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METABOLIC CHANGES UNDERLYING BOLD SIGNAL VARIATIONS AFTER ADMINISTRATION OF ZOLPIDEM

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Abstract

Zolpidem is a non-benzodiazepine drug belonging to the imidazopiridine class, which has selectivity for stimulating the effect of gamma aminobutyric acid [GABA] and is used for the therapy of insomnia. Nonetheless, several reports have been published over recent years about a paradoxical arousing effect of Zolpidem in patients with severe brain damage. We studied a PVS case using ^{1}H-MRS and BOLD signal, before and after Zolpidem administration. Significantly increased BOLD signal was localized in left frontal superior cortex, bilateral cingulated areas, left thalamus and right head of the caudate nucleus. A transient activation was observed in frontal cortex, comprising portions of anterior cingulate, medial, and orbito-frontal cortices. Additionally, significant pharmacological activation in sensory-motor cortex is observed 1 hour after Zolpidem intake. Significant linear correlations of BOLD signal changes were found with primary concentrations of NAA, Glx and Lac in the right frontal cortex. We discussed that when Zolpidem attaches to the modified GABA receptors of the neurodormant cells, dormancy is switched off, inducing brain activation. This might explain the significant correlations of BOLD signal changes and ^{1}H-MRS metabolites in our patient. We concluded that ^{1}H-MRS and BOLD signal assessment might contribute to study neurovascular coupling in PVS cases after Zolpidem administration. Although this is a report of a single case, considering our results we recommend to apply this methodology in series of PVS and MCS patients.

INTRODUCTION

Patients in a persistent vegetative state (PVS) are awake but are apparently unaware of themselves or their environment. The diagnosis of PVS has been made more difficult by recognition of the minimally conscious state (MCS) as a transitional phase in the partial recovery of self-awareness or environmental awareness as a patient emerges from PVS, leading to a relative high proportion of diagnostic errors.[1,2]

Zolpidem is a non-benzodiazepine drug belonging to the imidazopiridine class, which has selectivity for stimulating the effect of gamma aminobutyric acid (GABA) and is used for the therapy of insomnia.[3] Nonetheless, several reports have been published over recent years about a paradoxical "arousing" effect of Zolpidem in patients with severe brain damage.[4-7] Clauss et al., first reported Zolpidem use in brain injury in 2000 after an accidental prescription in a PVS case causing the patient to wake up and to speak to relatives within 15 minutes of receiving Zolpidem, the effect lasting for 3 hours.[8]

Functional neuroimaging has provided new insights for assessing cerebral function in PVS and MCS.[9] Improved perfusion in brain areas of hypoactivity, and at other brain sites such as physiologically suppressed cerebellum [cerebellar diaschisis], has been reported after Zolpidem[10]. Other case reports have been published in blepharospasm [11], Parkinson's disease,[12] spinocerebellar ataxia,[4], postanoxic spasticity,[13,14], and aphasia after stroke where SPECT imaging showed a 40% increase in cerebral blood flow (CBF) in affected areas.[15] Changes on 99mTc HMPAO Brain SPECT after Zolpidem are often accompanied by clinical improvement in severe brain damaged patients, such as awakening, release from brain injury symptoms and in sleep anomaly.[3,5,12,13,16] In summary, there is some evidence about the potential effect of Zolpidem to repeal metabolic, hemodinamic and electrical abnormal activity in severe brain injury.[3,17]

Advanced magnetic resonance methods, such as functional magnetic resonance imaging [fMRI] and proton magnetic resonance spectroscopy (^{1}H-MRS), have shown to be powerful tools to assess residual brain activity and outcome prediction in severe brain damaged patients.[18] fMRI and ^{1}H-MRS provide complementary information for investigating the human brain metabolism. fMRI based on blood oxygen level-dependent (BOLD) signal is related to a variety of physiological parameters as well as CBF. The magnitude of the BOLD change, due to elevated neuronal activity, is determined by decreased susceptibility effects resulting from the local increase in oxygenated haemoglobin.[19]

Localised ^{1}H-MRS has been used to monitor metabolic changes associated with several brain pathologies.[20] In PVS and MCS cases, ^{1}H-MRS has evidenced both local and diffuse biochemical impairment by quantifying several neurometabolites.[21] The potential of ^{1}H-MRS to provide specific brain metabolic information endorses its use for explaining the relative contribution of extravascular compartments to BOLD signal dynamics. [20, 21]

We studied a PVS case using ^{1}H-MRS and BOLD signal, according to our previous hypothesis that local changes in neurometabolites levels contribute to CBF increment, providing an index of neurovascular coupling after Zolpidem administration.

METHODS

Patient

We studied a 21 years old female patient (YOR), who suffered a stroke causing a top of the basilar artery syndrome, and who had been in PVS for 5 years. Previous MRI showed destruction of the rostral part of the pons, the mesencephalon, and both thalami. YOR showed circadian wakefulness, although she maintained longer periods of time with her eyes closed. With informed written consent of her parents, 10 mg of Zolpidem were administered through a percutaneous endoscopic gastrostomy. Ethical approval was obtained prior to data collection from the Institute of Neurology and Neurosurgery Ethics Committee. Throughout the session, clinical and physiological parameters were controlled.

Data collection.

Magnetic resonance data were collected on a Magnetom Symphony 1.5 Tesla MR system (Siemens, Earlangen, Germany), using a standard head coil for radio frequency transmission and signal reception. The patient remained in the same spatial location inside the scanner during the whole MRI study. The head was padded with foam to minimize head movement. For co-registration with functional images, a 3D whole-head MPRAGE image was collected. Furthermore, T2-weighted images in axial and coronal orientations were acquired to aid co-registration of the MRS voxel.

Functional image acquisition protocol involved six blocks of MRS and fMRI, with total duration of 10 min per block. The first images block acquisition started 10 min before Zolpidem administration. Five consecutive blocks were acquired after Zolpidem administration at +10, +25, +40, +55 and +70 minutes [for clarification of study timeline, see Figure 1].

BOLD sensitive functional images were acquired using an interleaved ascending EPI sequence, consisting of 36 axial slices with no interslice gap. Each run comprised 60 T2-weighted volumes, resulting in a total of 360 functional volumes.

In vivo ^{1}H-MRS spectra were acquired after manual shim adjustment by using a spin-echo single-voxel (SVS_SE) sequence, combined with the water suppression technique. Localization of the volume of interest (VOI = 2.0 cm^3) in the right frontal cortex was decided based on a previous EEG study of patient YOR, showing brain activation in that region after Zolpidem administration, in temporal concordance with autonomic and behavioral changes (Machado C. et al., in preparation). Details of acquisition sequences and parameters are available in Table 1.

Data processing and analysis

fMRI data were pre-processed and analysed using the SPM5 package (from the Wellcome Department of Cognitive Neurology, London, UK) running in Matlab 7.4 (Mathworks Inc., Sherborne, MA). Functional EPI images were spatially realigned using a least squares approach and a 6 parameter (rigid body) spatial transformation. Further pre-processing included spatial resampling at an isotropic voxel size (1mm×1mm×1mm) and slice-timing and spatial smoothing with an isotropic 8-mm full-width-at-half-maximum Gaussian kernel.

In the present report, the pre-medication fMRI run is considered as *baseline* and the term *activation* is used to represent the transient signal increase after Zolpidem administration. Student's *t*-tests were conducted at each voxel to detect Zolpidem-induced changes in BOLD time courses on each time point versus the baseline. Condition-specific effects were estimated using conventional statistical parametric mapping analysis. The resulting activation maps were checked subsequently for plausibility. All areas reaching significance on cluster level, with thresholds at a family-wise error (FWE) corrected probability of 0.05, will be reported. To further eliminate random noise, cluster filtering [cluster size >10 contiguous pixels] was applied to produce final statistical parametric maps (SPMs) of *t* deviates SPM(*t*), subsequently normalized to SPM(*Z)*.

The intensities of ^{1}H-MRS metabolites were estimated using the automated spectral fitting routine available in the commercial software Syngo MR A30 (Siemens, Erlangen, Germany) that included baseline correction. Prior to 2D Fourier transformation, the k-space imaging data were spatially smoothed with Hamming filtering (half width = 300 ms) to improve signal to noise ratio.

Metabolite concentrations were calculated for N-acetylaspartate (NAA), Glutamate / Glutamine (Glx), choline-containing compounds (Cho), creatine / phosphocreatine (Cr), myo-inositol (Ins) and lactate (Lac). Glutamatergic concentration was estimated from the combined signal of three resonance peaks. Metabolite concentration ratios were additionally calculated, using the sum of the Cr and phosphocreatine integrals as denominators.

The fMRI time series data were obtained from a cubic volume of interest (VOI) centred on the activation cluster in the right frontal cortex (Figure 2). For comparison with metabolites time spectra, the VOI mean signal intensity for each time point was calculated by averaging the time courses of all voxel in this VOI during the entire run [60 images]. Square Pearson product moment correlations were used to quantify relationships between Zolpidem-induced changes in BOLD signal and both primary and normalized metabolite levels.

RESULTS

BOLD changes

fMRI analysis revealed multiple areas of Zolpidem-induced activations. Figure 2 shows SPM(*t*) at the five conditions, with threshold at a *t*-value of 4.69 and superimposed on axial and sagittal high resolution anatomical slices. Significantly increased BOLD signal at $P<0.01$, FWE corrected for multiple comparisons, were localized in left frontal superior cortex, bilateral cingulated areas, left thalamus and right head of the caudate nucleus. All these findings were consistently reproducible throughout all conditions. A transient activation at $P<0.05$, FWE corrected, is observed in frontal cortex, comprising portions of anterior cingulate, medial, and orbitofrontal cortices. Additionally, significant pharmacological activation in sensory-motor cortex is observed 1 hour after Zolpidem intake. Table 2 presents the activated brain regions, showing the significant activation for each condition and the maximum Z score.

^{1}H-MRS Changes

Spectra were highly reproducible throughout the whole study. Information about resonance peaks is presented in Table 3. After 25 minutes of Zolpidem administration, NAA concentrations progressively augmented until 55 minutes, when concentrations of this metabolite began to gradually decrease. Glx and Lac concentrations showed a tendency to decrease after 40 minutes.

Coincidence in spatial location of the activated cluster and MRS voxel in right frontal cortex is shown in Figure 3. Time dependent behaviour of BOLD time course and metabolites concentrations were compared. Significant linear correlations ($p<0.05$) of BOLD signal changes were found with primary concentrations of NAA, Glx and Lac (Table 4). When BOLD amplitude change was compared with metabolite concentration ratios relative to Cr, no significant correlations were found.

DISCUSSION

After brain injury, an increment of excitatory and inhibitory neurotransmitters occurs, mostly glutamate and GABA.[3,17] Although Glutamate's excitatory action induces apoptosis in brain cells to absorb toxic metabolites, GABA's inhibitory effect predominates, suppressing cellular metabolism, which protects cells from unfavorable environment, leading to loss of consciousness. After some time GABA content diminishes due to increased usage and runs off from the brain into the blood. As GABA cannot be restored sufficiently in some brain regions, a secondary protective response is triggered, which converts GABA receptors hypersensitive, so that decreased levels of this neurotransmitter can preserve their suppressive effect, and uphold a trend of synchronized slow wave activity in the brain, termed neurodormant state.[17,22,23] Dormancy or hibernation of myocardium after an ischemic insult has been described in

cardiology,[24] and hence when applied to the brain is recognized as neurodormancy.[3,7,10,25-30]

When Zolpidem attaches to the modified GABA receptors of the neurodormant cells, the receptor structure is deformed and abnormal cell metabolism ceases, and hence dormancy is switched off. If dormancy involves large or important functional areas, clinical changes related to brain activation after Zolpidem administration can be dramatic. [3,8,10,17,31,32]

We found a significant correlation between transient changes of NAA, Glx and Lac primary concentration and BOLD signals. As NAA is considered a metabolic marker for neuronal density and function, the significant correlation of BOLD signal and NAA concentration increment might be related to dormancy switch-off. A CBF increment has been documented using 99mTc HMPAO SPECT or 18F FDG PET, indicating that those non-functioning areas start to function again after Zolpidem.[3,10,33]

At present, a convergent set of data points out that glutamate signaling on astrocytes provides a mechanism to strongly connect synaptic activity and glucose consumption.[29,34,35] According to Magistretti, astrocytes form the first cellular barrier encountered by glucose entering the brain parenchyma.[34] This metabolic pathway, often referred to as the astrocyte-neuron-lactate shuttle, is a clear example of cooperation between astrocytes and neurons. The basic mechanism in neurometabolic coupling is the glutamate stimulated aerobic glycolysis in astrocytes, which results in the release of lactate from astrocytes. [34,36-40] This could explicate a strengthening of brain activation in those areas previously governed by neurodormancy, [17,22] and it might also explain the strong correlation between Glx and BOLD signal after Zolpidem administration in our patient.

Clark et al. recently hypothesized that the abundant NAA in neuronal tissue can also serve as a large pool for replenishing Glx in periods of speedy or dynamic signaling demands and stress, helping to provide adequate levels of this metabolite.[40]

The initial GABA effect when CBF has not augmented yet after Zolpidem uptake could be explained by recent reports indicating that GABA does not couple inhibitory neuronal activity with glucose utilization, as does glutamate for excitatory neurotransmission, and suggests that GABA-mediated synaptic transmission does not contribute directly to brain imaging signals based on deoxyglucose.[41]

Another interesting result to discuss is the significant correlation we found between transient changes in Lac and BOLD signals. This might be also explained considering the metabolic pathway known as the astrocyte-neuron-lactate shuttle. As it was previously argued Glx stimulates aerobic glycolysis in astrocytes by a mechanism involving an activation of the Na+-K+ ATPase. This process results in a transient Lac overproduction, which is released from astrocytes to the extracellular space, followed by a recoupling phase during which time Lac would be oxidized by neurons into pyruvate and enters the tricarboxylic acid [TCA] cycle to

serve as an energy fuel.[34,36,37,39,42,43] ^{1}H-MRS in humans has demonstrated a transitory Lac peak in primary visual cortex, during physiologic activation of the visual system.[44] It has been discussed that the spatiotemporal window during a Lac peak which could be discriminated by ^{1}H-MRS would depend on the promptness and level of recoupling existing between astrocytic glycolysis and neuronal oxidative phosphorylation.[34]

When BOLD amplitude change was compared with metabolite concentration ratios relative to Cr, no significant correlations were found. The rationale for normalization is based on the concept that Cr is in chemical equilibrium, and that its regional concentration is not affected by neurodegenerative processes. However, Cr is roughly considered as a marker of energy metabolism and its metabolite resonance may be affected by BOLD changes in localized ^{1}H-MRS.[21,45]. In fact, in our case, Cr concentration varied in the same period when NAA augmented after Zolpidem intake.

We conclude that ^{1}H-MRS and BOLD signal assessment might contribute to study neurovascular coupling in PVS cases after Zolpidem administration. Although this is a report of a single case, considering our results we recommend to apply this methodology in series of PVS and MCS patients.

Acknowledgments

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Table 1. Detailed parameters of MRI acquisition sequences.

Sequence	*TE (ms)*	*TR (ms)*	*Matrix size*	*In-plane resolution (mm^2)*	*Slice thickness (mm)*	*Slices*
3D MPRAGE	1.33	14	256 x 256	1.0 x 1.0	1.0	160
T2	99	6220	256 x 256	0.5 x 0.5	2.0	30
EPI	50	3000	256 x 256	3.0 x 3.0	4.0	36
SVS_SE	30	5000	–	10 x 20	10	–

Table 2. Regions with Zolpidem – induced activations, normalized Z – values and significance levels

	$S_1>S_0$		$S_2>S_0$		$S_3>S_0$		$S_4>S_0$		$S_5>S_0$	
	Z-value	*P-value*	*Z-value*	*P-value*	*Z-value*	*P-value*	*Z-value*	*P-value*	*Z-value*	*P-value*
Frontal cortex L	4.59	0.054	4.74	0.028	4.99	<0.001	4.70	0.034	4.29	0.169
Frontal superior L	5.28	0.002	5.17	0.004	5.73	<0.001	5.10	0.006	3.94	0.483
Cingulate area L	6.07	<0.001	5.67	<0.001	5.86	<0.001	5.67	<0.001	4.83	0.019
Cingulate area R	6.89	<0.001	7.11	<0.001	6.11	<0.001	5.62	<0.001	5.19	0.004
Thalamus L	5.35	0.002	5.13	0.005	4.99	0.009	4.74	<0.001	4.34	0.140
Caudate R	6.86	<0.001	6.76	<0.001	5.67	<0.001	4.69	<0.001	5.03	0.008
Sensory-motor area	3.02	1.000	3.10	0.998	3.89	<0.001	4.97	0.011	4.35	0.133

P-values are corrected for family-wise error at the whole brain level. S_n indicates time point, where S_0 correspond to baseline.

Table 3. Metabolites of interest. Resonance peaks correspond to the maximum intensity of the correspondent metabolite.

Metabolites	*Abbreviation*	*Resonance peaks (ppm)*
N-acetylaspartate	NAA	2.02
Glutamate + Glutamine	Glx	2.14, 2.36 and 2.46
Choline-containing compounds	Cho	3.22
Creatine + phosphocreatine	Cr	3.03
Myo-inositol	Ins	3.54
Lactate	Lac	1.33

Table 4. Correlations of variation in metabolites concentrations with BOLD signal changes.

Metabolite	***R***	***p***
N-acetyl aspartate (NAA)	0.848	**0.03**
Creatine + phosphocreatine (Cr)	-0. 368	0.64
Choline (Cho)	0.117	0.82
Mioinositol (Ins)	-0.520	0.29
Glutamate + Glutamine (Glx)	0.913	**0.01**
Lactate (Lac)	0.905	**0.01**

R: Pearson product moment correlation coefficient

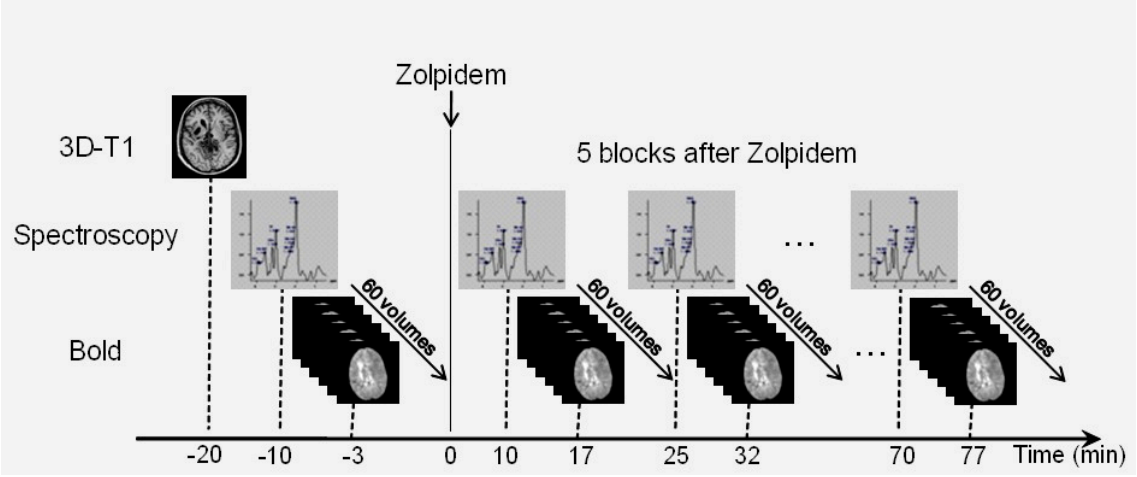

Figure 1. Scanning session time line. After a T1-weighted high resolution and a baseline acquisition block, patient received an acute dose of Zolpidem (10 mg). During the next 80 minutes, six consecutive blocks of single voxel ^{1}H-MRS and BOLD-fMRI were conducted.

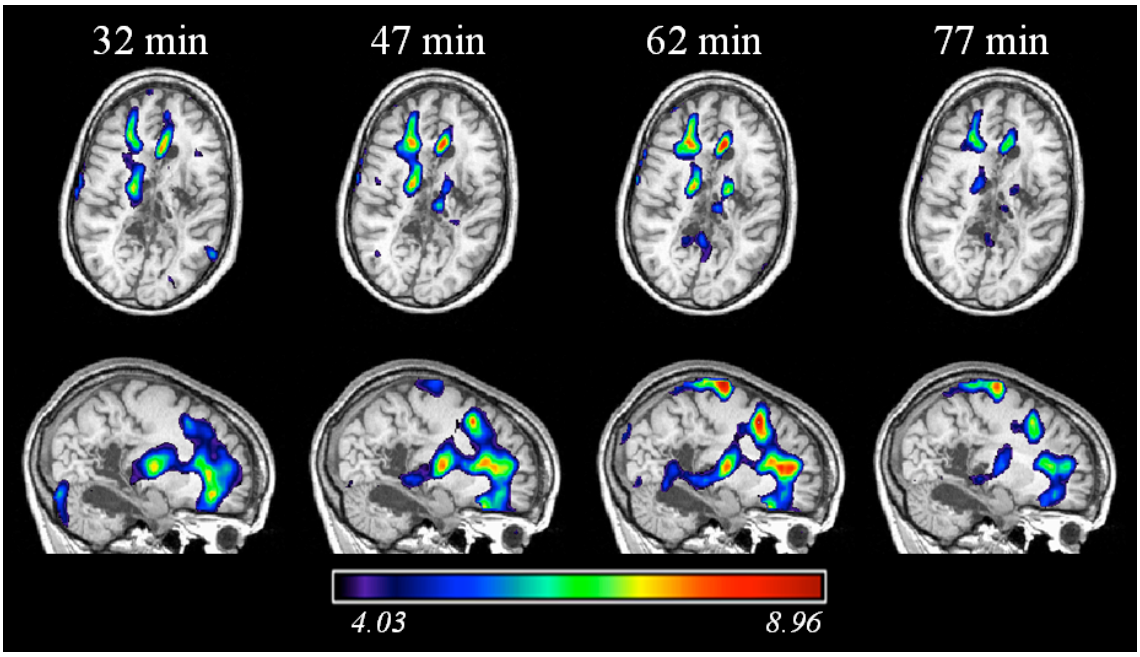

Figure 2. Statistically thresholded Zolpidem-induced BOLD activation in four time points, rendered on the patient'sT1-weighted axial and sagittal views. Results are thresholded for display at whole brain family wise error corrected (P<0.05). Colour scale represents t-values.

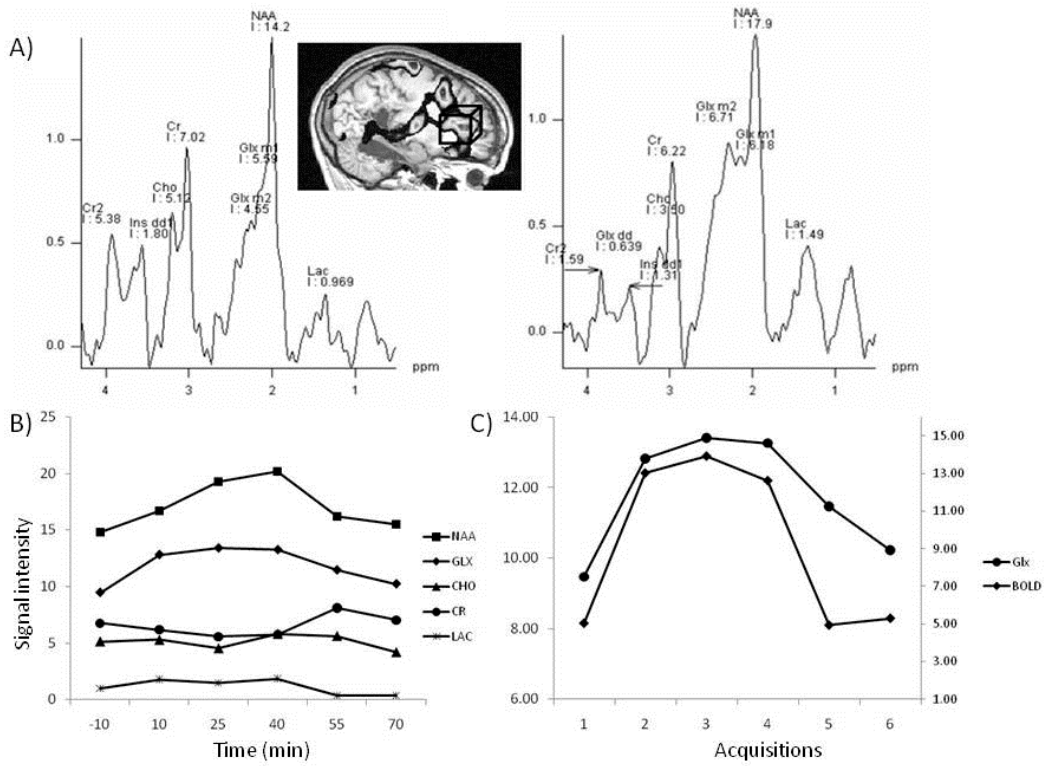

Figure 3. A): Representative MRS spectra before (left) and after (right) Zolpidem administration. The inset depicts the three-dimensionally localized MRS voxel (10 × 20 × 10 mm^3) in the activated frontal cortex. Note spatial correspondence with fMRI activation. B) Time courses of metabolites during the observation period, covering six acquisitions time points. C) Comparisons of Glx and BOLD time courses. NAA: N-acetylaspartate, Glx: glutamine/glutamate, Lac: lactate, Cho: choline, Cr: creatine/phosphocreatine.