced by pharmacologic manipulation of specific neural circuits (Wise and Tracey 2006). Abnormal neural drug responses identified by pharmacological fMRI may provide evoked phenotypes for further examination. Here we use the benzodiazepine alprazolam to probe potential abnormalities in the neural circuitry of emotion processing in first-degree relatives of patients with schizophrenia.

Schizophrenia is associated with prominent deficits in emotion processing. These include impaired identification of facial emotions (Heimberg et al. 1992; Kohler et al. 2009), which has been linked to broader social dysfunction (Addington et al. 2006; Meyer and Kurtz 2009) and prioritized as a target for translational research into negative symptoms of schizophrenia (Carter et al. 2009). First-degree relatives of individuals with schizophrenia exhibit similar but less severe cognitive, emotion-processing, and neurophysiological abnormalities (Gur et al. 2007b; MacDonald et al. 2009; Phillips and Seidman 2008). Investigation of family members offers a strategy to investigate neurobehavioral abnormalities related to schizophrenia risk without confounds such as antipsychotic medication effects, and can help clarify whether emotion-processing deficits reflect a schizotypal state, or only emerge with frank illness (Rasetti et al. 2009).

Neuroimaging may be more sensitive than behavior in revealing abnormalities related to genetic predisposition (Rasch et al. 2010). In patients with schizophrenia, abnormalities in amygdala and its interactions with other regions may mediate observed behavioral deficits (Gur et al. 2007a; Satterthwaite et al. 2010). However, the only prior fMRI study examining emotion-identification in family members reported normal behavioral and amygdala responses (Rasetti et al. 2009). Two fMRI studies of mood induction in family members reported functional abnormalities in amygdala and other brain regions during negative but not positive moods (Habel et al. 2004; Schneider et al. 2007). Neurobehavioral emotion-processing abnormalities in family members may thus be relatively subtle, condition-specific, and/or reduced by compensatory mechanisms. Therefore, the current study utilized two complementary emotion-processing tasks, facial emotion identification and facial emotion memory, in conjunction with drug challenge, to evaluate emotion-processing performance and probe amygdala function.

Prior imaging studies of facial emotion processing in schizophrenia have reported decreased (Li et al. 2009), increased (Hall et al. 2009; Holt et al. 2006), and normal amygdala activation which may in part reflect different analytic approaches (Anticevic et al.). Previously, we reported a link between higher amygdala activation and negative symptom severity (Gur et al. 2007a). Abnormal amygdala activation could relate to aberrant GABAergic signaling, with deficient GABAergic inhibition producing hyperactivation. GABAergic deficits in schizophrenia, best characterized in prefrontal cortex (Hashimoto et al. 2008), are also found in medial temporal lobe where they are hypothesized to dysregulate amygdalo-hippocampal interactions (Berretta et al. 2001; Simpson et al. 1989; Spokes et al. 1980), potentially contributing to emotion-processing abnormalities. The GABAergic deficit in schizophrenia may be primarily presynaptic, with compensatory post-synaptic upregulation of specific benzodiazepine-sensitive GABA receptors (Lewis et al. 2005). This could increase sensitivity to changes in GABA signaling, consistent with heightened lorazepam effects in an fMRI study of working memory in patients with schizophrenia (Menzies et al. 2007).

These considerations suggest that GABAergic drugs such as benzodiazepines could differentially impact amygdala function in family members, providing mechanistic insight

into emotion-processing endophenotypes. Anxiolytic effects of benzodiazepines depend on GABAergic inhibition of amygdala and related limbic structures (Davis 1992).

Benzodiazepines impair emotion identification and emotional memory in healthy subjects, an effect that may also depend on amygdala inhibition (Buchanan et al. 2003; Coupland et al. 2003). Consistent with this mechanism, the two fMRI studies examining benzodiazepine effects on facial emotion processing in healthy people both reported reductions in amygdala recruitment (Del-Ben et al.; Paulus et al. 2005). However, effects of benzodiazepines on emotion-processing tasks have not yet been investigated in schizophrenia patients or their relatives.

Given limited evidence of normal amygdala activation during emotion processing in family members (Rasetti et al. 2009), we expected our results to shed additional light on this important issue. If baseline amygdala function indeed proved normal, alprazolam challenge could help distinguish between two competing interpretations. One interpretation is that amygdala dysfunction seen in schizophrenia during emotion processing occurs only as a consequence of frank illness or antipsychotic effects (Rasetti et al. 2009). Alternatively, normal baseline amygdala responses could reflect successful compensatory mechanisms. By unmasking the hypothesized compensatory increase in GABAergic sensitivity, alprazolam challenge could provide evidence of an underlying primary abnormality. Thus we predicted that regardless of whether family members showed amygdala activation abnormalities under placebo, they would demonstrate more robust reductions in amygdala activity by alprazolam.

Methods

Participants

The sample included 20 healthy adults with a first-degree relative affected by schizophrenia and 27 healthy controls without a family history of psychosis. Groups were matched on demographic and clinical variables (Table 1). Data analysis was limited to subjects with usable data from both study days for at least one fMRI task (20 family, 25 control). Because of technical problems affecting some scans (see Supplementary Methods), final analyzed samples for imaging varied slightly across different imaging components: 20 family, 24 control for emotion identification; 19 family, 25 control for emotion memory; 19 family, 25 control for perfusion.

Study procedures were approved by the University of Pennsylvania IRB. Participants underwent standard medical, neurological, psychiatric, and neurocognitive evaluations (detailed in Supplementary Methods). After complete description of the study to the subjects, written informed consent was obtained.

Study design and pharmacological challenge

Each subject underwent two identical fMRI sessions approximately 1 week apart. On one day they received 1mg oral alprazolam, and on the other day an identical-appearing placebo, in a balanced double-blind within-subject crossover design. Drug was administered under direct staff observation 1 hour before fMRI, so that alprazolam levels and effects were near their peak (Greenblatt et al. 1988) during scanning.

Immediately following the MRI sessions, blood was drawn and stored for later measurement of alprazolam plasma levels (HPLC assay with LLOQ 5ng/mL performed by NMS Labs, Willow Grove, PA). Blood samples could not be obtained from 2 controls and 1 family member. Subjects then provided numerical ratings (0–10 Likert scale) of changes in subjective states of sedation (“sleepy”) and relaxation (“relaxed”) that might result from benzodiazepine administration. Blood pressure and pulse were measured while seated.

Subjects and research staff also guessed whether they had taken alprazolam or placebo that day, to allow assessment of blinding efficacy (Perlis et al. 2010).

Imaging tasks

During both MRI sessions, subjects completed an emotion identification task followed by an emotion memory task (Fig. 1). To control for practice effects, two equivalent forms of both tasks were administered in a counterbalanced order. The study design with two identical test days required an explicit rather than implicit memory task; subjects were informed ahead of time that they would be asked to remember the emotional faces they saw during the emotion identification task, and practiced both tasks briefly before scanning. Stimuli were color pictures of human faces conveying emotional expressions, selected from a larger database (Gur et al. 2002). In the emotion identification task, participants viewed 60 unique faces and decided which emotion (happy, sad, anger, fear, or neutral) was expressed on each face. There were 12 presentations for each of the 5 expressions. In the emotion memory task, participants viewed faces of the same individuals seen in the identification task, but each stimulus displayed three emotional expressions for that individual: one was the same expression seen in the preceding emotion identification task, and two were foil expressions. Subjects were instructed to choose the expression that matched the previously seen expression. There were 12 trials from each of the 5 target emotion categories; each emotion was also used as a foil 24 times. In both tasks, faces were displayed for 5.5 seconds, followed by a variable (0.5–18.5s) interval during which subjects fixed their gaze on a complex crosshair that was matched to faces on perceptual qualities. Both tasks contained 60 trials in an event-related design, pseudorandomized using optseq2 (D. Greve, <http://surfer.nmr.mgh.harvard.edu/optseq/>) (Dale 1999). Each task lasted 10.5 minutes, one run per task, with a 2-minute delay between tasks.

Image acquisition and processing

Images were acquired with a Siemens Trio 3T (Erlangen, Germany) system, in the following order: structural, resting perfusion, identification BOLD, memory BOLD. Acquired BOLD volumes spanned the temporal lobe and inferior frontal and occipital lobes, this slab was chosen to allow both good coverage of ventral regions of interest (Fig. S1) and high spatial resolution (2×2×2mm). To ensure the slab included the same anatomical regions across sessions and subjects we utilized an automated FOV-prescription algorithm (ImScribe, <http://www.mmrrcc.upenn.edu/mediawiki/index.php/ImScribe>, see Supplementary Methods).

BOLD fMRI data were preprocessed and analyzed using FEAT (FMRI Expert Analysis Tool, v. 5.9), part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). Pulsed Arterial Spin Label (PASL) perfusion images were processed and quantified using SPM5 software according to standard procedures (Wang et al. 2008). See Supplementary Methods for further acquisition and processing details.

Individual subject image analysis

Subject-level timeseries analysis was carried out using FILM (FMRIB's Improved General Linear Model) (Woolrich et al. 2001). All event conditions were modeled in the GLM as 5.5 second boxcars convolved with a canonical hemodynamic response function; temporal derivatives for each condition and 6 motion parameters were included as nuisance regressors. For the identification task, each of the 5 emotions (correct responses only) was modeled as a separate regressor; errors and non-responses across all emotions were modeled as separate nuisance regressors. In the memory task three different emotions (1 target and 2 foil) were present in each trial; therefore, the model included only three regressors: correct responses, incorrect responses, and nonresponses. For both tasks, the first-level contrast of

interest reflected activation to correct responses regardless of emotion relative to fixation baseline.

Group-level region of interest image analysis

For analysis of group-level effects, a region of interest (ROI) approach was used to test *a priori* hypotheses (Fig. S1). The primary ROI was the bilateral amygdala (Supplementary Methods detail ROI definitions). Bilateral hippocampus was selected as a secondary ROI, given its close functional and spatial relationship to the amygdala, and the amnesic effects of benzodiazepines (Barbee et al. 1992). We also examined two additional task-activated control regions in order to assess the regional specificity of any observed effects (Fig. S2 shows voxelwise task-activation maps). Right fusiform cortex was chosen as a task-activated sensory cortical region, and bilateral orbitofrontal cortex was chosen as a control emotion-processing region. For each of these four ROIs, individual subject activation (percent signal change) was extracted for offline mixed model analysis (see below). As our primary focus was on the single *a priori* amygdala ROI, we did not correct statistical comparisons for the use of the other three ROIs.

Group-level exploratory voxelwise analyses

In addition to ROI-based group analyses, we conducted exploratory voxelwise analyses to uncover effects outside predicted regions. Subject-level statistical maps were transformed into MNI space and resampled to 2mm isotropic voxels. Within-subject fixed effects were calculated across both scan days, including the drug-placebo difference, as well as an average activation contrast across drug conditions. These second-level within-subject contrasts were then subjected to group-level analyses examining group differences in average activation (group effect), drug-placebo differences common to both groups (drug effect), and group $\times$ drug interactions (group differences in the drug effect). Group-level random effects analyses were performed in FSL, using FMRIB Local Analysis of Mixed Effects (FLAME1) (Woolrich et al. 2004). All voxelwise analyses were corrected for multiple comparisons by cluster correction within FSL based on Gaussian Random Field Theory, using voxel height $Z > 2.33$ and cluster extent $p > 0.05$ (minimum significant cluster extent 195 $2 \times 2 \times 2$ mm voxels).

Mixed model analysis and other statistical tests

Effects of group, drug, and their interaction were examined using mixed-model analysis of variance (MIXED procedure) implemented in SAS (Gary, Indiana), with group (control vs. family) and drug (alprazolam vs. placebo) as fixed effects and subjects as a random factor. Age, gender, test day, and task form were included as covariates in statistical models and retained if they explained a significant amount of variance. Effects of age and day are reported only where significant; gender and task form did not have a significant effect on any analyzed study outcome. Assessment of potential confound variables was conducted by inclusion one at a time in the above mixed models, even if they did not explain a significant amount of variance.

Descriptive statistics and uncorrected post-hoc t-tests or nonparametric equivalents were used to explicate significant results from the mixed model. For between-group comparisons of demographic and clinical variables, Student's t-tests were used. Categorical variables (gender, handedness, and smoking status) were investigated with Fisher's Exact Test. For non-normal variables (age, anxiety), the Wilcoxon's Rank Sum Test was applied. For all statistical tests, significance was determined using an alpha criterion of $p < 0.05$, two-tailed.

Behavioral performance analysis

For behavioral data, efficiency was used as an overall measure of performance. Each subject's efficiency was calculated as the proportion of correct responses divided by response time (within-subject median reaction time for correct responses). Error rates (the proportion of responses which were incorrect) were used to describe performance accuracy. Behavioral data was tested for group and drug effects using a mixed model analysis as above.

Results

Behavioral performance

Family members showed relatively normal behavioral performance in both tasks, with the exception of slower and less accurate identification of sad emotions (Table S1, Fig. S3). Alprazolam significantly decreased efficiency by 7% during emotion identification [$F(1,42)=11.52$; $p=0.002$] and by 20% during emotion memory [$F(1,41)=22.10$; $p<0.0001$]. Neither task showed a main effect of group, or group $\times$ drug interaction (all p 's >0.5). Older subjects were less efficient in emotion identification [$F(1,42)=8.11$; $p=0.007$; see Fig. S4] but not emotion memory ($p=0.203$). Efficiency was increased on the second test day for emotion identification [$F(1,42)=11.16$; $p=0.002$], but was lower on the second day for emotion memory [$F(1,41)=5.16$; $p=0.028$]. Neither age nor test day showed interactions with drug or group.

Emotion identification fMRI

Amygdala response during emotion identification was blunted by alprazolam only in family members [Fig. 2; group $\times$ drug interaction $F(1,42)=5.82$; $p=0.020$]. There was no main effect of group [$F(1,42)=0.90$; $p=0.349$] or drug [$F(1,42)=2.21$; $p=0.144$]. Left and right amygdala showed qualitatively similar effects. Examining the placebo data alone, amygdala activation did not differ between the two groups ($p=0.527$).

Effects in hippocampus were similar to those found in amygdala, with a significant group $\times$ drug interaction [$F(1,42)=4.39$; $p=0.042$] but no drug ($p=0.772$) or group ($p=0.107$) effects. As predicted, no significant effects were found in either fusiform cortex (drug, $p=0.867$; group $p=0.144$; drug $\times$ group, $p=0.549$) or orbitofrontal cortex (drug, $p=0.485$; group, $p=0.089$; drug $\times$ group $p=0.690$).

Exploratory voxelwise analysis revealed a significant effect of alprazolam across groups in cerebellum and thalamus (Table S2). No other significant drug effects, group effects, or group $\times$ drug interactions were found.

Emotion memory fMRI

Bilateral amygdala activation during emotion memory was strongly reduced by alprazolam [Fig. 3; $F(1,42)=11.97$; $p=0.001$], without any effect of group ($p=0.695$) or group $\times$ drug interaction ($p=0.783$). The same pattern was also present in bilateral hippocampus, with a main effect of drug [$F(1,42)=8.49$; $p=0.006$], without any group ($p=0.346$) or group $\times$ drug interaction ($p=0.595$). No significant effects were observed in fusiform (drug, $p=0.177$; group $p=0.093$; drug $\times$ group, $p=0.650$) or orbitofrontal cortex (drug, $p=0.283$; group $p=0.394$; drug $\times$ group, $p=0.899$). Across groups, drug-induced reductions in hippocampal activation correlated with drug-induced reductions in memory performance efficiency ($r=0.37$, $p=0.014$; see Fig. S5).

Exploratory voxelwise analysis revealed that alprazolam reduced activation for the emotion memory task across both groups in the right amygdala and hippocampus, with subthreshold

effects on the left (Fig. 3, Table S2). Significant drug effects were also seen in the left lateral occipital cortex and left inferior frontal gyrus. No other significant main effects or interactions were found.

Potential confounds

We carefully evaluated important potential confounds that might bias our results. As detailed below, the above findings were largely unaffected by potential confounding factors, including anxiety, sedation, motion artifact, unblinding, drug levels and cardiovascular drug effects.

Anxiety—As expected, trait anxiety and pre-medication state anxiety were unaffected by alprazolam (no main effect or group $\times$ drug interaction, p 's > 0.2). Consistent with prior studies reporting a lack of anxiety reduction measured with the STAI in healthy subjects following benzodiazepine challenge (Monteiro et al. 1990; Paulus et al. 2005; Schunck et al. 2010), there was no significant main or interaction effect of alprazolam on measures of state anxiety after drug administration (pre-scan and post-scan STAI scores as a percentage of pre-medication scores, p 's > 0.1). Alprazolam did produce a significant increase in subjective relaxation, which was greater in family members (main effect of drug $p = 0.008$, group $\times$ drug interaction $p = 0.019$). Including STAI scores or subjective relaxation in the model rendered the group $\times$ drug interaction in the hippocampus during emotion identification nonsignificant. However, all other behavioral and imaging results were unaffected (including amygdala effects in both tasks, and hippocampal effects during the recognition task).

Sedation—Alprazolam increased subjective sedation (“sleepy”) significantly in both groups ($p < 0.001$), with a trend towards a group $\times$ drug interaction ($p = 0.051$) due to a trend toward larger effects in family members (post-hoc Tukey-Kramer corrected $p = 0.081$). Missed responses were used as an objective measure of in-scanner sedation; this revealed a significant drug effect ($p < 0.001$ for both tasks) without any group or interaction effect (p 's > 0.5). However, fMRI models limited the effect of sedation by focusing on correct responses only, and amygdala activation from these models showed no relationship to subjective or objective measures of sedation. The main effect of reduced hippocampal activation under alprazolam during the recognition task was reduced to trend significance ($p = 0.080$) by inclusion of subjective sedation. All other imaging and behavioral results remained significant after controlling for sedation.

Motion-related image artifact—Alprazolam reduced temporal signal-to-noise ratio (SNR) in BOLD data during both tasks (p 's < 0.02) without any significant group or group $\times$ drug interaction effects (all p 's > 0.1). SNR was strongly related to in-scanner motion ($r = -0.79$) but SNR was more sensitive to drug effects than motion parameters. SNR showed no significant relationship to amygdala activation in either task (p 's > 0.29), and its inclusion in the statistical models did not change the significance of reported amygdala results. SNR did relate significantly to hippocampal activation during recognition ($p = 0.047$) but not identification ($p = 0.294$). Including SNR in the statistical models did not alter the reported results of drug in the recognition task but did slightly reduce the significance of the hippocampus group $\times$ drug interaction during emotion identification to trend levels ($p = 0.060$). Notably, the group $\times$ drug interaction in the amygdala was unaffected.

Unblinding—There was partial unblinding of subjects, with 70.3% correct guesses regarding drug condition. Subjects were significantly more likely to guess they had received alprazolam during the alprazolam condition than the placebo condition ($p < 0.001$). This is not surprising given that subjective side effects differed by drug condition. However,

unblinding did not differ between groups ($p>0.4$), and did not relate to reported performance or BOLD activation measures; nor did inclusion of subjects' guesses alter the significance of any reported findings. Examination of experimenter guesses yielded very similar levels of unblinding, and did not relate to or alter reported results.

Unblinding was prominent on Day 2 but not Day 1, suggesting that comparison of subjective feelings on Day 2 vs. Day 1 made it easier to identify treatment condition on Day 2. Notably, on Day 1 subjects' accuracy was at chance levels (53.3% correct guesses). We therefore analyzed the successfully-blinded Day 1 data separately. Critically, despite reduced statistical power, reported findings for group $\times$ drug interaction in amygdala during identification remained significant in analysis of Day 1 data alone. The group $\times$ drug effect in hippocampus during identification and the drug effect in amygdala and hippocampus during recognition remained qualitatively similar but were no longer statistically significant.

Alprazolam blood levels—Based on prior literature (Streeter et al. 1998; Volkow et al. 1995), we did not expect strong relationships between acute alprazolam levels and our outcome measures, but examined drug level as a potential confounding variable. Blood levels after alprazolam administration did not differ by group, test day, age or gender ($p's>0.2$). Inclusion of drug levels in statistical models did not alter results reported above. For the identification task, in the alprazolam condition there was no effect of blood level nor any group $\times$ level interaction effect on behavioral efficiency or activation of amygdala or hippocampus. In the memory task, higher blood levels related to lower activation in hippocampus ($p=0.049$) but not amygdala ($p>0.70$), with a trend towards lower behavioral efficiency ($p=0.066$). Two control subjects had undetectable levels of alprazolam despite supervised pill administration, which could reflect assay error or differences in absorption and metabolism. Exclusion of these two subjects did not alter these confound analysis results nor the drug, group, or drug $\times$ group interaction effects reported above.

Cardiovascular effects—Alprazolam produced small but statistically significant reductions in seated systolic blood pressure (2%, $p<0.020$) and increases in heart rate (6%, $p<0.001$). There were no significant effects of group or group $\times$ drug interactions on these physiological measures ($p's>0.17$). There was no relationship between reported fMRI results and these physiological measures ($p's>0.14$), nor did inclusion of physiological measures in the statistical models affect the significance of reported results.

Discussion

We used alprazolam challenge during fMRI to examine the effects of GABAergic modulation on potential emotion-processing abnormalities in first-degree relatives of individuals with schizophrenia. During emotion identification, alprazolam reduced amygdala activation only in family members, indicating that benzodiazepines may unmask an abnormality in amygdala function that could relate to GABAergic hypersensitivity.

Implications for benzodiazepine response as a schizophrenia endophenotype

This cohort of family members did not show robust deficits in emotion processing, as assessed by both behavior and neural activation; abnormalities were only revealed by the pharmacologic challenge. Family members demonstrated differential suppression of amygdala responses by alprazolam during emotion identification, an effect not seen in controls or other face- or emotion-processing brain regions. Family members showed relatively normal behavioral performance, except for greater difficulty identifying sad expressions; this sample did not show the broad behavioral deficits observed in larger samples (Gur et al. 2007b). In the placebo condition amygdala activation during emotion

identification was also normal in family members. While specific task features or power limitations may contribute, this result is consistent with a recent study using a similar fMRI task (Rasetti et al. 2009), which concluded that abnormal amygdala activation during emotion processing in schizophrenia reflects the disease state rather than a schizotypal predisposition.

Amygdala sensitivity to GABAergic modulation provides a potential schizophrenia endophenotype. To date, no studies have evaluated benzodiazepine effects on emotion processing or amygdala function in schizophrenia. However, the single fMRI study examining benzodiazepine effects in schizophrenia (Menzies et al. 2007) did report greater lorazepam-induced abnormalities in fronto-parietal activation during a working memory task in patients compared to controls.

The observed augmentation of alprazolam's effect on the amygdala may therefore reflect widespread abnormalities in GABAergic neurotransmission, with regional selectivity arising from specific task demands. Lewis and colleagues have identified GABAergic abnormalities in multiple cortical areas in schizophrenia and hypothesize an inhibitory deficit (Hashimoto et al. 2008). More limited investigation also supports GABAergic deficits in amygdala and hippocampus (Berretta et al. 2001; Simpson et al. 1989; Spokes et al. 1980). If a milder form of this deficit is present in family members, post-synaptic compensatory mechanisms (Lewis et al. 2005) might produce the enhanced sensitivity to GABAergic stimulation seen here.

Our results implicate a distinctive pattern of responses to alprazolam in family members, rather than a general increase in drug response that might occur secondary to more rapid blood-brain absorption or other nonspecific mechanisms. We would expect a nonspecific increase in drug responses to lead to greater effects on task performance in family members, which was not observed. Furthermore, the absence of effects on global perfusion in this study (Fig. S6) makes it unlikely that our findings reflect global alterations in perfusion due to cardiovascular or other nonspecific drug effects (Roy-Byrne et al. 1993; Wise and Tracey 2006). Finally, in the memory task, the drug-induced reduction in medial temporal lobe BOLD responses was equivalent between groups and correlated with behavioral impairment. This is consistent with the interpretation that the memory task activated amygdalo-hippocampal circuits that were sensitive to the amnesic effects of benzodiazepines (Barbee et al. 1992), but without any increased sensitivity among family members.

Implications for pharmacological fMRI

In the emotion identification task, abnormal responses to alprazolam in family members were found in fMRI activation but not task performance, supporting claims that fMRI can enhance sensitivity beyond behavioral measures alone (Rasch et al. 2010). In contrast, during the emotion memory task, both task performance and medial temporal lobe activation were robustly suppressed by alprazolam. These results highlight the strong dependence of pharmacological fMRI measures on the psychological tasks employed. Examination of drug effects across multiple tasks that engage distinct psychological processes and neural circuits may be useful in assessing novel agents. As seen here, such an approach allows simultaneous evaluation of the neurobehavioral mechanisms of both desirable and undesirable effects. For example, such an approach may aid in the development of more selective GABAergic medications (Lewis et al. 2008) that impact anxiety without producing sedation, incoordination, or amnesia. Selective serotonin reuptake inhibitors effective in treating anxiety disorders reduce amygdala activation during facial emotion processing (Arce et al. 2008; Harmer et al. 2006; Windischberger et al. 2010), as do some other drugs with anxiolytic effects including MDMA ("ecstasy") and tetrahydrocannabinol (Bedi et al. 2009; Phan et al. 2008). Pharmacological fMRI comparing agents with common clinical

effects but distinct molecular mechanisms could help identify critical neural targets and biomarkers of therapeutic drug response.

Limitations

The current study had several limitations. We did not include a group of patients with schizophrenia, and do not know if the specific alprazolam-induced abnormality seen here in family members is present to a similar or even greater extent in patients, as would be expected for an endophenotype. Therefore, the possibility that abnormalities observed here in family members reflect a schizophrenia risk endophenotype remains speculative, requiring further investigation. Our group of family members also included a mix of both parents and siblings of affected patients, which likely increases heterogeneity related to age, education, and generational factors. While this may limit sensitivity, it is unlikely to explain observed group effects given that groups were well-matched on demographic factors. Also, our family members did not show significantly elevated levels of schizotypy, perhaps because of the self-selection of voluntary participation in a two-day pharmacological fMRI study. In future studies, explicit inclusion of subjects with higher levels of schizotypy together with more detailed measures of negative symptoms and social function could yield stronger effects, and inclusion of a schizophrenia group could help establish whether alprazolam effects can identify useful endophenotypes. Finally, alprazolam produced effects on sedation, motion-related fMRI artifact, heart rate and blood pressure, and partial unblinding, which could all potentially impact neurobehavioral outcomes. However, careful analysis of these potential confounds strengthened the study, and the key findings in the amygdala remain robust after taking these into account.

Summary and conclusions

We report the first study examining benzodiazepine effects on emotion processing and amygdala function in unaffected first-degree relatives of patients with schizophrenia. In contrast to their normal amygdala responses under placebo, family members exhibit exaggerated drug-induced reductions in amygdala activation during emotion identification. GABAergic activation by benzodiazepines may unmask amygdala dysfunction during emotion identification that reflects an endophenotypic marker of schizophrenia. These results should encourage further use of such pharmacological fMRI paradigms in developing and testing drugs that target specific neural circuits in order to treat or prevent schizophrenia or other psychiatric disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Addington J, Saeedi H, Addington D. Facial affect recognition: a mediator between cognitive and social functioning in psychosis? *Schizophr Res*. 2006; 85:142–150. [PubMed: 16678388]
2. Anticevic A, Van Snellenberg JX, Cohen RE, Repovs G, Dowd EC, Barch DM. Amygdala Recruitment in Schizophrenia in Response to Aversive Emotional Material: A Meta-analysis of Neuroimaging Studies. *Schizophr Bull*. 2010 e-pub ahead of print 28 December.
3. Arce E, Simmons AN, Lovero KL, Stein MB, Paulus MP. Escitalopram effects on insula and amygdala BOLD activation during emotional processing. *Psychopharmacology (Berl)*. 2008; 196:661–672. [PubMed: 18058090]
4. Barbee JG, Black FW, Todorov AA. Differential effects of alprazolam and buspirone upon acquisition, retention, and retrieval processes in memory. *J Neuropsychiatry Clin Neurosci*. 1992; 4:308–314. [PubMed: 1498583]
5. Bedi G, Phan KL, Angstadt M, de Wit H. Effects of MDMA on sociability and neural response to social threat and social reward. *Psychopharmacology (Berl)*. 2009; 207:73–83. [PubMed: 19680634]
6. Berretta S, Munno DW, Benes FM. Amygdalar activation alters the hippocampal GABA system: "partial" modelling for postmortem changes in schizophrenia. *J Comp Neurol*. 2001; 431:129–138. [PubMed: 11169955]
7. Buchanan TW, Karafin MS, Adolphs R. Selective effects of triazolam on memory for emotional, relative to neutral, stimuli: differential effects on gist versus detail. *Behav Neurosci*. 2003; 117:517–525. [PubMed: 12802880]
8. Carter CS, Barch DM, Gur R, Pinkham A, Ochsner K. CNTRICS final task selection: social cognitive and affective neuroscience-based measures. *Schizophr Bull*. 2009; 35:153–162. [PubMed: 19011231]
9. Coupland NJ, Singh AJ, Sustrik RA, Ting P, Blair R. Effects of diazepam on facial emotion recognition. *J Psychiatry Neurosci*. 2003; 28:452–463. [PubMed: 14631456]
10. Dale AM. Optimal experimental design for event-related fMRI. *Hum Brain Mapp*. 1999; 8:109–114. [PubMed: 10524601]
11. Davis M. The role of the amygdala in fear and anxiety. *Annu Rev Neurosci*. 1992; 15:353–375. [PubMed: 1575447]
12. Del-Ben CM, Ferreira CA, Sanchez TA, Alves-Neto WC, Guapo VG, de Araujo DB, Graeff FG. Effects of diazepam on BOLD activation during the processing of aversive faces. *J Psychopharmacol*. 2010 e-pub ahead of print 24 November.
13. Greenblatt DJ, Harmatz JS, Dorsey C, Shader RI. Comparative single-dose kinetics and dynamics of lorazepam, alprazolam, prazepam, and placebo. *Clin Pharmacol Ther*. 1988; 44:326–334. [PubMed: 3138056]
14. Gur RC, Sara R, Hagendoorn M, Marom O, Hughett P, Macy L, Turner T, Bajcsy R, Posner A, Gur RE. A method for obtaining 3-dimensional facial expressions and its standardization for use in neurocognitive studies. *J Neurosci Methods*. 2002; 115:137–143. [PubMed: 11992665]
15. Gur RE, Loughhead J, Kohler CG, Elliott MA, Lesko K, Ruparel K, Wolf DH, Bilker WB, Gur RC. Limbic activation associated with misidentification of fearful faces and flat affect in schizophrenia. *Arch Gen Psychiatry*. 2007a; 64:1356–1366. [PubMed: 18056543]
16. Gur RE, Nimgaonkar VL, Almasy L, Calkins ME, Ragland JD, Pogue-Geile MF, Kanes S, Blangero J, Gur RC. Neurocognitive endophenotypes in a multiplex multigenerational family study of schizophrenia. *Am J Psychiatry*. 2007b; 164:813–819. [PubMed: 17475741]
17. Habel U, Klein M, Shah NJ, Toni I, Zilles K, Falkai P, Schneider F. Genetic load on amygdala hypofunction during sadness in nonaffected brothers of schizophrenia patients. *Am J Psychiatry*. 2004; 161:1806–1813. [PubMed: 15465977]
18. Hall J, Whalley HC, McKirdy JW, Sprengelmeyer R, Santos IM, Donaldson DI, McGonigle DJ, Young AW, McIntosh AM, Johnstone EC, Lawrie SM. A common neural system mediating two different forms of social judgement. *Psychol Med*. 2009; 1–10. [PubMed: 19335938]

19. Harmer CJ, Mackay CE, Reid CB, Cowen PJ, Goodwin GM. Antidepressant drug treatment modifies the neural processing of nonconscious threat cues. *Biol Psychiatry*. 2006; 59:816–820. [PubMed: 16460693]
20. Hashimoto T, Bazmi HH, Mirnics K, Wu Q, Sampson AR, Lewis DA. Conserved regional patterns of GABA-related transcript expression in the neocortex of subjects with schizophrenia. *Am J Psychiatry*. 2008; 165:479–489. [PubMed: 18281411]
21. Heimberg C, Gur RE, Erwin RJ, Shtasel DL, Gur RC. Facial emotion discrimination: III. Behavioral findings in schizophrenia. *Psychiatry Res*. 1992; 42:253–265. [PubMed: 1496057]
22. Holt DJ, Kunkel L, Weiss AP, Goff DC, Wright CI, Shin LM, Rauch SL, Hootnick J, Heckers S. Increased medial temporal lobe activation during the passive viewing of emotional and neutral facial expressions in schizophrenia. *Schizophr Res*. 2006; 82:153–162. [PubMed: 16377154]
23. Kohler CG, Walker JB, Martin EA, Healey KM, Moberg PJ. Facial Emotion Perception in Schizophrenia: A Meta-analytic Review. *Schizophr Bull*. 2009; 36:1009–1019. [PubMed: 19329561]
24. Lewis DA, Cho RY, Carter CS, Eklund K, Forster S, Kelly MA, Montrose D. Subunit-selective modulation of GABA type A receptor neurotransmission and cognition in schizophrenia. *Am J Psychiatry*. 2008; 165:1585–1593. [PubMed: 18923067]
25. Lewis DA, Hashimoto T, Volk DW. Cortical inhibitory neurons and schizophrenia. *Nat Rev Neurosci*. 2005; 6:312–324. [PubMed: 15803162]
26. Li H, Chan RC, McAlonan GM, Gong QY. Facial Emotion Processing in Schizophrenia: A Meta-analysis of Functional Neuroimaging Data. *Schizophr Bull*. 2009; 36:1029–1039. [PubMed: 19336391]
27. MacDonald AW 3rd, Thermenos HW, Barch DM, Seidman LJ. Imaging genetic liability to schizophrenia: systematic review of fMRI studies of patients' nonpsychotic relatives. *Schizophr Bull*. 2009; 35:1142–1162. [PubMed: 18556667]
28. Menzies L, Ooi C, Kamath S, Suckling J, McKenna P, Fletcher P, Bullmore E, Stephenson C. Effects of gamma-aminobutyric acid-modulating drugs on working memory and brain function in patients with schizophrenia. *Arch Gen Psychiatry*. 2007; 64:156–167. [PubMed: 17283283]
29. Meyer MB, Kurtz MM. Elementary neurocognitive function, facial affect recognition and social-skills in schizophrenia. *Schizophr Res*. 2009; 110:173–179. [PubMed: 19328653]
30. Monteiro MG, Schuckit MA, Irwin M. Subjective feelings of anxiety in young men after ethanol and diazepam infusions. *J Clin Psychiatry*. 1990; 51:12–16. [PubMed: 2295585]
31. Paulus MP, Feinstein JS, Castillo G, Simmons AN, Stein MB. Dose-dependent decrease of activation in bilateral amygdala and insula by lorazepam during emotion processing. *Arch Gen Psychiatry*. 2005; 62:282–288. [PubMed: 15753241]
32. Perlis RH, Ostacher M, Fava M, Nierenberg AA, Sachs GS, Rosenbaum JF. Assuring that double-blind is blind. *Am J Psychiatry*. 2010; 167:250–252. [PubMed: 20194487]
33. Phan KL, Angstadt M, Golden J, Onyewuenyi I, Popovska A, de Wit H. Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. *J Neurosci*. 2008; 28:2313–2319. [PubMed: 18322078]
34. Phillips LK, Seidman LJ. Emotion processing in persons at risk for schizophrenia. *Schizophr Bull*. 2008; 34:888–903. [PubMed: 18644853]
35. Rasch B, Papassotiropoulos A, de Quervain DF. Imaging genetics of cognitive functions: Focus on episodic memory. *Neuroimage*. 2010; 53:870–877. [PubMed: 20060913]
36. Rasetti R, Mattay VS, Wiedholz LM, Kolachana BS, Hariri AR, Callicott JH, Meyer-Lindenberg A, Weinberger DR. Evidence that altered amygdala activity in schizophrenia is related to clinical state and not genetic risk. *Am J Psychiatry*. 2009; 166:216–225. [PubMed: 19074979]
37. Roy-Byrne P, Fleishaker J, Arnett C, Dubach M, Stewart J, Radant A, Veith R, Graham M. Effects of acute and chronic alprazolam treatment on cerebral blood flow, memory, sedation, and plasma catecholamines. *Neuropsychopharmacology*. 1993; 8:161–169. [PubMed: 8471128]
38. Satterthwaite TD, Wolf DH, Loughhead J, Ruparel K, Valdez JN, Siegel SJ, Kohler CG, Gur RE, Gur RC. Association of enhanced limbic response to threat with decreased cortical facial recognition memory response in schizophrenia. *Am J Psychiatry*. 2010; 167:418–426. [PubMed: 20194482]

39. Schneider F, Habel U, Reske M, Toni I, Falkai P, Shah NJ. Neural substrates of olfactory processing in schizophrenia patients and their healthy relatives. *Psychiatry Res.* 2007; 155:103–112. [PubMed: 17532193]
40. Schunck T, Mathis A, Erb G, Namer IJ, Demazieres A, Luthringer R. Effects of lorazepam on brain activity pattern during an anxiety symptom provocation challenge. *J Psychopharmacol.* 2010; 24:701–708. [PubMed: 19460871]
41. Simpson MD, Slater P, Deakin JF, Royston MC, Skan WJ. Reduced GABA uptake sites in the temporal lobe in schizophrenia. *Neurosci Lett.* 1989; 107:211–215. [PubMed: 2616032]
42. Spokes EG, Garrett NJ, Rossor MN, Iversen LL. Distribution of GABA in post-mortem brain tissue from control, psychotic and Huntington's chorea subjects. *J Neurol Sci.* 1980; 48:303–313. [PubMed: 6449563]
43. Streeter CC, Ciraulo DA, Harris GJ, Kaufman MJ, Lewis RF, Knapp CM, Ciraulo AM, Maas LC, Ungeheuer M, Szulewski S, Renshaw PF. Functional magnetic resonance imaging of alprazolam-induced changes in humans with familial alcoholism. *Psychiatry Res.* 1998; 82:69–82. [PubMed: 9754450]
44. Volkow ND, Wang GJ, Hitzemann R, Fowler JS, Pappas N, Lowrimore P, Burr G, Pascani K, Overall J, Wolf AP. Depression of thalamic metabolism by lorazepam is associated with sleepiness. *Neuropsychopharmacology.* 1995; 12:123–132. [PubMed: 7779240]
45. Wang Z, Aguirre GK, Rao H, Wang J, Fernandez-Seara MA, Childress AR, Detre JA. Empirical optimization of ASL data analysis using an ASL data processing toolbox: ASLtbx. *Magn Reson Imaging.* 2008; 26:261–269. [PubMed: 17826940]
46. Windischberger C, Lanzenberger R, Holik A, Spindelegger C, Stein P, Moser U, Gerstl F, Fink M, Moser E, Kasper S. Area-specific modulation of neural activation comparing escitalopram and citalopram revealed by pharmacofMRI: a randomized cross-over study. *Neuroimage.* 2010; 49:1161–1170. [PubMed: 19833214]
47. Wise RG, Tracey I. The role of fMRI in drug discovery. *J Magn Reson Imaging.* 2006; 23:862–876. [PubMed: 16649197]
48. Woolrich MW, Behrens TE, Beckmann CF, Jenkinson M, Smith SM. Multilevel linear modelling for FMRI group analysis using Bayesian inference. *Neuroimage.* 2004; 21:1732–1747. [PubMed: 15050594]
49. Woolrich MW, Ripley BD, Brady M, Smith SM. Temporal autocorrelation in univariate linear modeling of FMRI data. *Neuroimage.* 2001; 14:1370–1386. [PubMed: 11707093]

Fig. 1.

The *experimental paradigm* is illustrated above. Subjects initially performed an *emotion identification task* (a) in which they identified the facial affect displayed. Five emotional labels were available: happy, sad, anger, fear, and neutral. During the *emotion memory task* (b) that followed, subjects were asked to remember which expression had been previously displayed during the emotion identification task. One target emotion and two foils were displayed. In both tasks, faces were displayed for 5.5 seconds, followed by a variable (0.5–18.5s) interval during which subjects fixed their gaze on a complex crosshair that was matched to faces on perceptual qualities. Both tasks contained 60 trials in an event-related design; each task lasted 10.5 minutes, with a 2-minute delay between tasks

Fig. 2. Emotion identification imaging results: Family members of patients with schizophrenia displayed a significant reduction of amygdala activity during face identification when given alprazolam (ALP) relative to placebo (PLC). Controls did not demonstrate this effect. Error bars indicate standard error of the mean

Fig. 3. Emotion memory imaging results: Relative to placebo (PLC), alprazolam (ALP) produced a significant reduction of activity during emotional face memory in the amygdala and hippocampus in both controls and family members. Error bars indicate standard error of the mean

Table 1

Demographic and clinical variables

Variable	CONTROLS (n=25)		FAMILY (n=20)		p-value
	Percentage	Proportion	Percentage	Proportion	
Gender (% F)	52.0%	13F/12M	55.0%	11F/9M	0.99
Handedness (% R)	92.0%	23R/2L	80.0%	16R/4L	0.38
Smoke (% N)	80.0%	20N/5Y	80.0%	16N/4Y	1
	Mean (SD)	Range	Mean (SD)	Range	
Age (yrs)	39.0 (10.7)	22.3–56.3	42.3 (14.8)	20.9–59.4	0.28
Education (yrs)	14.8 (1.9)	11–18	14.8 (2.3)	11–20	0.95
Parental Education	13.4 (3.1)	7.5–20	13.9 (2.7)	9.5–18	0.62
Height (in)	67.3 (3.7)	61–74	67.6 (4.3)	60–73	0.79
Weight (lb)	173.3 (32.0)	115–225	176 (34)	118–250	0.83
BMI (lb/in ² *in)	27.0 (4.9)	20.4–36.8	27.0 (4.5)	18.7–33.9	0.99
Trait Anxiety	28.6 (6.7)	20–48	30.2 (8.9)	20–58	0.73
Schizotypy (SIS) Total	11.9 (7.2)	1–29	15.0 (7.2)	7–39	0.17
Alpraz. Level (ng/mL)	7.2 (4.1)	0–13	7.8 (4.1)	0.97–14.0	0.66

Abbreviations: F female, M male, R right, L left, N nonsmoker, Y smoker, SD standard deviation, BMI body mass index, SIS Structured Interview for Schizotypy