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# Temporal profile of brain response to alprazolam in patients with generalized anxiety disorder

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ABSTRACT

This study investigated the temporal pattern of brain response to emotional stimuli during 28 days of alprazolam treatment among patients with generalized anxiety disorder (GAD) randomized 2:1 to drug or placebo in a double-blind design. Functional magnetic resonance imaging scans obtained during an emotion face matching task (EFMT) and an affective stimulus expectancy task (STIMEX) were performed at baseline, one hour after initial drug administration and 28 days later. Alprazolam significantly reduced scores on the Hamilton Anxiety Scale and the Penn State Worry Questionnaire after one week and 28 days of treatment. Brain activation in the amygdala during the EFMT and in the insula during the STIMEX was reduced one hour after alprazolam administration but returned to baseline levels at Day 28. Exploratory analyses revealed significant treatment differences in brain activity during the STIMEX on Day 28 in frontal lobe, caudate nucleus, middle temporal gyrus, secondary visual cortex, and supramarginal gyrus. These results are consistent with the notion that the neural mechanisms supporting sustained treatment effects of benzodiazepines in GAD differ from those underlying their acute effects.

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## 1. Introduction

Alprazolam (ALZ) is indicated for treatment of panic disorder and generalized anxiety disorder (Verster and Volkerts, 2004; Bereza et al., 2012). In clinical trials, the anxiolytic effects of ALZ observed within the first week can be maintained up to two months (Verster and Volkerts, 2004). Like other benzodiazepines, ALZ presumably attenuates the core symptoms of generalized anxiety disorder (GAD) by indirectly promoting gamma-amino butyric acid (GABA) activity (Davidson et al., 2010). In addition to stable anxiolytic effects, ALZ is associated with dynamic neurobehavioral changes, appearing as temporally linked side effects and dose tolerance (Barbarito et al., 1996; Verster and Volkerts, 2004). The dynamic side effects of ALZ observed among patients with anxiety disorders has an analog in neurobehavioral studies of healthy volunteers, where impaired visuomotor performance and alertness after administration of ALZ often dissipate after one to three weeks of drug treatment (Bourin et al., 1998; Verster and Volkerts, 2004). It has been hypothesized that benzodiazepine induced tolerance is due to GABA(A) receptor down regulation or reduced coupling between GABA(A) receptor/chloride channel gating and benzodiazepine receptor binding (Schoch et al., 1993; Galici et al., 1998). The presence of GABA(A) receptors in the amygdala along with the contribution of the amygdala to fear conditioning supports the claim that perturbations of GABA (A) receptors fundamentally contribute to the genesis of anxiety (Graeff et al., 1993; Tomaz et al., 1993). The hypothesis that dynamic changes of GABA(A) function contribute to the treatment tolerance and to other time-linked neurobehavioral changes associated with benzodiazepine use is a natural extension of the GABAergic anxiogenesis claim. In support of this hypothesis, 24 days of oral ALZ administered to healthy human subjects decreased GABA(A) receptor density in whole brain from day 3 to day 10, before returning to baseline levels by day 17 (Fujita et al., 1999).

Whether dynamic changes in the brain's responsiveness to emotional stimuli occur among patients with anxiety disorders during the first month of treatment remains is an open question. Because of its non-invasive nature and its repeatability, blood oxygen level dependent (BOLD) functional magnetic resonance

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imaging (fMRI) is especially useful in the study of dynamic brain changes during treatment (Paulus and Stein, 2007). Studies performed to date show that BOLD fMRI may be sensitive to treatment changes in anxiety disorders. Open-label pre–post-studies have shown that fMRI signals either predict treatment effects or sensitively monitor treatment with a variety of anti-anxiety drugs (Hoehn-Saric et al., 2004; Whalen et al., 2008; Nitschke et al., 2009; Phan et al., 2013). Among the studies reviewed above, only the Whalen et al. study assessed brain activation at more than two time points, limiting the dynamic information that could be gleaned from these designs.

The current study is a double-blind, placebo-controlled, subchronic ALZ treatment study in patients with GAD, involving both fMRI and clinical outcomes. Three fMRI sessions were performed: baseline, one hour after the first ALZ administration (acute) and 28 days later (subchronic). In each session, two fMRI tasks were presented. One task investigated brain activation following presentation of pictures of emotional faces, whereas the other investigated activation during an anticipatory task, where the presentation of adverse or pleasant pictures was cued. We analyzed functional brain imaging data in the amygdala and rostral anterior cingulate – *a priori* regions of interest (ROIs) that are core neural substates of anxiety circuits and which have been found to be involved in all anxiety disorders including GAD (Duval et al., 2015; Holzschneider and Mulert, 2011). We also investigated the effect of ALZ on brain activity in the insula, another component of the anxiety circuit that responds to pharmacotherapy or cognitive-behavioral therapy in GAD patients (Fonzo et al., 2014; Hoehn-Saric et al., 2004). Within each ROI we specifically investigated the questions: (1) Does ALZ response occur in study ROIs after one hour of ALZ administration? (2) Is there an ALZ treatment response in study ROIs that is detectable after one month of treatment?

## 2. Patients and methods

### 2.1. Study design

In this double-blind, placebo-controlled, randomized, parallel group study subjects were treated with ALZ (starting dose of 0.5 mg bid and escalated to 1 mg bid on day 3 unless not tolerated) or placebo for 28 days. Following a two-week washout period of psychotropic drugs (five weeks for fluoxetine), 32 subjects were randomized to a treatment group with twice the number of subjects targeted to receive active treatment than placebo. Investigators remained blind to each participant's condition throughout the duration of the study. Four subjects (3 on ALZ and 1 on placebo) did not escalate to 1 mg because of mild adverse events. One subject withdrew before treatment was initiated due to claustrophobia during the training MRI scan. A subject in the ALZ group was withdrawn early due to moderate fatigue, leaving 19 participants receiving ALZ and 11 receiving placebo.

### 2.2. Participants

Written informed consent was obtained from all study subjects. Right-handed individuals 18–64 years of age were recruited from San Diego County through advertisements posted on radio, in newspapers, or online. Subjects pre-screened by telephone were invited to a full screening visit that included collection of medical and medication history, a physical examination performed by study physicians (OS, MP, MS), psychological interview and tests and laboratory assessments including electrocardiogram.

Diagnoses were based on symptoms derived from the Mini-International Neuropsychiatric Interview for DSM-IV (Sheehan et al., 1998) administered by licensed clinical psychologists [GB, CW]. Subjects were required to have GAD according to DSM-IV criteria (exception: at least 3 months of symptoms) as determined by a consensus diagnostic group led by the study psychiatrists [MBS, MP]. GAD participants also had to have a Hamilton Anxiety Rating Scale score at screening $\geq 20$ and a Montgomery–Asberg Depression Rating Scale (MADRS) score at screening $< 25$ (Hamilton, 1959; Montgomery and Asberg, 1989). Participants needed to be in good general health as determined by the medical evaluation described above. Key exclusion criteria were an Axis I disorder including current depressive episode or dysthymia if the MADRS at screening exceeded 25; current treatment of GAD; drug or alcohol dependence in the past six months; medications that could interfere either with the fMRI or with clinical assessments; smoking more than 10 cigarettes per day; a body mass index $\geq 32.5$ kg/m$^2$. Intact mental status was confirmed by the Dementia Rating Scale – 2 (Jurica et al., 2004).

### 2.3. Clinical endpoints

Prior to dosing, subjects completed the trait portion of the Spielberger State Trait Anxiety Inventory (STAI) (Spielberger et al., 1983), the HAM-A, the Quick Inventory of Depressive Symptomatology—Self Report 16 items (QIDS-SR16) (Rush et al., 2003), and the Penn State Worry Questionnaire (PSWQ) (Meyer et al., 1990). During screening, subjects were asked to undergo an abbreviated functional magnetic resonance imaging (fMRI) session to familiarize them with the procedure. On days 7 and 28, the HAM-A, PSWQ, and QIDS-SR16 were performed in order to assess clinical efficacy of the study medication. Subjects received the Karolinska Sleepiness Scale (KSS) before and after each imaging session (Åkerstedt and Gillberg, 1990).

### 2.4. FMRI methods

Subjects underwent full imaging sessions at baseline (i.e. within a week prior to the first dose of study medication), on day 1 (start of imaging approximately 60 minutes after taking the 0.5 mg dose of ALZ or placebo) and on day 28. Imaging task parameters and the subject level image analysis plan were developed in a three session reliability study to assess the stability of individual differences in BOLD response. Reliability improved when two runs of each task were averaged and when all faces types were aggregated as described below for the EFMT (unpublished data).

#### 2.4.1. Paradigms for BOLD imaging

*2.4.1.1. Emotional Faces Matching Task (EFMT).* The EFMT robustly activates the amygdala (Paulus et al., 2005; Arce et al., 2008). Blocks of 5 s trials consisted of presentation of a target face and two probe faces. Participants were instructed to select the probe with the same emotional expression as the target by pressing the left or right key on a button box. A block of stimuli consisted of six consecutive trials in which the target face was consistently angry, fearful, or happy. During shape matching control blocks, subjects were presented with 5 s trials of ovals or circles in an analogous configuration and were instructed to match the shape of the probe to the target. Four versions of the task were created involving different faces and different ordering of face and shape matching trials. The task duration was about 8.5 min with two versions of the task presented to each subject during each session.

*2.4.1.2. Stimulus Expectancy Task (STIMEX).* The STIMEX is a continuous performance task that has reliably activated the anterior insula in prior studies (Simmons et al., 2006; Aupperle et al., 2011). The STIMEX assesses the impact of anticipating affective stimuli on attention to a response signal and on the subsequent response