Published in final edited form as:

Prog Neuropsychopharmacol Biol Psychiatry. 2011 August 15; 35(7): 1645–1652. doi:10.1016/j.pnpbp.2011.05.015.

Zolpidem reduces the blood oxygen level-dependent signal during visual system stimulation

Stephanie C. Licata^a, Steven B. Lowen^{a,b}, George H. Trksak^{a,c}, Robert R. MacLean^a, and Scott E. Lukas^{a,b,c}

Stephanie C. Licata: slicata@mclean.harvard.edu; Steven B. Lowen: lowen@mclean.org; George H. Trksak: gtrksak@mclean.harvard.edu; Robert R. MacLean: rrmaclean@gmail.com; Scott E. Lukas: lukas@mclean.harvard.edu

^a Behavioral Psychopharmacology Research Laboratory, McLean Hospital/Harvard Medical School, 115 Mill Street, Belmont MA, 02478, USA

^b Brain Imaging Center, McLean Hospital/Harvard Medical School, 115 Mill Street, Belmont MA, 02478, USA

^c Sleep Research Laboratory, McLean Hospital/Harvard Medical School, 115 Mill Street, Belmont MA, 02478, USA

Abstract

Zolpidem is a short-acting imidazopyridine hypnotic that binds at the benzodiazepine binding site on specific GABA_A receptors to enhance fast inhibitory neurotransmission. The behavioral and receptor pharmacology of zolpidem has been studied extensively, but little is known about its neuronal substrates *in vivo*. In the present within-subject, double-blind, and placebo-controlled study, blood oxygen level-dependent functional magnetic resonance imaging (BOLD fMRI) at 3 Tesla was used to assess the effects of zolpidem within the brain. Healthy participants (n=12) were scanned 60 minutes after acute oral administration of zolpidem (0, 5, 10, or 20 mg), and changes in BOLD signal were measured in the visual cortex during presentation of a flashing checkerboard. Heart rate and oxygen saturation were monitored continuously throughout the session. Zolpidem (10 and 20 mg) reduced the robust visual system activation produced by presentation of this stimulus, but had no effects on physiological activity during the fMRI scan. Zolpidem's modulation of the BOLD signal within the visual cortex is consistent with the abundant distribution of GABA_A receptors localized in this region, as well as previous studies showing a relationship between increased GABA-mediated neuronal inhibition and a reduction in BOLD activation.

Keywords

zolpidem; hypnotic; BOLD fMRI; GABA_A; visual cortex

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Corresponding author: Stephanie C. Licata, Ph.D., McLean Hospital/Harvard Medical School, 115 Mill Street, Neuroimaging Center, Belmont MA, 02478, USA, Phone: (+001) 617-855-2738, Fax: (+001) 617-855-3711, slicata@mclean.harvard.edu.

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1. Introduction

Zolpidem (Ambien®) is a short-acting imidazopyridine hypnotic that increases GABA-mediated inhibition via positive allosteric modulation at the benzodiazepine binding site on specific GABA_A receptors (Tallman et al., 1978). Although the typical sedative-hypnotic effects of zolpidem have been well documented in both laboratory animals (e.g., Sanger et al., 1987) and humans (see review by Rush, 1998), only a handful of studies have employed imaging to understand zolpidem's neurobiological substrates *in vivo*. Positron emission tomography (PET) and single-photon emission computed tomography (SPECT) have shown that in neurologically abnormal humans (Brefel-Courbon et al., 2007; Clauss et al., 2000, 2004; Cohen et al., 2004; Hall et al., 2010) and baboons (Clauss et al., 2001, 2002), zolpidem increased cerebral perfusion in areas that were hypoactive as a result of brain injury. Otherwise, frontal reductions in brain metabolism (Brefel-Courbon et al., 2007) or an absence of drug-induced changes in brain perfusion (Clauss et al., 2001) were observed. In healthy humans during sleep, zolpidem reduced cerebral glucose metabolism (Gillin et al., 1996) and cerebral blood flow (CBF; Finelli et al., 2000) in a number of frontal regions including white matter and anterior cingulate, as well as basal ganglia, insula, and hippocampus. Simultaneously, zolpidem administration also increased CBF to regions such as parietal and occipital cortices, parahippocampal gyrus, and cerebellum (Finelli et al., 2000), collectively suggesting a complex action *in vivo*.

To date, no studies have used functional magnetic resonance imaging (fMRI) to investigate zolpidem's effect on brain function in awake, healthy humans. Like PET and SPECT, fMRI provides indirect measures of neuronal activity depending on cerebral oxygenation, blood flow, and blood volume. Typically, changes in the blood oxygen level-dependent (BOLD) signal are used to visualize the relationship between the brain's hemodynamic response to a stimulus and presumed changes in neuronal activity. In the present study, fMRI correlates of zolpidem's action were investigated. It was hypothesized that based on previous studies showing a relationship between increased GABA-mediated neuronal inhibition and a reduction in the BOLD signal (Chen et al., 2005; Donahue et al., 2010; Muthukumaraswamy et al., 2009, 2011; Northoff et al., 2007), zolpidem would reduce BOLD activation similarly due to its GABA-enhancing pharmacological effects. Specifically, BOLD signal changes were measured during presentation of a flashing checkerboard to healthy zolpidem-naïve volunteers following acute oral administration of zolpidem (0, 5, 10, and 20 mg). This stimulus produces robust activation of the visual cortex (Kwong et al., 1992), and it has been shown to be sensitive to the activation-reducing effects of other positive GABA_A receptor modulators such as alcohol (Levin et al., 1998) or pentobarbital (Martin et al., 2000).

2. Methods

2.1 Participants

Twelve healthy zolpidem-naïve male (6) and female (6) right-handed non-smoking volunteers between the ages of 21–35 (mean $\pm$ SD age was 24.2 ± 2.3 years) participated in this study. Volunteers reported ≤ 10 lifetime recreational experiences with drugs of abuse, no family history of alcoholism, no personal history of diagnosis of a DSM-IV Axis I or neurological disorder, no scanning contraindications, and no regular medications. Also, volunteers were required to tolerate the high dose of zolpidem during a [pilot] laboratory visit (i.e., they did not vomit or report nausea following acute oral administration of 20 mg zolpidem) in order to be invited to participate further in the full study. Participants were instructed not to eat breakfast and they were required to abstain from alcohol and caffeine for at least 12 hr prior to all study visits. Upon arriving at the laboratory on each visit, participants were screened for drug use (QuickTox® urine screen kits, Branan Medical Corporation; Irvine, CA) and breath alcohol level (AlcoSensor, Intoximeter; Saint Louis,

MO). Female participants underwent a QuPID® urine pregnancy test (Stanbio Laboratory; Boerne, TX); pregnancy was a contraindication in this study. Participants testing positive on any screen would have been rescheduled and sent home (although none were). A standard breakfast was provided to all participants to control for stomach contents on the rate of drug absorption. All participants were transported to and from the laboratory via taxicab. This study was reviewed and approved by the McLean Hospital Institutional Review Board, and it was in accord with the Declaration of Helsinki. All volunteers provided verbal and written informed consent and they were compensated for their participation.

2.2 Study design

There were four separate fMRI scanning visits in this randomized, double-blind, placebo-controlled, within-subject study (for a total of 48 scanning sessions). Each BOLD scan that took place during passive visual stimulation began approximately 60 min (57.56 ± 1.04 min, mean $\pm$ SEM) following oral administration of one of four treatment conditions (0, 5, 10, or 20 mg zolpidem). BOLD scanning also occurred during a key press task that followed directly after the passive visual stimulation. Heart rate and oxygen saturation were recorded throughout the 35-min scanning session.

2.2.1 BOLD scans—Stimuli for both the passive visual stimulation and key press scans were presented to participants using a rear-projection system running Presentation® software (Version 11.0; Neurobehavioral Systems, Inc.; Albany, CA). A mirror positioned on the head coil at a 45-degree angle and approximately 13 cm from the participants' eyes permitted participants to view stimuli on a screen positioned 173 cm away from the mirror. Prior to beginning all BOLD scans participants were engaged in conversation to ensure they were awake, and they were instructed to focus on the screen.

A flashing radial checkerboard (100% contrast at 8 Hz) was employed as a passive visual stimulus; the checkerboard subtended a field of 12 degrees (vertical) by 17 degrees (horizontal). The average gray value did not change over time, and the largest contiguous region of the same color was less than two degrees. Therefore, no change over time would be detected through closed eyelids. The 56 images (3 sec each, 168 sec total) were divided into seven blocks of eight images (24 sec) each; blocks alternated between a fixation point and the checkerboard, starting with the fixation point. The passive visual stimulation BOLD scan (56 TRs, 174 sec) followed a spontaneous activity resting state scan and preceded a key press task scan, the former of which will be reported on elsewhere. The key press task (144 TRs, 7:18) required participants to press one button on a fiber-optic response device (Current Designs, Inc.; Philadelphia, PA) when odd numbers were presented and the opposite button for even numbers. Numbers were presented during half of the total TRs (for 1 sec, followed by 2 sec fixation) while a fixation point was presented during the other half (for 3 sec).

2.2.2. Data acquisition—Scans were performed on a 3 Tesla Siemens Trio MR imaging system (Siemens AG, Erlangen, Germany). A 3-plane scout scan (conventional FLASH sequence with isotropic voxels of 2.8 mm) was acquired and used for prescription of the fMRI image stack: gradient echo EPI, TR/TE= 3000/30 ms, 224×224 mm FOV, 41 3.5-mm interleaved axial slices starting from the spinal cord covering the entire brain, no gap, AP readout, 64×64 pixel, full k-space acquisition, no SENSE acceleration; pulse sequence-enhanced version of the Siemens epibold, yielding isotropic 3.5 mm voxels. Each BOLD scan included an additional two images (6 sec) at the beginning that were not acquired, in order to ensure steady-state magnetization. Automatic second order shimming was performed over the fMRI imaging volume prior to acquisition. Other scans were inserted between the visual stimulation and key press task functional runs to serve as inter-trial

breaks. A conventional T1 scan was performed on the same functional prescription; therefore it had identical susceptibility distortion (“matched-warped”; identical geometry to the fMRI scans except for 256×256 pixels; Rohan et al., 2001). A standard T1 weighted MP-RAGE3D scan (FOV= $256 \times 256 \times 170$ mm, $256 \times 256 \times 128$) also was collected.

2.2.3 Data pre-processing—Except where noted, data processing was performed with FSL release 4.1 (FMRIB Analysis Group; Oxford University, United Kingdom). Pre-processing procedures included nonlinear de-spiking filtering (using an in-house program), motion estimation and correction with six degrees of freedom, slice timing, removal of non-brain regions, spatial filtering with a 5 mm full width half maximum Gaussian kernel, global (4D) normalization, and high pass temporal filtering with a cut-off of 48 sec. Functional results were aligned with the matched-warped scan with six degrees of freedom, which then was aligned to the high-resolution MP-RAGE scan with 12 degrees of freedom. In the final registration step, the MP-RAGE scan was aligned with the MNI152 standard brain with 12 degrees of freedom. Rendering of the functional results in MNI space was performed once, following concatenation of the three alignments into a single matrix. A summary of this registration was monitored for each run of each participant. Finally, for the tensor independent component analysis only, the data were re-sampled to (4 mm³) resolution.

2.2.4 Statistical modeling: GLM—For generalized linear model (GLM) processing, regularized autocorrelation functions were estimated independently for each voxel using temporal Tukey prewhitening (Woolrich et al., 2001). The regressor of interest was set to 1 during volumes when the flashing checkerboard was visible and 0 when the uniform gray background appeared, and was demeaned. This regressor then was subjected to a linear filter modeling the default hemodynamic response function (gamma impulse response, width of 3 sec, and mean lag of 6 sec), as well as the same temporal filter that was applied to the data. Demeaned versions of the six motion estimates obtained from motion correction (rotation and translation about all three axes) were used as nuisance regressors, without temporal filtering.

Single-scan results were combined in a mixed-effects analysis with automatic outlier detection (Woolrich, 2008). Differences between each of the active doses and placebo formed the three regressors of interest which were combined to yield an ANOVA test. A nuisance regressor was included for each participant, and it was set to unity for the four scans for that participant and zero for other scans. All results were converted first to Z scores, and thresholded to a significance level of $p < 0.01$ (uncorrected). Using Gaussian random field theory, clusters achieving a cluster-wide significance level of $p < 0.05$ were identified, and family-wise error-corrected for multiple comparisons over the whole brain. Two-tailed *post-hoc* tests were carried out for all twelve comparisons across dose for voxels that exhibited significant ANOVA results. The significance level was Bonferroni-corrected for these six tests to $p < 0.05/6 = 0.00833$ uncorrected. A functional region of interest (ROI) was determined by setting the Z score threshold to 4 and selecting the main resulting cluster in the averaged response over all participants and doses. This ROI was used to generate a binary mask. Percent change of BOLD response was averaged over this mask for each scan, and changes across dose were assessed with an ANOVA.

2.2.5 Statistical modeling: TICA—Pre-processed data were analyzed using MELODIC (c.f., Beckmann and Smith, 2004), which implemented a tensor independent component analysis (TICA). This method decomposes the data from all 48 scans into components that are spatially independent. Each component in TICA has three parts: a spatial map displaying the activation associated with that component as it is distributed over the brain, a temporal waveform that describes the time progression of that component, and a scan-specific number describing the strength of the component for that particular participant and drug treatment

condition. TICA is particularly well-suited for experiments that employ the same stimulus for each scan, thereby permitting application of the same temporal component to all scans. Although GLM analysis has been the standard method for analyzing data with external stimuli that are identical over all scans (Friston et al., 1995), this approach necessitates specification of the temporal response to the stimuli. In contrast, TICA estimates the temporal response directly from the data. MELODIC uses a principal component analysis as a first stage of data reduction. Automatic dimension estimation yielded 33 components, but the visual system response was split into many components, making it difficult to obtain meaningful data. The number of components was set to 10 in order to combine responses to derive a single response covering the visual cortex. Variance normalization was used to de-emphasize the amplitude of the fluctuations of each voxel over time. Finally, the temporal waveform of each component was compared with the expected response to the stimulus in the visual cortex, taking into account the hemodynamic response. This approach permits the selection of components likely to represent visual system activation without requiring detailed *a priori* knowledge of the resulting temporal dynamics. Differences across dose in the TICA values obtained for each scan per participant were assessed using ANOVA.

2.2.6 Physiological monitoring—Physiologic data were collected using MRI-compatible equipment (In Vivo Research; Orlando, FL), and included heart rate and oxygen saturation. Although these measures were collected every second throughout the scan, they were computed as 5-min averages over the 35-min scanning session.

2.3 Statistical analyses

Visual stimulation data were analyzed using one-way repeated measures ANOVA to examine the effect of drug treatment on activation of the functional ROI within the visual cortex as well as on tensor strength of the activated components chosen from the visual cortex. Key press behavioral data (percentage of correct responses and time to press the key) also were analyzed using one-way repeated measures ANOVA to examine the effect of drug treatment on behavioral response. Heart rate and oxygen saturation were analyzed using two-way repeated measures ANOVAs examining the within-subjects factors of treatment by time. The voxelwise ANOVA was computed within FSL, but all other data were analyzed using standard statistical software (SigmaStat 3.1; Systat Software, Inc.; San Jose, CA and SPSS 17.0 for Windows; SPSS Inc.; Chicago, IL) and with alpha set at 0.05. When appropriate, post hoc tests were performed to assess treatment effects using Bonferroni *t*-tests for multiple comparisons.

3. Results

3.1 BOLD fMRI

No visual stimulation scan had motion that exceeded a Euclidean distance equal to the voxel size (3.5 mm), and registration appeared normal for all 48 scans.

3.1.2 Visual Stimulation GLM—The GLM analysis showed robust activation of the visual cortex produced by the flashing checkerboard when all four treatment conditions were averaged together (cluster $p < 10^{-20}$). Visual system activation was significantly reduced (cluster $p < 10^{-23}$) following the highest dose of zolpidem (20 mg) compared to placebo (Figure 1), while the lower doses resulted in less extensive reductions in activation (data not shown). The voxel-based ANOVA yielded a single cluster in the right hemisphere with a volume of 4.768 ml (cluster $p < 0.005$) that comprised lingual gyrus, occipital fusiform gyrus, and the inferior division of the lateral occipital cortex (Figure 2). For each of the 596 voxels within the cluster, between one and four post-hoc tests were significant, including 587 voxels (98%) for the test assessing differences between placebo and 20 mg. All such

post-hoc tests revealed reduced activation following administration of the highest dose and are shown in Figure 2 where color represents the number of significant post-hoc tests. BOLD activation differed significantly across dose in lateral occipital cortex, inferior division, occipital fusiform gyrus, lingual gyrus, and occipital pole.

The functional ROI within the visual cortex had a volume of 134 ml. Within this specified ROI, the average (mean $\pm$ SEM) percent change from placebo was -31.75 ± 20.27 for 5 mg, -21.38 ± 28.06 for 10 mg, and -65.48 ± 24.43 for 20 mg zolpidem (Figure 3; shown in black bars). While zolpidem treatment had a significant effect on activation in the visual cortex during presentation of the flashing checkerboard [$F_{(3,33)} = 3.626$, $p = 0.023$], the *post-hoc* analysis revealed an effect only of the high dose (20 mg; $p = 0.007$).

3.1.3 Visual Stimulation TICA—The TICA procedure yielded 10 components that together captured 65% of the variance, for a data compression ratio of 0.36%. Of these components, seven had significant ($p < 0.05/10 = 0.005$) correlation with the expected temporal response to presentation of the flashing checkerboard (Table 1). Of those, one exhibited significant activation in V1 and V2, the expected loci of activation within the visual system (1st component; TICA-1), while another component was localized to the cuneus and calcarine cortex (4th component; TICA-4). Although both exhibited highly significant correlations ($p < 10^{-8}$) with the expected response to the visual stimulus, the analysis was focused on TICA-1 due to the variability associated with TICA-4. Not only did TICA-4 explain much less of the variance in the analysis relative to TICA-1 (6.9 % vs. 21.30 %; see Table 1), but within the TICA-4 dataset there was a great deal of variability among activation values as determined by the error measurements across the treatment conditions (average SEM = 109 for TICA-4 vs. average SEM = 13 for TICA-1). Thus, scan-specific values for the TICA-1 component, one per participant per dose, were extracted and analyzed as described above.

Figure 4 shows the time course and the spatial distribution of the 1st independent component (TICA-1). In addition to the actual time course the expected response to the visual stimulus also is shown, which takes into account the hemodynamic response in the brain as well as the pre-processing temporal filter. There was excellent agreement between the actual time course and the expected time course (Pearson's $r > 0.986$). The spatial extent of this component and its relative restriction to V1 and V2 also is shown in Figure 4. Within this specified component, the average percent change (mean $\pm$ SEM) from placebo was -33.74 ± 12.94 for 5 mg, -34.53 ± 12.69 for 10 mg, and -63.47 ± 11.54 for 20 mg zolpidem (Figure 3; shown in gray bars). Zolpidem treatment had a significant effect on activation in this component within the visual cortex during presentation of the flashing checkerboard [$F_{(3,33)} = 7.200$, $p < 0.001$], such that the *post-hoc* analysis revealed an effect of the high dose (20 mg; $p < 0.001$), the middle dose (10 mg; $p = 0.032$), and a trend toward a significant effect with the low dose (5 mg; $p = 0.064$).

Considering the potentially soporific effects of the drug, the data were examined for a distribution pattern that may have differentiated scans performed during an eyes open state (i.e., awake) from those during an eyes closed state. A pilot test in which eyes were open for three scans and closed for three scans yielded tensor strengths of 3.26 ± 0.30 and 0.18 ± 0.14 (mean $\pm$ SEM), respectively. Therefore a bimodal distribution could be expected such that an eyes closed state would result in activation values centered on zero, while the eyes open state would result in values centered on another larger value. In contrast, examination of the experimental tensor strength values per participant were indistinguishable from a uniform distribution ($p = 0.587$; Kolmogorov-Smirnov test), arguing against the possibility that observed results were a function of participants having their eyes closed during the flashing checkerboard scan.

3.2 Key press task

Performance during the key press task was affected by zolpidem. The maximum amount of time allotted to press the key after stimulus presentation (i.e., reaction time) was 3 s, and only data from participants who made no responses at all were entered as 3 s. One such entry was made for the 5 mg dose, two for 10 mg, and three for 20 mg of zolpidem. Otherwise the time to press a key ranged from 0.6266 s to 1.662 s. Time to press the correct key showed a dose effect [$F_{(3,33)} = 3.619$, $p = 0.023$] such that the 20 mg dose increased the reaction time relative to placebo [$p = 0.034$; Bonferroni-corrected for multiple comparisons]. When null responses (those coded as 3 s) were removed from the analysis, a significant treatment effect still was apparent [$F_{(3,21)} = 6.688$, $p = 0.002$]. Similar to reaction time, the percentage of correct responses made decreased with increasing dose [$F_{(3,33)} = 4.849$, $p = 0.007$] and was significantly reduced by the 20 mg dose [$p = 0.004$; Bonferroni-corrected for multiple comparisons].

3.3 Physiological measurements

Out of the 12 participants who completed this study, full sets of heart rate and oxygen saturation data (i.e., data for all four treatment conditions) from the scanning session were collected for only eight participants due to equipment failures. Statistical analysis of both the heart rate and oxygen saturation data indicated no effects of treatment, time, or any interactions between those factors (data not shown).

4. Discussion

Acute oral administration of zolpidem reduced the robust visual system activation produced by presentation of the flashing checkerboard. The greatest impact on the BOLD signal was observed following administration of the highest dose (20 mg), but the therapeutic dose (10 mg) also reduced the BOLD response. Although zolpidem dose had a differential on the BOLD signal, a previous report of subjective drug effects in this study cohort indicated that participants were able to detect effects of all three doses relative to placebo (Licata et al., 2011). To model the data, we employed three different statistical methodologies (both voxelwise and functional ROI for GLM as well as TICA) in order to take advantage of their individual strengths. While the ROI and TICA approaches met the expectation of being more robust than the voxelwise approach due to aggregation of the signal over a larger volume, all methods yielded similar results. The observed results agree with previous fMRI studies showing that other positive GABA_A receptor modulators such as alcohol (Levin et al., 1998) or pentobarbital (Martin et al., 2000) reduced activation in visual cortex during a stimulation paradigm.

Similar to the benzodiazepines, zolpidem increases fast inhibitory neurotransmission by enhancing the affinity of GABA_A receptors for GABA (Macdonald and Olsen, 1994; Perrais and Ropert, 1999). However, unlike benzodiazepines, zolpidem exhibits relatively selective affinity for $\alpha 1$ -containing GABA_A receptors (Benavides et al., 1988; Sanna et al., 2002). Of the highly concentrated population of GABA_A receptors within the visual cortex (Albin et al., 1991; Eickhoff et al., 2007; Hendry et al., 1994), more than half of them are the zolpidem-sensitive $\alpha 1$ -containing subtype (Hendrickson et al., 1994). This receptor specificity may explain why previous findings demonstrating that acute challenge with non-GABAergic drugs such as nicotine (Marutle et al., 1998) or cocaine (Kufahl et al., 2005) had little effect on BOLD signal stability during pharmacological MRI in a similar experimental paradigm (Gollub et al., 1998; Jacobsen et al., 2002; Lowen et al., 2009).

How synaptic inhibition manifests itself as a decrease in BOLD response during fMRI has been a matter of intense study due to the fact that inhibition is an active synaptic process

requiring glucose and oxygen consumption (Jueptner and Weiller, 1995), together which should increase the BOLD signal (Ogawa et al., 1990). Recent work employing fMRI combined with magnetic resonance spectroscopy has probed the link between inhibition and BOLD signal change to show that increased GABA levels were associated with a reduction in positive BOLD responses (Chen et al., 2005; Donahue et al., 2010; Muthukumaraswamy et al., 2009, 2011; Northoff et al., 2007). While it is tempting to attribute the observed zolpidem-induced reduction in BOLD to inhibitory action potentials generated via enhanced GABA_A receptor activity, another consideration is zolpidem's effect on vascular tone within the brain. Not only has application of exogenous GABA or GABA agonists to isolated cerebral blood vessels (Edvinsson and Krause, 1979) or brain slices (Fergus and Lee, 1997) resulted in vasodilatory effects that could increase CBF, but zolpidem previously has been shown to increase regional CBF within the occipital cortex (Finelli et al., 2000). Although increased blood flow theoretically should lead to a larger BOLD response, it is important to note that the relationship between CBF and BOLD is complicated by the confounding effects of drug influence on resting perfusion. Specifically, it has been shown that manipulations (such as drug administration in the present study) that increase resting CBF subsequently decrease the BOLD signal during a behavioral challenge (Brown et al., 2003; Cohen et al., 2002). The absence of a CBF measurement is an important limitation to overcome in future fMRI investigations of zolpidem's neurobiological substrates (Iannetti and Wise, 2007), but if the assumption is made that based on previous work zolpidem would increase resting perfusion (Finelli et al., 2000), then an argument can be made for a zolpidem-induced reduction in the BOLD signal.

Another confounding factor in the interpretation of the dose-related effects of zolpidem on the BOLD signal is the absence of objective data regarding which individuals may have closed their eyes during the task. Employing an eye-tracking device or incorporating an auditory stimulus in addition to the visual stimulus could have circumvented this confound. However, examination of the present data for any inconsistencies indicating that maybe not all participants saw the flashing checkerboard due to zolpidem-induced sedation with corresponding eye closure suggested this was not the case. In addition, previous work has shown that during early sleep there is an increase in CBF in visual association cortices (Kjaer et al., 2002) and an increase in the occipital cortex with zolpidem specifically (Finelli et al., 2000), both of which are contrary to the present results. Furthermore, in agreement with other studies demonstrating that zolpidem has little to no effect on blood pressure, heart rate, or respiration (Evans et al., 1990; Licata et al., 2008; McCann et al., 1993), physiological activity (heart rate and oxygen saturation) did not change with any dose of zolpidem relative to placebo. Thus respiration was unchanged, further suggesting that sleep did not occur.

Zolpidem-associated reductions in BOLD response within the visual system also could be attributed to reduced attentional capacity due to drug treatment. It has been established that zolpidem's effects include reducing attention (Mattila et al., 1998), and attention modulates the BOLD signal within visual cortex such that the temporal profiles of attention onset and V1 BOLD response are similar (e.g., Smith et al., 2006). Although specific measures of attention were not obtained during presentation of the flashing checkerboard, behavioral results from the key press task following the visual stimulation scan may lend support to an attention-related hypothesis. Longer reaction times and/or reductions in the percentage of correct responses made in concert with increasing doses of zolpidem suggest that participants may have become increasingly inattentive to the stimuli being presented. Alternatively, these behavioral data may indicate that participants succumbed to the motor-impairing effects of zolpidem (Licata et al., 2009), particularly because manual dexterity has been shown to decline following a high dose of zolpidem (Desager et al., 1988). Additional evidence against attentional deficits underlying the decrease in BOLD signal was reported in

conference proceedings indicating that although diverting attention away from a visual stimulus during passive viewing induces a widespread negative BOLD response in visual areas outside of V1, it has little effect on BOLD contrast within V1 (Zhang et al., 2005). Taken together, a reduction in the BOLD signal driven by zolpidem-induced attentional deficits cannot be ruled out, but future studies could incorporate a behavioral measure during the visual stimulation in order to determine empirically if drug effects on attention can confound BOLD results.

The functional significance of a zolpidem-induced reduction in BOLD response within the visual system during stimulation is not known, but it could be related to reported distortions in visual perception attributed to the drug. Although this hypothesis is speculative, zolpidem was associated with an increase in blurred vision compared to a comparable short-acting benzodiazepine hypnotic (Mintzer et al., 1998), while a number of case reports have described zolpidem-induced hallucinatory experiences (Ansseau et al., 1992; de Haas et al., 2007; Huang et al., 2003; Iruela et al., 1993). Anecdotally, several participants reported such experiences during experimental sessions (particularly after the highest dose of zolpidem but not to the extent of qualifying as an adverse event), but the mechanism by which zolpidem mediates this type of sensory effect in healthy individuals is unknown. Although some studies have demonstrated a role for hypoperfusion within the visual system and associated regions as a consistent factor correlated with hallucinations (e.g., Kuo et al., 1998; Matsui et al., 2006; Nagahama et al., 2010), such effects have been observed primarily in individuals suffering from illness. It can be hypothesized that administering zolpidem under those circumstances might actually be instrumental in reducing hallucinations because it has been shown to increase cerebral perfusion preferentially in the presence of brain injury versus healthy tissue (Brefel-Courbon et al., 2007; Clauss et al., 2000, 2001, 2002, 2004; Cohen et al., 2004; Hall et al., 2010). As mentioned previously, future studies using acute zolpidem administration in conjunction with other imaging paradigms such as arterial spin labeling and/or magnetic resonance spectroscopy may be helpful for elucidating further the biological relevance of the present observations.

In summary, the present study demonstrated that acute oral challenge with zolpidem has measurable effects on the BOLD signal, as evidenced by a drug-induced reduction in the robust visual cortical activation produced by presentation of a flashing checkerboard. Based on the existing literature regarding zolpidem's mechanism of action, similar effects observed with other positive GABA_A receptor modulators, and findings demonstrating a GABA-mediated inhibition-associated reduction in the BOLD response, it is proposed that the observed effects in healthy volunteers likely resulted from synaptic inhibitory activity, although drug effects on neurovascular tone cannot be ruled out at this time. Future studies should explore these mechanisms more fully in order for zolpidem to be used as a pharmacological tool in conjunction with fMRI to probe the neuronal substrates of sedative/hypnotic action.

Acknowledgments

This study was funded by the National Institute on Drug Abuse grant K01 DA023659 (SCL). The funding source had no role in the study design, in the collection, analysis, or interpretation of the data, in the writing of this report, or in the decision to submit the report for publication. The authors thank Dr. Lisa Nickerson for helpful discussion regarding data analyses.

Abbreviations

BOLD fMRI	blood oxygen level-dependent functional magnetic resonance imaging
CBF	cerebral blood flow

GLM	generalized linear model
PET	positron emission tomography
ROI	region of interest
SPECT	single-photon emission computed tomography
TICA	tensor independent component analysis

References

- Albin RL, Sakurai SY, Markowicz RL, Higgins DS, Young AB, Penney JB. Excitatory amino acid, GABA(A), and GABA(B) binding sites in human striate cortex. *Cereb Cortex*. 1991; 1:499–09. [PubMed: 1668367]
- Anseau M, Pitchot W, Hansenne M, Gonzalez Moreno A. Psychotic reactions to zolpidem. *Lancet*. 1992; 339:809. [PubMed: 1347827]
- Beckmann CF, Smith SM. Probabilistic independent component analysis for functional magnetic resonance imaging. *IEEE Trans Med Imaging*. 2004; 23:137–52. [PubMed: 14964560]
- Beckmann CF, DeLuca M, Devlin JT, Smith SM. Investigations into resting-state connectivity using independent component analysis. *Philos Trans R Soc Lond B Biol Sci*. 2005; 360:1001–13. [PubMed: 16087444]
- Benavides J, Peny B, Dubois A, Perrault G, Morel E, Zivkovic B, Scatton B. In vivo interaction of zolpidem with central benzodiazepine (BZD) binding sites (as labeled by [3H]Ro 15–1788) in the mouse brain. Preferential affinity of zolpidem for the omega 1 (BZD1) subtype. *J Pharmacol Exp Ther*. 1998; 245:1033–41. [PubMed: 2838599]
- Brefel-Courbon C, Payoux P, Ory F, Sommet A, Slaoui T, Raboyeau G, Lemesle B, Puel M, Montastruc JL, Demonet JF, Cardebat D. Clinical and imaging evidence of zolpidem effect in hypoxic encephalopathy. *Ann Neurol*. 2007; 62:102–05. [PubMed: 17357126]
- Brown GG, Eyler Zorrilla LT, Georgy B, Kindermann SS, Wong EC, Buxton RB. BOLD and perfusion response to finger-thumb apposition after acetazolamide administration: differential relationship to global perfusion. *J Cereb Blood Flow Metab*. 2003; 23:829–837. [PubMed: 12843786]
- Chen Z, Silva AC, Yang J, Shen J. Elevated endogenous GABA level correlates with decreased fMRI signals in the rat brain during acute inhibition of GABA transaminase. *J Neurosci Res*. 2005; 79:383–91. [PubMed: 15619231]
- Clauss RP, Guldenpfennig WM, Nel HW, Sathekge MM, Venkannagari RR. Extraordinary arousal from semi-comatose state on zolpidem. A case report. *S Afr Med J*. 2000; 90:68–72. [PubMed: 10721397]
- Clauss RP, Dormehl IC, Oliver DW, Nel WH, Kilian E, Louw WK. Measurement of cerebral perfusion after zolpidem administration in the baboon model. *Arzneimittelforschung*. 2001; 51:619–22. [PubMed: 11556120]
- Clauss RP, Dormehl IC, Kilian E, Louw WK, Nel WH, Oliver DW. Cerebral blood perfusion after treatment with zolpidem and flumazenil in the baboon. *Arzneimittelforschung*. 2002; 52:740–44. [PubMed: 12442636]
- Clauss R, Sathekge M, Nel W. Transient improvement of spinocerebellar ataxia with zolpidem. *N Engl J Med*. 2004; 351:511–12. [PubMed: 15282364]
- Cohen ER, Ugurbil K, Kim SG. Effect of basal conditions on the magnitude and dynamics of the blood oxygenation level-dependent fMRI response. *J Cereb Blood Flow Metab*. 2002; 22:1042–1053. [PubMed: 12218410]
- Cohen L, Chaaban B, Habert MO. Transient improvement of aphasia with zolpidem. *N Engl J Med*. 2004; 350:949–50. [PubMed: 14985499]
- Cole DM, Smith SM, Beckmann CF. Advances and pitfalls in the analysis and interpretation of resting-state FMRI data. *Front Syst Neurosci*. 2010; 4:1–15. [PubMed: 20204156]

- de Haas S, Dingemanse J, Hoever P, Cohen A, van Gerven J. Pseudohallucinations after zolpidem intake: a case report. *J Clin Psychopharmacol*. 2007; 27:728–30. [PubMed: 18004155]
- Desager JP, Hulhoven R, Harvenge C, Hermann P, Guillet P, Thiercelin JF. Possible interactions between zolpidem, a new sleep induce and chlorpromazine, a phenothiazine neuroleptic. *Psychopharmacology*. 1988; 96:63–66. [PubMed: 3147477]
- Donahue MJ, Near J, Blicher JU, Jezzard P. Baseline GABA concentration and fMRI response. *Neuroimage*. 2010; 53:392–98. [PubMed: 20633664]
- Edvinsson L, Krause DN. Pharmacological characterization of GABA receptors mediating vasodilation of cerebral arteries in vitro. *Brain Res*. 1979; 173:89–97. [PubMed: 226209]
- Eickhoff SB, Rottschy C, Zilles K. Laminar distribution and co-distribution of neurotransmitter receptors in early human visual cortex. *Brain Struct Funct*. 2007; 212:255–67. [PubMed: 17828418]
- Evans SM, Funderburk FR, Griffiths RR. Zolpidem and triazolam in humans: behavioral and subjective effects and abuse liability. *J Pharmacol Exp Ther*. 1990; 255:1246–55. [PubMed: 2262904]
- Fergus A, Lee KS. GABAergic regulation of cerebral microvascular tone in the rat. *J Cereb Blood Flow Metab*. 1997; 179:992–1003. [PubMed: 9307613]
- Finelli LA, Landolt HP, Buck A, Roth C, Berthold T, Borbély AA, Achermann P. Functional neuroanatomy of human sleep states after zolpidem and placebo: a H215O-PET study. *J Sleep Res*. 2000; 9:161–73. [PubMed: 10849243]
- Friston KJ, Holmes AP, Worsley KJ, Poline JB, Frith C, Frackowiak RSJ. Statistical parametric maps in functional imaging: A general linear approach. *Hum Brain Mapp*. 1995; 2:189–10.
- Gillin JC, Buchsbaum MS, Valladares-Neto DC, Hong CC, Hazlett E, Langer SZ, Wu J. Effects of zolpidem on local cerebral glucose metabolism during non-REM sleep in normal volunteers: a positron emission tomography study. *Neuropsychopharmacology*. 1996; 15:302–13. [PubMed: 8873114]
- Gollub RL, Breiter HC, Kantor H, Kennedy D, Gastfriend D, Mathew RT, Makris N, Guimaraes A, Riorden J, Campbell T, Foley M, Hyman SE, Rosen B, Weisskoff R. Cocaine decreases cortical cerebral blood flow but does not obscure regional activation in functional magnetic resonance imaging in human subjects. *J Cereb Blood Flow Metab*. 1998; 18:724–34. [PubMed: 9663502]
- Hall SD, Yamawaki N, Fisher AE, Clauss RP, Woodhall GL, Stanford IM. GABA(A) alpha-1 subunit mediated desynchronization of elevated low frequency oscillations alleviates specific dysfunction in stroke-A case report. *Clin Neurophysiol*. 2010; 121:549–55. [PubMed: 20097125]
- Hendrickson A, March D, Richards G, Erickson A, Shaw C. Coincidental appearance of the alpha 1 subunit of the GABA-A receptor and the type I benzodiazepine receptor near birth in macaque monkey visual cortex. *Int J Dev Neurosci*. 1994; 12:299–14. [PubMed: 7976485]
- Hendry SH, Huntsman MM, Viñuela A, Möhler H, de Blas AL, Jones EG. GABAA receptor subunit immunoreactivity in primate visual cortex: distribution in macaques and humans and regulation by visual input in adulthood. *J Neurosci*. 1994; 14:2383–01. [PubMed: 8158275]
- Huang CL, Chang CJ, Hung CF, Lin HY. Zolpidem-induced distortion in visual perception. *Ann Pharmacother*. 2003; 37:683–86. [PubMed: 12708947]
- Iannetti GD, Wise RG. BOLD functional MRI in disease and pharmacological studies: room for improvement? *Magn Reson Imaging*. 25:978–988. [PubMed: 17499469]
- Iruela LM, Ibanez-Rojo V, Baca E. Zolpidem-induced macropsia in anorexic woman. *Lancet*. 1993; 342:443–44. [PubMed: 8101949]
- Jacobsen LK, Gore JC, Skudlarski P, Lacadie CM, Jatlow P, Krystal JH. Impact of intravenous nicotine on BOLD signal response to photic stimulation. *Magn Reson Imaging*. 2002; 20:141–45. [PubMed: 12034334]
- Jueptner M, Weiller C. Review: does measurement of regional cerebral blood flow reflect synaptic activity? Implications for PET and fMRI. *Neuroimage*. 1995; 2:148–56. [PubMed: 9343597]
- Kjaer TW, Law I, Wiltshjötz G, Paulson OB, Madsen PL. Regional cerebral blood flow during light sleep-a H2¹⁵O-PET study. *J Sleep Res*. 2002; 11:201–07. [PubMed: 12220315]
- Kuo YT, Chiu NC, Shen EY, Ho CS, Wu MC. Cerebral perfusion in children with Alice in Wonderland syndrome. *Pediatr Neurol*. 1998; 19:105–08. [PubMed: 9744628]

- Kwong KK, Belliveau JW, Chesler DA, Goldberg IE, Weisskoff RM, Poncelet BP, Kennedy DN, Hoppel BE, Cohen MS, Turner R, Cheng H-M, Brady TJ, Rosen BR. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proc Natl Acad Sci USA*. 1992; 89:5675–79. [PubMed: 1608978]
- Kufahl PR, Li Z, Risinger RC, Rainey CJ, Wu G, Bloom AS, Li SJ. Neural responses to acute cocaine administration in the human brain detected by fMRI. *Neuroimage*. 2005; 28:904–14. [PubMed: 16061398]
- Levin JM, Ross MH, Mendelson JH, Kaufman MJ, Lange N, Maas LC, Mello NK, Cohen BM, Renshaw PF. Reduction in BOLD fMRI response to primary visual stimulation following alcohol ingestion. *Psychiatry Res*. 1998; 82:135–46. [PubMed: 9754438]
- Licata SC, Penetar DM, Dunlap S, Lukas SE. A therapeutic dose of zolpidem has limited abuse-like effects in drug-naïve females: a pilot study. *Eur J Pharmacol*. 2008; 598:64–67. [PubMed: 18831970]
- Licata SC, Platt DM, Cook JM, Van Linn ML, Rowlett JK. Contribution of alpha1 subunit-containing gamma-aminobutyric acidA (GABAA) receptors to the motor-impairing effects of benzodiazepines in squirrel monkeys. *Psychopharmacology*. 2009; 203:539–546. [PubMed: 19031072]
- Licata SC, Mashhoon Y, MacLean RR, Lukas SE. Modest abuse-related subjective effects of zolpidem in drug-naïve volunteers. *Behav Pharmacol*. 2011; 22:160–166. [PubMed: 21301324]
- Lowen SB, Nickerson LD, Levin JM. Differential effects of acute cocaine and placebo administration on visual cortical activation in healthy subjects measured using BOLD fMRI. *Pharmacol Biochem Behav*. 2009; 92:277–82. [PubMed: 19138702]
- Macdonald RL, Olsen RW. GABAA receptor channels. *Annu Rev Neurosci*. 1994; 17:569–02. [PubMed: 7516126]
- Martin E, Thiel T, Joeri P, Loenneker T, Ekatodramis D, Huisman T, Hennig J, Marcar VL. Effect of pentobarbital on visual processing in man. *Hum Brain Mapp*. 2000; 10:132–39. [PubMed: 10912592]
- Marutle A, Warpman U, Bogdanovic N, Nordberg A. Regional distribution of subtypes of nicotinic receptors in human brain and effect of aging studied by (+/-)-[3H]epibatidine. *Brain Res*. 1998; 801:143–49. [PubMed: 9729344]
- Matsui H, Nishinaka K, Oda M, Hara N, Komatsu K, Kubori T, Udaka F. Hypoperfusion of the visual pathway in parkinsonian patients with visual hallucinations. *Mov Disord*. 2006; 21:2140–44. [PubMed: 17029272]
- Mattila MJ, Vanakoski J, Kalska H, Seppälä T. Effects of alcohol, zolpidem, and some other sedatives and hypnotics on human performance and memory. *Pharmacol Biochem Behav*. 1998; 59:917–923. [PubMed: 9586849]
- McCann CC, Quera-Salva MA, Boudet J, Frisk M, Barthouli P, Borderies P, Meyer P. Effect of zolpidem during sleep on ventilation and cardiovascular variables in normal subjects. *Fundam Clin Pharmacol*. 1993; 7:305–10. [PubMed: 8406294]
- Mintzer MZ, Frey JM, Griffiths RR. Zolpidem is differentiated from triazolam in humans using a three-response drug discrimination procedure. *Behav Pharmacol*. 1998; 9:545–59. [PubMed: 9862080]
- Muthukumaraswamy SD, Edden RA, Jones DK, Swettenham JB, Singh KD. Resting GABA concentration predicts peak gamma frequency and fMRI amplitude in response to visual stimulation in humans. *Proc Natl Acad Sci USA*. 2009; 106:8356–61. [PubMed: 19416820]
- Muthukumaraswamy SD, Evans CJ, Edden RA, Wise RG, Singh KD. Individual variability in the shape and amplitude of the BOLD-HRF correlates with endogenous GABAergic inhibition. *Hum Brain Mapp*. 2011 [Epub ahead of print].
- Nagahama Y, Okina T, Suzuki N, Matsuda M. Neural correlates of psychotic symptoms in dementia with Lewy bodies. *Brain*. 2010; 133 (Pt 2):557–67. [PubMed: 19920063]
- Northoff G, Walter M, Schulte RF, Beck J, Dydak U, Henning A, Boeker H, Grimm S, Boesiger P. GABA concentrations in the human anterior cingulate cortex predict negative BOLD responses in fMRI. *Nat Neurosci*. 2007; 10:1515–17. [PubMed: 17982452]

- Ogawa S, Lee TM, Kay AR, Tank DW. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc Natl Acad Sci USA*. 1990; 87:9868–72. [PubMed: 2124706]
- Perrais D, Ropert N. Effect of zolpidem on miniature IPSCs and occupancy of postsynaptic GABA_A receptors in central synapses. *J Neurosci*. 1999; 19:578–88. [PubMed: 9880578]
- Rohan, M.; Killgore, W.; Eskesen, J.; Renshaw, P.; Yurgelun-Todd, D. Match-warped EPI anatomic images and the amygdala: Imaging in hard places. International Society for Magnetic Resonance in Medicine 9th Scientific Meeting; 2001; Glasgow, United Kingdom.
- Rush CR. Behavioral pharmacology of zolpidem relative to benzodiazepines: a review. *Pharmacol Biochem Behav*. 1998; 61:253–69. [PubMed: 9768560]
- Sanger DJ, Perrault G, Morel E, Joly D, Zivkovic B. The behavioral profile of zolpidem, a novel hypnotic drug of imidazopyridine structure. *Physiol Behav*. 1987; 41:235–40. [PubMed: 3324120]
- Sanna E, Busonero F, Talani G, Carta M, Massa F, Peis M, Maciocco E, Biggio G. Comparison of the effects of zaleplon, zolpidem, and triazolam at various GABA(A) receptor subtypes. *Eur J Pharmacol*. 2002; 451:103–10. [PubMed: 12231378]
- Smith AT, Cotillon-Williams NM, Williams AL. Attentional modulation in the human visual cortex: the time-course of the BOLD response and its implications. *Neuroimage*. 2006; 29:328–334. [PubMed: 16054845]
- Tallman JF, Thomas JW, Gallager DW. GABAergic modulation of benzodiazepine binding site sensitivity. *Nature*. 1978; 274:383–85. [PubMed: 27722]
- Woolrich MW, Ripley BD, Brady M, Smith SM. Temporal autocorrelation in univariate linear modeling of FMRI data. *Neuroimage*. 2001; 14:1370–86. [PubMed: 11707093]
- Woolrich MW. Robust group analysis using outlier inference. *Neuroimage*. 2008; 41:286–01. [PubMed: 18407525]
- Zhang X, Ferrera J, Hood DC, Hirsch J. The effect of attention and contrast on the BOLD response in V1 and beyond. *J Vis*. 2005; 5:681.

Highlights

- Zolpidem is a short-acting non-benzodiazepine hypnotic acting via GABA_A receptors.
- The neuronal substrates of zolpidem within the brain are unknown.
- We examined how zolpidem modulates visual system activation during BOLD fMRI.
- Zolpidem reduced robust activation produced by a flashing checkerboard.
- Effects were consistent with GABA-related reductions in the BOLD signal.

Fig. 1.

Group results for the GLM analysis showing coronal (top row), axial (middle row), and sagittal (bottom row) projections near the center of activation $[(x, y, z) = (6, -84, 0 \text{ mm})]$ in response to drug treatment. Colors represent significance values converted to a Gaussian distribution ranging from $z = 2.3$ (red; $p = 0.01$) to $z = 8.7$ (yellow; $p = 10^{-18}$), with cluster significance ($p < 0.05$) corrected across the whole brain. Column A shows a large region of activation that includes the visual cortex following administration of placebo (0 mg), while column B shows the reduced activation following the highest dose of zolpidem (20 mg). The contrast showing regions for which the activation was significantly less for zolpidem (20 mg) than for placebo is depicted in column C.

Fig. 2.

Series of axial slices showing the cluster of voxels ($n=596$) for which activation varied significantly across dose (F test, $p=0.004$). The color was determined by the number of significant two-tailed t -tests ($p<0.05/6$) for each voxel within the cluster (red=1, yellow=4). The 20 mg dose of zolpidem reduced BOLD activation significantly in 587 of the voxels within the cluster.

Fig. 3.

Average percentage of the control BOLD signal within the visual cortex in response to stimulation with the flashing checkerboard. Bars represent average percentage of the control activation as determined by the GLM analysis (GLM) and the 1st component of the TICA procedure (TICA-1) across the range of doses of zolpidem. All values are means $\pm$ SEM and asterisk represents significant difference from placebo ($p < 0.05$).

Fig. 4.

Time course and spatial distribution of TICA-1. Panel A shows the time course of the expected response to presentation of the visual stimulus (dotted line) as well as the actual time course of the 1st independent component (solid line). Both curves are normalized to zero mean and unit variance. The expected time courses take into account the effects of the hemodynamic response in the brain as well as the pre-processing temporal filter. Note the close agreement between the curves. Panel B shows the thresholded independent component map displaying the spatial extent of TICA-1 and its inclusion of V1 and V2. Color scale represents value of the z statistic, displayed over a background of T1-weighted anatomical scans averaged over all 12 participants. Red corresponds to a probability $p > 0.5$ of a voxel belonging to the component, based on a Gaussian/gamma mixture model, yellow indicates the highest z statistic in this component, and blue shading into cyan represents increasing activity of the opposite sign within the component.

Table 1

Tensor independent component analysis of visual stimulus.

Component	% Variance	P value	Brain regions
1	21.30	5×10^{-22}	V1, V2, precuneus*, right angular gyrus*
2	8.72	1.0×10^{-7}	Bilateral superior cortex, lateral temporal cortex
3	6.96	5.1×10^{-6}	Ring around superior areas (motion artifact)
4	6.90	3.5×10^{-9}	Cuneus, calcarine cortex
5	5.96	2.4×10^{-6}	Cerebellum
6	4.68	3.0×10^{-5}	Dorsal visual stream (Beckmann et al., 2005)
7	4.41	0.095	Brainstem*
8	3.17	0.43	Frontal regions, cerebellum, including white matter*
9	2.46	6.5×10^{-7}	Default mode network (Cole et al., 2010)
10	0.88	0.28	White matter

Components are those that were significantly correlated with the expected temporal response to presentation of the flashing checkerboard stimulus. % Variance refers to the amount of variance explained by that component; P value refers to the *p* value for fitting that component to the expected response; Asterisks represent brain regions in which the activity change was deactivation rather than activation.