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In vivo proton magnetic resonance spectroscopic examination of benzodiazepine action in humans

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ABSTRACT

In an examination of the effect of benzodiazepines on brain chemistry, 44 healthy controls underwent a short echo-time proton magnetic resonance spectroscopy (^{1}H MRS) session after induced sedation with intravenous midazolam (0.03 mg/kg) plus fentanyl (2 μg/kg). The regions of interest were the anterior cingulate cortex, right basal ganglia, right frontal lobe, and right hippocampus. Twenty-five of these subjects underwent the second ^{1}H MRS session while awake. The measured ^{1}H MRS metabolites included *N*-acetyl-aspartate, creatine-containing compounds (PCr+Cr), choline-containing compounds, *myo*-inositol, and glutamate plus glutamine, which were quantified both as absolute values and metabolite/PCr+Cr ratios. The results were analyzed using independent group *t* tests and repeated measures analysis of variance (ANOVA, with alpha values set at 0.025 to minimize the risk of false-positive findings arising from multiple comparisons. No significant difference between subjects under midazolam plus fentanyl induced sedation and awake could be detected with unpaired analyses. Paired comparisons by ANOVA with repeated measures found that neither drug (midazolam plus fentanyl) nor the drug by time (interval between two scan times) interaction had a significant effect on the quantified metabolites. These findings encourage utilization of benzodiazepine-induced brief sedation during in vivo ^{1}H MRS experiments of the brain, and may help with elucidation of state-dependent neurochemical alterations during the course of bipolar and schizoaffective disorders.

1. Introduction

Pathophysiological mechanisms underlying expression of psychiatric disorders are yet to be elucidated. Proton magnetic resonance spectroscopy (^{1}H MRS) allows assessment of neurochemical metabolites in vivo such as (i) *N*-acetylaspartate (NAA), which is a widely accepted marker for neuronal density, viability and function that is involved in myelin lipid turnover and mitochondrial energy production; (ii) phosphorylcholine plus glycerophospocholine (GPC+PC), which primarily reflects cell membrane phospholipids; (iii) phosphocreatine plus creatine (PCr+Cr), which represents cell energy metabolism; (iv) *myo*-inositol (mI), which participates in phospholipid metabolism and signal transduction; and (v) glutamate plus glutamine (Glu+Gln), which provides a window into the integrity of the glutamatergic synapse and neuronal–glial coupling (Auer et al., 2000; Yildiz-Yesiloglu and Ankerst, 2006; Moffett et al., 2007; Fountoulakis et al., 2008; Öngür et al., 2008; Scherk et al., 2009). Thus, along with 31P and 13C studies, hold considerable promise to illuminate brain mechanisms involved in severe psychiatric conditions such as bipolar and schizoaffective disorders (Kato et al., 1998; Glitz et al., 2002).

One of the major limitations of conducting in vivo MRS studies is the difficulty of scanning psychiatric patients when they are excited, agitated, or psychotic as in the case of bipolar or schizoaffective disorders in manic, mixed, or at times depressive states. During an MR scanning session, we need the patient subject to stay calm, quiet, and motionless for a reliable assessment of brain metabolites. However, patients in manic or mixed states or with psychotic symptoms are usually uncooperative. Considering presentation of bipolar or schizoaffective disorders in three, and unipolar depression in two different mood states, each with a potentially distinct neurochemical profile, better understanding of the pathophysiology would only be possible if we could obtain reliable and comparable information about

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brain chemistry during the mood states of mania and/or depression where accompanying psychosis and/or agitation is not uncommon.

Moderate sedation often induced by benzodiazepines is a level of anesthesia in which uncooperative patients are able to tolerate procedures requiring them not to move. Benzodiazepines interact with a specific binding site in the brain, which is an integral part of the gamma-aminobutyric acid$_A$ ($GABA_A$) receptor complex (Nutt and Malizia, 2001; Rudolph and Antkowiak, 2004). The ongoing level of neuronal activity is regulated by the balance between excitatory inputs (mostly glutamatergic) and inhibitory GABAergic activity. The $GABA_A$–benzodiazepine receptors, which are ligand-gated chloride channels, comprise five protein sub-units arranged around a central pore (Nutt and Malizia, 2001; Prommer, 2008). Benzodiazepine binding allosterically changes the receptor complex to increase the efficiency of GABA, so enabling the GABAergic circuits to produce a greater inhibitory effect. Compared with barbiturates, chloral hydrate, and ethanol, which can directly open the chloride channel, benzodiazepines are safer since the vital brain circuits cannot be inhibited over and above the level that would be achieved by the natural GABAergic effects (Nutt and Malizia, 2001).

Very few studies to date examined whether acute sedation with benzodiazepines could cause significant changes in the in vivo brain MR spectra. Brambilla et al. (2002) using ^{1}H MRS, and Deicken et al. (1992) using ^{31}P MRS have shown that benzodiazepines when administered orally do not change the human brain chemistry. We performed this study to examine whether parenteral administration of benzodiazepines does affect the human brain metabolite levels as measured by ^{1}H MRS. We examined the anterior cingulate cortex (ACC), right basal ganglia, right frontal lobe, and right hippocampus because earlier ^{1}H MRS studies of bipolar and schizoaffective disorders have reported changes suggestive of neuronal dysfunction in these brain regions (Strakowski et al., 1994; Soares and Mann, 1997; Yildiz-Yesiloglu and Ankerst, 2006; Moffett et al., 2007; Öngür et al., 2008; Scherk et al., 2009). The underlying *a priori* hypothesis was that the healthy subjects with and without benzodiazepine-induced transient sedation would have similar brain metabolite concentrations.

2. Methods

2.1. Subjects

Forty-four healthy human subjects who had no DSM-IV axis I disorder, as determined by the SCID-IV non-patient version (SCID-NP) were enrolled and underwent a ^{1}H MRS session under midazolam (0.03 mg/kg) plus fentanyl (2 μg/kg) intravenous (iv) administration. Of these 25 subjects, 44 underwent a second ^{1}H MRS session awake without administration of any drugs within the same study year (time interval between the two scans, mean ± standard deviation [SD]: 121.9 ± 86.7 days). They did not have any current medical problems or history of psychiatric disorders among their first-degree relatives. The study was approved by the Dokuz Eylül University, Institutional Ethics Committee. Written informed consent was obtained from all subjects.

2.2. Induction of sedation

The subjects who were scheduled for ^{1}H MRS under 'moderate sedation' fasted 6 h prior to the examination. For all subjects a venous route was opened with a 20-gauge iv cannula and a 0.9% normal saline infusion was initiated at the time of the scheduled MR scan. A study anesthesiologist who stayed with the subject throughout the procedure, administered midazolam 0.03 mg/kg iv, which was followed by fentanyl 2 μg/kg iv at a 1-min interval, just before the initiation of the MR session. Sedation was followed by the Ramsey Sedation Score and 0.015 mg/kg additional midazolam was administered until a Ramsey Sedation Score of 3 was reached (Ramsey et al., 1974). During the ^{1}H MRS sessions 6 L/min oxygen was applied via a face mask. Heart rate, respiratory rate, and Ramsey Sedation Score were monitored. At the end of the MRS session subjects with a Ramsey Sedation Score of 2 were transferred to the recovery room and post-anesthesia discharge scores were monitored at 10-min intervals. Subjects with a post-anesthesia score of 9 or higher were discharged. The mean discharge time was 1 h (Alderete Score) (Blanshard and Chung, 1999).

2.3. ^{1}H MRS procedures

In vivo ^{1}H MRS was conducted on a Philips System, at field strength of 1.5 T. The subjects were provided with earplugs to reduce noise disturbances, and the subject's head was positioned comfortably in the quadrature head radiofrequency coil with foam cushioning for motion stability. A set of sagittal, transverse and coronal T2 weighted images was obtained to verify subject position, image quality, and voxel positioning. To ensure consistent voxel placement, all scanner operators (who were certified MR technologists) were trained for this study and followed a standard prescription based on anatomic boundaries and reference atlas images for each region of interest. Anatomic (T2 weighted) images showing voxel placement were reviewed for consistency and accuracy for each region.

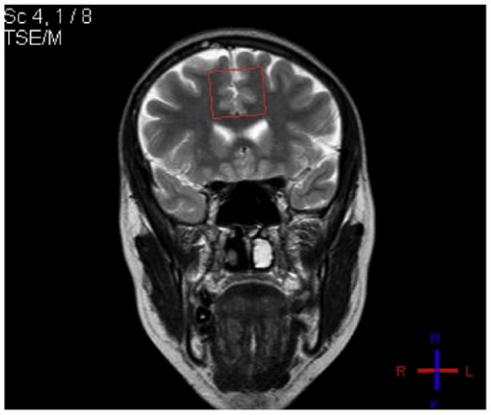
The single voxel short TE MRS data were collected with a PRESS sequence (TE = 31 ms, TR = 2000 ms, spectral bandwidth 1 kHz, 1024 complex data points, 128 acquisitions). Single voxel spectra were obtained from four different brain regions with voxel dimensions of 20 × 30 × 25 mm (15 cm^3) for the ACC; 15 × 30 × 20 mm (9 cm^3) for the right basal ganglia; 20 × 20 × 20 mm (8 cm^3) for the right frontal lobe; and 30 × 20 × 15 mm (9 cm^3) for the right hippocampus (Fig. 1). We used the commercial spectral-fitting package LC Model (version 6.1-4E) to measure metabolite peak integrals (Provencher, 1993). Unsuppressed water reference spectra were acquired for all acquisitions and used for both eddy-current correction and water-scaling to estimate absolute metabolite concentrations. Metabolite ratios, which are somewhat less sensitive to partial volume and relaxation effects, were also calculated for reference with the existing literature. T1 and T2 relaxation times were not measured. A long TR (2000 ms) and a short TE (31 ms) will significantly attenuate T1 and T2 relaxation effects, respectively. Only the ^{1}H MRS metabolites with reasonable precision for quantification (Stanley et al., 1995) were reported in the results (i.e., NAA, GPC+PC, mI, Glu+Gln PCr+Cr), and were expressed as absolute values, as well as metabolite/PCr+Cr ratios.

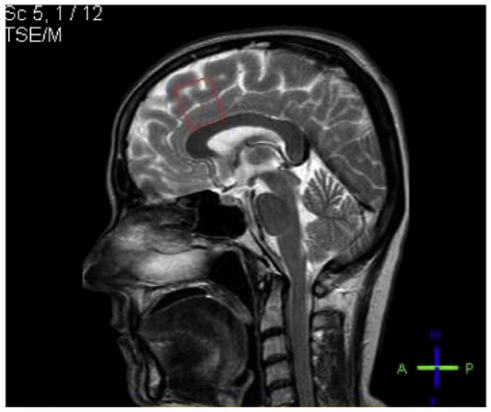
2.4. Data analysis

A two-pass quality assessment was made for all spectra. First, a global qualitative assessment of the spectral fit and baseline for each spectrum was made. Of the 276 potential in vivo proton brain spectra (four brain regions for 44 healthy subjects with 25 going through a 2nd scan), 241 were judged to be of adequate quality to undergo quantitative analysis. Following spectral fitting with the LC (linear combinations) Model, individual metabolite results were accepted/rejected based on specific "goodness of fit" criteria discussed below. We set a CRLB (Cramer-Rao lower bounds) threshold of less than 20% for NAA, GPC+PC, and PCr+Cr but allowed a larger uncertainty for Glu+Gln and mI: CRLB<40% due to the greater difficulty of fitting these metabolites.

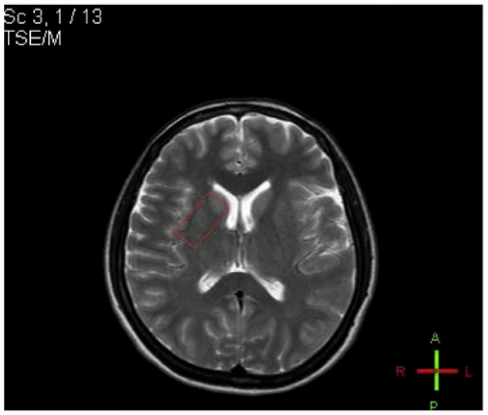
The LC Model uses *a priori* knowledge (optimized linear combinations of single metabolite basis set spectra) for fitting *in vivo* spectral peaks which correspond to these basis spectra, and allows resolution of some cases of overlapping spectral peaks which can be accurately separated due to curve fitting and linear-combination constraints derived from fitting other satellite peaks (at different ppm) for each metabolite. In these cases the data would be reported and included in

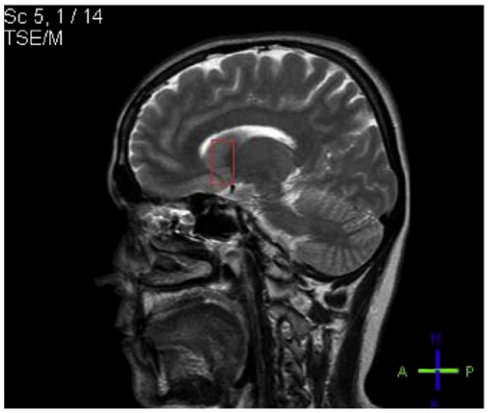
a) The voxel of interest in the anterior cingulate cortex (2 x 3 x 2.5–15 cm^3) in coronal and sagittal magnetic resonance images.



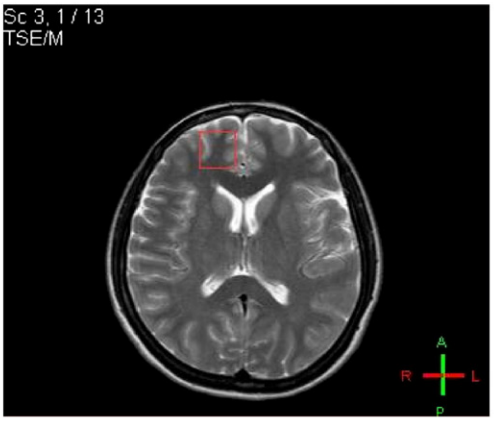


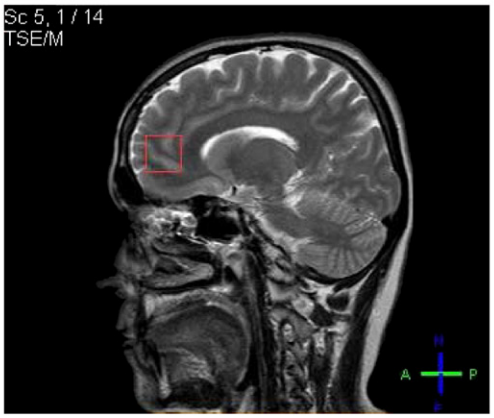
b) The voxel of interest the right basal ganglia (1.5 x 3 x 2–9 cm^3) in axial and sagittal magnetic resonance images.



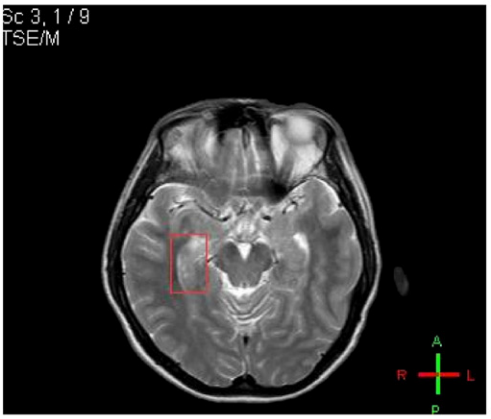


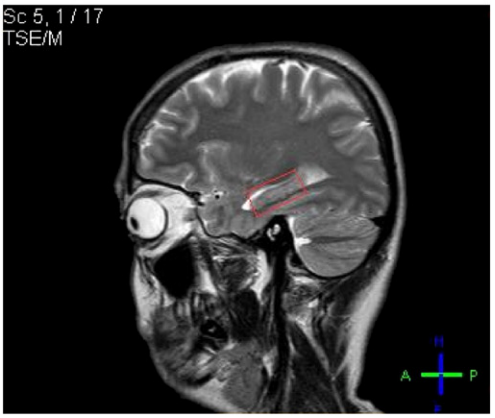
c) The voxel of interest in the right frontal lobe (2 x 2 x 2–8 cm^3) in axial and sagittal magnetic resonance images.





d) The voxel of interest the right hippocampus (3 x 2 x 1.5–9 cm^3) in axial and sagittal magnetic resonance images.





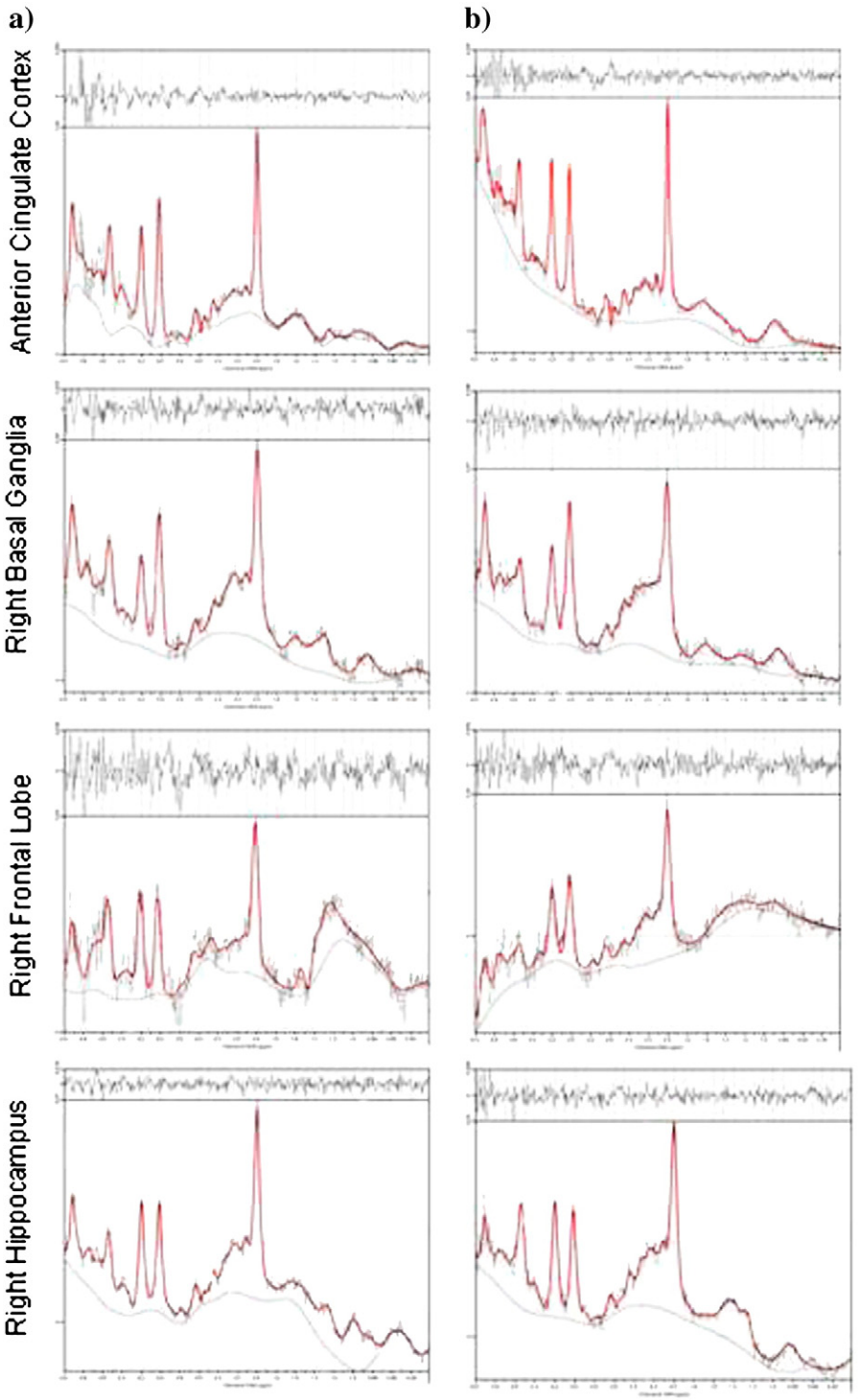


Fig. 2. Representative 1H MR spectra for a healthy human subject with (a), and without (b) midazolam plus fentanyl induced sedation.

the analysis using CRLB criteria. With the LC Model algorithm, spectral peak fits are characterized by CRLB thresholds, which estimate the percent uncertainty for each metabolite concentration. While spectral overlap can be a significant source of uncertainty and reduced precision when attempting to resolve individual metabolite peaks, elimination of one or more individual spectral peak fits due to CRLB cutoffs does not require that the entire spectrum must be rejected. Where the LC (linear combination) algorithm fails to accurately resolve overlapping peaks, that uncertainty is numerically apportioned to all individual spectral quantities that derive from that.

Fig. 1. The red box represents the location of the voxel of interest in the brain region studied. a) The voxel of interest in the anterior cingulate cortex ($2\times3\times2.5$–15 cm^3) in coronal and sagittal magnetic resonance images. b) The voxel of interest the right basal ganglia ($1.5\times3\times2$–9 cm^3) in axial and sagittal magnetic resonance images. c) The voxel of interest in the right frontal lobe ($2\times2\times2$–8 cm^3) in axial and sagittal magnetic resonance images. d) The voxel of interest the right hippocampus ($3\times2\times1.5$–9 cm^3) in axial and sagittal magnetic resonance images.

Table 1
^{1}H MRS metabolite concentrations of the subjects with and without midazolam plus fentanyl induced sedation in the anterior cingulate cortex (ACC), right basal ganglia, right frontal lobe, and right hippocampus. SD: Standard deviation. Unpaired comparisons made by independent samples *t* test with two-tailed α set at 0.025. †Not significant after correction for multiple comparisons.

	ACC				Right basal ganglia				Right frontal lobe				Right hippocampus			
	N	Mean±SD	t_{df}	*p*	*N*	Mean±SD	t_{df}	*p*	*N*	Mean±SD	t_{df}	*p*	*N*	Mean±SD	t_{df}	*p*
NAA																
With	40	6.41±0.63	−0.6459	0.519	37	6.56±1.19	−0.2154	0.833	34	6.14±1.32	−0.6753	0.503	43	5.03±0.73	−0.6864	0.496
Without	24	6.49±0.40			19	6.62±0.81			21	6.39±1.25			23	5.15±0.63		
GPC+PC																
With	39	1.25±0.26	−0.7661	0.445	33	1.53±0.52	0.5851	0.559	29	1.31±0.28	−1.1746	0.246	41	1.37±0.22	0.2962	0.769
Without	23	1.30±0.24			20	1.45±0.44			19	1.42±0.40			23	1.35±0.18		
mI																
With	38	4.66±1.38	0.5558	0.585	31	5.22±3.13	1.3546	0.182	31	4.99±1.83	1.0549	0.299	42	4.59±1.04	−0.9862	0.328
Without	22	4.47±1.01			17	4.10±1.87			20	4.45±1.73			22	4.86±1.02		
Glu+Gln																
With	37	7.35±2.29	−1.4158	0.164	33	10.6±3.25	−2.0348	0.048†	25	10.8±3.20	−0.0838	0.932	35	6.71±2.23	0.0156	0.985
Without	23	8.20±2.22			17	12.4±2.20			15	10.9±4.52			23	6.70±2.29		
PCr+Cr																
With	38	4.83±0.67	−1.2248	0.227	35	6.28±1.58	−0.0554	0.955	34	4.44±1.06	−0.7952	0.432	41	4.27±0.78	−0.7563	0.453
Without	23	5.04±0.63			21	6.30±1.40			20	4.68±1.22			24	4.42±0.75		
NAA/PCr+Cr																
With	38	1.34±0.24	1.1859	0.242	35	1.03±0.22	0.3954	0.693	34	1.51±0.50	2.1753	0.034†	41	1.16±0.22	0.5664	0.576
Without	23	1.27±0.18			21	1.01±0.16			21	1.24±0.32			25	1.12±0.24		
GPC+PC/PCr+Cr																
With	39	0.27±0.06	0.5961	0.533	34	0.24±0.63	0.3252	0.745	29	0.30±0.06	0.2646	0.794	40	0.31±0.03	1.4763	0.145
Without	24	0.26±0.05			20	0.23±0.06			19	0.29±0.09			25	0.30±0.03		
mI/PCr+Cr																
With	38	0.91±0.26	0.2058	0.842	32	0.89±0.52	1.5247	0.134	31	1.15±0.48	1.2450	0.220	39	1.05±0.21	−1.2859	0.203
Without	22	0.90±0.26			17	0.67±0.39			21	0.99±0.42			22	1.13±0.22		
Glu+Gln/PCr+Cr																
With	39	1.60±0.55	0.1159	0.907	33	1.75±0.54	−1.4648	0.149	26	2.57±0.84	1.8042	0.078	35	1.60±0.52	0.6456	0.521
Without	22	1.59±0.40			17	1.98±0.51			18	2.12±0.75			23	1.51±0.46		

Consequently, all spectral quantities that are numerically derivative of a poorly resolved overlap will also correspondingly have increased CRLBs reflecting that uncertainty. This is internally consistent and essential to the LC algorithm. Well-resolved individual spectral peaks with very low CRLBs may co-exist (in the same spectrum) with other peaks, at different ppm, which are less well resolved (for a variety of reasons) and which have significantly higher CRLBs. Relying on this background, we have excluded (rejected) poor metabolite fits while retaining more accurate fits in each spectrum which has resulted in some variation in the sample sizes of NAA, GPC+PC, mI and PCr+Cr, and somewhat larger variation in the sample size of Glu+Gln (as expected due to lower precision measurements due to overlapping resonances at 1.5 T). Both absolute metabolite concentrations and metabolite/Cr ratios were compared using independent *t* tests and repeated measures ANOVA with the factor time interval between the two scan times (days). To limit risk of false-positive (type I) errors, we corrected two-tailed $\alpha = 0.05$ by dividing it with the number of comparisons (independent *t* tests and repeated measures ANOVA), requiring $p \leq 0.025$ (0.05/2) to establish statistical significance. Computed group means were shown with their SD. All statistical tests were performed using SPSS, statistical software version 15.0.

3. Results

Forty-four healthy volunteers (20 men [45%]; age ± SD: 32.0 ± 12.1, range: 17–57 years; 37 right-, 7 left [16%]-handed) underwent ^{1}H MRS under midazolam plus fentanyl sedation. Twenty-five (10 men [40%]; age ± SD: 28.8 ± 9.7, range: 17–54 years; 21 right-, 4 left [16%]-handed) of the initial 44 subjects were also scanned awake without the administration of any drugs. A representative spectrum obtained with the utilized method of short TE ^{1}H MRS is shown in Fig. 2, which illustrates the spectra of a single subject under midazolam plus fentanyl sedation and awake without administration of any hypnotic sedative agent.

3.1. Comparisons by independent t test

Table 1 illustrates means (SD), and unpaired comparisons (via *t* test) of the quantified metabolite concentrations for the whole sample under midazolam plus fentanyl induced sedation (With) and awake (Without), in the brain regions of ACC, right basal ganglia, right frontal lobe, and right hippocampus. Subjects with and without benzodiazepine-induced sedation had similar metabolite concentrations in the brain regions of ACC, and right hippocampus (Table 1). In the right basal ganglia, the absolute Glu+Gln concentration of 33 subjects with benzodiazepine-induced sedation was 10.6 ± 3.25; and that value for 17 subjects without sedation was 12.4 ± 2.20, yielding a nonsignificant difference after correction for multiple comparisons ($t_{df=48} = -2.03$, $p = 0.048$). All the other absolute metabolite concentrations and metabolite/PCr+Cr ratios in the right basal ganglia were similar between subjects with and without benzodiazepine-induced sedation (Table 1). In the right frontal lobe all the metabolite concentrations of the subjects with and without benzodiazepine-induced sedation were similar, except for the NAA/PCr+Cr ratio, which was nonsignificantly different between subjects with benzodiazepine-induced sedation and awake (1.51 ± 0.50 and 1.24 ± 0.32, respectively; $t_{df=53} = 2.17$, $p = 0.034$, Table 1).

3.2. ANOVA with repeated measures

For paired comparisons of the subjects scanned twice with and without midazolam plus fentanyl-induced sedation, we used ANOVA with repeated measures by considering the time interval (number of days) between the two scan times as a factor. Summary results of these paired analyses are demonstrated in Table 2. Neither benzodiazepine-induced sedation nor time had an apparent effect on the metabolites of interest in the brain regions of the ACC and the right basal ganglia (Table 2). A slight decrease in the right frontal lobe GPC+PC/PCr+Cr ratio of 0.03 (from 0.31 ± 0.05 to 0.28 ± 0.05) with benzodiazepine-induced sedation for 14 subjects revealed an $F_{df=1}$ of

Table 2
^{1}H MRS metabolite concentrations of the subjects scanned twice, under midazolam plus fentanyl induced sedation (With), and awake (Without), in the anterior cingulate cortex (ACC), right basal ganglia, right frontal lobe, and right hippocampus. SD: Standard deviation. Paired comparisons made by ANOVA repeated measures; time interval between the two ^{1}H MRS sessions was assigned as a factor; two-tailed α set at 0.025. †Not significant after correction for multiple comparisons.

	ACC						Right basal ganglia						Right frontal lobe						Right hippocampus					
	N	Mean±SD	F_{df}	*p*	F_{df}	p_{time}	*N*	Mean±SD	F_{df}	*p*	F_{df}	p_{time}	*N*	Mean±SD	F_{df}	*p*	F_{df}	p_{time}	*N*	Mean±SD	F_{df}	*p*	F_{df}	p_{time}
NAA																								
With	22	6.44 ± 0.57	1.41_1	0.249	2.74_1	0.113	18	6.65 ± 1.23	0.23_1	0.632	0.13_1	0.717	18	6.15 ± 1.36	0.45_1	0.510	0.21_1	0.653	23	5.08 ± 0.72	2.49_1	0.129	3.42_1	0.078
Without	22	6.48 ± 0.37					18	6.55 ± 0.77					18	6.37 ± 1.20					23	5.04 ± 0.69				
GPC+PC																								
With	20	1.22 ± 0.25	0.56_1	0.464	0.57_1	0.814	15	1.53 ± 0.55	0.12_1	0.731	0.87_1	0.367	13	1.26 ± 0.25	3.30_1	0.096	0.94_1	0.352	22	1.29 ± 0.21	0.58_1	0.453	0.001_1	0.988
Without	20	1.27 ± 0.23					15	1.41 ± 0.33					13	1.54 ± 0.38					22	1.36 ± 0.18				
mI																								
With	21	4.62 ± 1.30	0.12_1	0.730	0.02_1	0.873	13	5.06 ± 1.94	2.61_1	0.134	0.12_1	0.735	16	4.62 ± 1.41	1.55_1	0.233	1.66_1	0.218	23	4.63 ± 0.94	0.45_1	0.508	0.001_1	0.971
Without	21	4.50 ± 1.03					13	3.71 ± 1.33					16	4.37 ± 1.68					23	4.98 ± 1.16				
Glu+Gln																								
With	21	7.43 ± 2.24	1.84_1	0.190	0.79_1	0.383	12	11.5 ± 3.56	0.34_1	0.569	1.37_1	0.269	10	10.1 ± 2.68	1.31_1	0.285	1.12_1	0.320	21	6.92 ± 2.44	0.91_1	0.351	0.37_1	0.548
Without	21	8.14 ± 2.15					12	12.3 ± 1.76					10	9.44 ± 2.94					21	6.39 ± 2.09				
PCr+Cr																								
With	21	4.94 ± 0.64	2.99_1	0.100	2.87_1	0.106	17	6.64 ± 1.47	0.09_1	0.757	0.72_1	0.407	19	4.57 ± 1.09	1.63_1	0.218	1.44_1	0.245	23	4.06 ± 0.70	0.01_1	0.921	2.55_1	0.125
Without	21	5.05 ± 0.61					17	6.33 ± 0.99					19	4.75 ± 1.30					23	4.44 ± 0.76				
NAA/PCr+Cr																								
With	19	1.35 ± 0.21	1.08_1	0.311	0.06_1	0.796	19	0.99 ± 0.24	0.47_1	0.502	0.32_1	0.575	18	1.45 ± 0.49	3.07_1	0.099	1.40_1	0.253	22	1.24 ± 0.23	4.57_1	0.045†	0.10_1	0.755
Without	19	1.25 ± 0.15					19	1.02 ± 0.15					18	1.29 ± 0.32					22	1.09 ± 0.21				
GPC+PC/PCr+Cr																								
With	21	0.26 ± 0.06	0.002_1	0.961	0.08_1	0.769	16	0.24 ± 0.07	1.17_1	0.297	2.72_1	0.121	14	0.28 ± 0.05	1.10	0.314	5.07	0.044†	23	0.31 ± 0.03	0.39_1	0.534	3.40_1	0.079
Without	21	0.25 ± 0.04					16	0.23 ± 0.04					14	0.31 ± 0.05					23	0.30 ± 0.03				
mI/PCr+Cr																								
With	20	0.90 ± 0.28	0.79_1	0.385	0.58_1	0.454	13	0.84 ± 0.35	0.52_1	0.484	0.004_1	0.949	16	1.07 ± 0.50	4.19_1	0.060	3.94_1	0.067	20	1.06 ± 0.18	0.131	0.715	0.15_1	0.703
Without	20	0.95 ± 0.26					13	0.71 ± 0.35					16	0.94 ± 0.34					20	1.07 ± 0.25				
Glu+Gln/PCr+Cr																								
With	21	1.58 ± 0.54	1.45_1	0.242	2.09_1	0.164	12	1.72 ± 0.51	0.85_1	0.378	2.30_1	0.160	12	2.67 ± 0.88	0.60_1	0.456	0.04_1	0.845	22	1.80 ± 0.62	2.38_1	0.138	0.005_1	0.946
Without	21	1.60 ± 0.40					12	1.79 ± 0.41					12	2.26 ± 0.71					22	1.44 ± 0.46				

5.07, and p_{time} of 0.044, without reaching the level of statistical significance. Neither benzodiazepine-induced sedation nor time had an apparent effect on the other metabolites quantified in this brain region (Table 2). The difference observed between the right hippocampal NAA/PCr+Cr ratio of 1.24 ± 0.23 with benzodiazepine-induced sedation and 1.09 ± 0.21 awake did not reach the adjusted level of statistical significance ($F_{df=1}=4.57$, $p=0.045$; $F_{df=1}=0.10$, $p_{time}=0.755$). Neither benzodiazepine-induced sedation nor time caused any other visible changes in the quantified metabolites of the right hippocampus (Table 2).

4. Discussion

This study of 44 healthy individuals found that induced transient sedation with iv administration of midazolam plus fentanyl does not significantly alter the values of the major metabolites measured by in vivo ^{1}H MRS in the brain regions of ACC, right basal ganglia, right frontal lobe, and right hippocampus. In an in vivo MRS study, involving 10 healthy human subjects scanned twice, Brambilla et al. (2002) found similar ^{1}H-metabolites (absolute values and ratios) before and 1 h after administration of a single oral dose of lorazepam (2 mg) in the left dorsolateral prefrontal cortex. Deicken et al. (1992) in an vivo ^{31}P MRS study found no significant changes in membrane phospholipids or high-energy phosphate metabolites in white and subcortical gray matter regions of 10 healthy human subjects 1 h after the oral administration of diazepam up to 20 mg. Burau et al. (1997) applied have applied iv midazolam (0.05 mg/kg) to 23 healthy individuals and reported only transient increases in lactate and mI levels, which decreased to initial values by the end of the study in the striatal region, but no changes were found for the other ^{1}H-metabolites. Similarly, we did not find any significant change of mI and mI/Cr values in any of the brain regions studied. These findings, though in need of further confirmation, encourage use of benzodiazepines to induce transient sedation during MRS experiments involving subjects with severe psychiatric conditions.

Paired comparisons in the right hippocampus ($p=0.045$), and unpaired comparisons in the right frontal lobe ($p=0.034$) found a trend for higher NAA/PCr+Cr ratios under midazolam plus fentanyl induced sedation (Tables 1 and 2). By virtue of its exceptionally high concentrations in the human brain, NAA gives off a powerful signal in water-suppressed ^{1}H MRS (Moffett et al., 2007). While the peak at 2.02 ppm is prominently attributable to NAA, this signal includes smaller contributions from *N*-acetylaspartylglutamate (NAAG); thus, besides neuronal viability and mitochondrial functioning, it is an essential component of neuron↔glia signaling (Baslow, 2000; Moore and Galloway, 2002; Yildiz-Yesiloglu and Ankerst, 2006; Moffett et al., 2007). An early investigation reported that anesthetics and GABA administration caused NAA levels to increase in rodent brains (Buniatian et al., 1965); but these findings have not been confirmed elsewhere (Moffett et al., 2007). Anesthesia is associated with a dose-dependent general decrease in whole-brain activity; as such, if an alteration is in place, a decrease in NAA levels may be more likely (Heinke and Schwarzbauer, 2002). Moreover, a nonsignificant increase in the NAA/PCr+Cr ratios with benzodiazepine-induced sedation was not supported by parallel changes in the absolute NAA concentrations.

Considering the effect of benzodiazepines on $GABA_A$ receptors and the findings from a recent study where a benzodiazepine (clonazepam per oral 0.5 mg) was shown to decrease occipital cortex GABA levels and increase glutamate levels in healthy human subjects (Goddard et al., 2004), one could expect a change in Glu+Gln levels with benzodiazepines. Among 16 comparisons (paired and unpaired comparisons in four brain regions) made for Glu+Gln and Glu+Gln/PCr+Cr, a weak signal for a decrease of Glu+Gln levels with benzodiazepine-induced sedation appeared in the right basal ganglia ($p=0.048$, Table 1). No alterations in glutamatergic transmissions could be detected with paired comparisons (Table 2). At low field strengths such as 1.5 T the resonances originating from glutamate, glutamine, and GABA result in an overlapping signal in ^{1}H MRS (Auer et al., 2000; Stanley et al., 2000). Therefore, opposing changes in these metabolites might have neutralized each other, resulting in a false observation of no change in the Glu+Gln peak. Yet, if such a masking effect occurred in this study, it had to have occurred in all of the four different brain regions studied. Previous ^{1}H MRS studies on the effect of benzodiazepines in the brain could not detect any changes of the Glu+Gln concentrations, as well (Burau et al., 1997; Brambilla et al., 2002).

4.1. Limitations

There are several imitations of the present study. Despite our efforts to obtain data from multiple brain regions, for consistency we elected to study the right side of the brain, and the reported results do not apply to the left side. One might consider the removal of a certain metabolite level or ratio in the analysis due to not meeting the CRLB threshold and not rejecting all levels or ratios from that spectrum as a limitation or may claim that LC Model does not use *a priori* knowledge for baseline fitting and this poses an independent source of uncertainty in spectral quantitation. It has been demonstrated, however, that this method is robust and accurate within stipulated constraints. The LC Model's baseline fitting algorithm works in combination with the linear combination method which extracts the individual spectral peaks from which the corresponding baseline is subtracted. The LC Model uses "a nearly model-free constrained regularization method, which attempts to optimize between empirical baseline models with too few parameters (leading to bias) and too many fitting parameters which can lead to numerical instabilities and artifacts in the analysis" (Provencher, 1993). Within the stipulated constraints indicated in the data analysis section, satisfied by our data, the LC Model algorithm attempts to find the smoothest baseline consistent with the data and makes no other restrictive assumptions. It handles the residual water signal, artifacts and spectral peaks corresponding to substances not present in the basis set of the model metabolite spectra without significant degradation of fitting accuracy. Consistent with LC Model criteria, residuals for our LC Model spectra are reasonably randomly scattered about zero, our calculated baselines are smooth and do not move away from the data. Yet, the LC Model algorithm has been shown to be robust and accurate even in the context of moderate "dips and humps" in the baseline.

The time interval for the subjects scanned twice was considerable (122 ± 87 days), and this might have caused variability in the metabolite measurements. However, most of the accumulated ^{1}H MRS data in mood disorders are from cross-sectional exploratory studies; and longitudinal studies, where patients are followed up for treatment effects and/or changing mood states, are much needed. Such longitudinal studies would also be influenced by similar time-related variations on the metabolite measurements. As this study itself was conducted tagged onto such a longitudinal ^{1}H MRS study of bipolar disorder, healthy control subjects were scanned in a comparable time-frame. Nonetheless, in the ANOVA for repeated measures, time was assigned as a factor and had no significant impact on the measured metabolites of interest. The possibility of type II error, arising from small sample size for detection of minor differences, is a major problem in most of the in vivo MRS studies, as well as ours (Brambilla et al., 2002; Yildiz-Yesiloglu and Ankerst, 2006; Scherk et al., 2009). Even with our sample, which is the largest study conducted to date on the effects of benzodiazepines on human brain metabolites, an effect size of 0.77 or larger would be required to achieve a power ≥85% for detecting significance at two-tailed $\alpha=0.05$. Thus, alterations of the metabolites with medium effect sizes could have been undetected. Nevertheless, most prior ^{1}H MRS studies that detected significant alterations in NAA, GPC+PC, mI, Glu+Gln, and PCr+Cr in bipolar disorder had total sample sizes of 38, 30, 38, 16,

and 30, respectively (Yildiz-Yesiloglu and Ankerst, 2006), suggesting that with MRS studies it may be wiser to pursue strong signals rather than weak ones. Besides, to achieve an adequate power for most of the ^{1}H-measured metabolites, more than 100 subjects would need to be scanned, which might not be feasible in view of the expense of performing MRS experiments. The unfeasibility of performing larger experiments makes meta-analysis an appealing approach for combining data from individual MRS studies. Accordingly, in the worst case scenario that a type II error is in place, the present data may be incorporated in future meta-analysis. Finally, to limit the risk of false-positive (type I) errors, we applied a modest correction for multiple comparisons. Considering the above explained issues on type II error, although there were 16 comparisons (paired and unpaired, absolute values and ratios in four brain regions) for each metabolite of interest, we only applied correction for paired and unpaired comparisons.

4.2. Safety considerations and adverse events

The American Society of Anesthesiologists—ASA Task Force has developed practice guidelines for 'sedation and analgesia' by non-anesthesiologists and approved the use of 'sedation and analgesia' for uncooperative adults going through procedures that are not particularly uncomfortable but that require that the patient not move. 'Sedation and analgesia' comprise a continuum of states ranging from minimal sedation (anxiolysis) through general anesthesia (ASA Task Force, 2002). Definition of 'moderate sedation' (frequently called 'conscious sedation'), as adopted by the ASA, is a drug-induced depression of consciousness during which patients respond purposefully to verbal commands, either alone or accompanied by light tactile stimulation. In applications of 'moderate sedation' as in this study, no interventions are required to maintain a patent airway, and spontaneous ventilation is adequate. Cardiovascular function is usually well maintained (ASA Task Force, 2002).

As we were the first to administer 'moderate sedation' for uncooperative manic patients undergoing MR scan (healthy subjects reported here were matched controls to those manic patients), to enhance the effect of benzodiazepines, our study anesthesiologist applied fentanyl following midazolam as defined in the study protocol. Recent literature and ASA consultants suggest that combining a sedative with an opioid provides effective moderate sedation; yet it is equivocal regarding whether the combination may be more effective than a sedative or an opioid alone in providing adequate moderate sedation (ASA Task Force, 2002). Given that and the potential confounding effect of fentanyl on the observed changes, we suggest use of a benzodiazepine alone in future studies.

In this study sedation was induced and terminated by an anesthesiologist. No subjects needed assisted ventilation during the procedure; in all cases ventilation was achieved spontaneously with an oxygen mask. No cardiovascular complications were observed. Administration of 5 mg midazolam over 24 h via syringe driver has been reported to induce extrapyramidal side effects (Prommer, 2008). None of our subjects experienced extrapyramidal or any other adverse effects with iv administration of midazolam 0.03 mg/kg and fentanyl 2 μg/kg. Although opioids, when administered repeatedly, can have mood-elevating effects (Schaffer et al., 2007), neither the healthy subjects reported here nor bipolar patients scanned using the same protocol have experienced any mood elevation. Owing to the iv administration route and short half-life, the study subjects were not prone to any carry on effects of benzodiazepines.

4.3. Comment and conclusion

Patients suffering from major psychiatric disorders are often excited and agitated, and therefore not able to remain still in a scanner. For such patients in vivo ^{1}H MRS investigations of the brain have been difficult or impossible, resulting in an insufficiency of available data for conditions such as mania with or without psychotic features, severe, agitated or psychotic depression, and schizoaffective disorder in exacerbated states. Reported findings suggest that transient induction of sedation with iv midazolam can be utilized, if needed, to acutely sedate such psychiatric patients during ^{1}H MRS investigations.

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