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ARTICLE

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Proton MR spectroscopy of the hippocampus at 3 T in patients with unipolar major depressive disorder: correlates and predictors of treatment response

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Abstract

Various lines of research suggest that neurotrophic processes in the hippocampus are key mechanisms in major depressive disorder and are of relevance for response to antidepressive treatment. We performed proton magnetic resonance spectroscopy (^{1}H-MRS) of the hippocampus at 3 T in 18 unmedicated subjects with unipolar major depressive episodes and in 10 age- and gender-matched healthy volunteers. Thirteen patients underwent a second examination after 8 wk treatment with either citalopram ($n=7$) or nortriptyline ($n=6$). Of these patients, 11 MRS datasets could be used for the assessment of treatment correlates. In the cross-sectional comparison, we observed a significant reduction of the metabolic ratios Glx/Cr (Glx = glutamine, glutamate and gamma-aminobutyric acid) and glutamine (Gln)/Cr in the patient group. The Gln/Glx ratio also showed a trend towards significant reduction. The individual effect of treatment correlated with an increase in the absolute concentrations of *N*-acetylaspartate (NAA) and of choline compounds (Cho). Low baseline NAA and Cho levels predicted positive treatment effects. There was no difference in any clinical or metabolic measure, either at baseline or at follow-up between the two treatment groups (citalopram, nortriptyline). Our data provide first evidence for a reduction of Gln in the hippocampus of subjects with major depression. Furthermore, we provide first evidence in patients with major depression for neurorestorative effects in the hippocampus by pharmacological treatment expressed by a correlation of NAA and Cho increases with treatment response. This accounts in particular for those patients with low NAA and Cho baseline levels.

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Introduction

Current research has highlighted the crucial role of the hippocampus in the pathophysiology of major depression. Reduced hippocampal neurogenesis and restoration after antidepressive treatment is considered a key event and has been demonstrated in different animal models (Becker and Wojtowicz, 2007; Henn and Vollmayr, 2004; Santarelli et al., 2003). In humans, post-mortem studies of hippocampal tissue have revealed reduced levels of cellular growth factors (e.g. Castren et al., 2007; Turner et al., 2006); and smaller hippocampal volumes have been observed in many structural magnetic resonance imaging (MRI) studies in patients with major depression (Videbech and Ravnkilde, 2004). However, evidence for the neurorestorative effects of antidepressive treatment in humans is still rare.

Proton magnetic resonance spectroscopy (^{1}H-MRS) provides information about the biochemical composition of brain tissue. The most prominent signal obtained with ^{1}H-MRS reflects the concentration of the amino acid *N*-acetylaspartate (NAA). NAA is highly concentrated in neurons. It is a relevant component in myelinization and neuronal energy metabolism

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(Moffett et al., 2007). The NAA level is interpreted as an indicator for neuronal density and viability. The majority of studies in depression have not found differences of NAA in comparison with control subjects (Yildiz-Yesiloglu and Ankerst, 2006). A few studies have investigated the hippocampus. Here, Blasi et al. (2004) reported a reduced NAA/(phospho)creatine (Cr) ratio in depressed patients with psychotic symptoms. Michael et al. (2003) reported an increase of NAA in the amygdala and adjacent anterior hippocampus in patients who responded to electroconvulsive treatment (ECT). However, Ende et al. (2000) did not find any changes of NAA in the hippocampus after ECT treatment.

Choline-containing compounds (Cho) also provide a prominent ^{1}H-MRS signal. The major components of the Cho signal are unbound phosphocholine and glycerophosphocholine, which are indicators of membrane metabolism. Accordingly, elevated Cho is interpreted as evidence for increased membrane turnover. ^{1}H-MRS studies of the hippocampus in major depression have provided ambiguous results with increased, reduced and unchanged Cho levels (Ende et al., 2007; Mervaala et al., 2000; Michael et al., 2003). Ende et al. (2000) reported an increase of hippocampal Cho after ECT treatment. This increase vanished over time (Obergriesser et al., 2003). The rise of choline in the hippocampus after ECT has been replicated in rats (Sartorius et al., 2003).

The Cr signal indicates the (phospho-)creatine concentration, reflecting cellular energy metabolism. Myo-inositol (MI), which provides a resonance peak at short echo times, is highly concentrated in astrocytes and is considered a glial marker. Both, Cr and MI have mostly been found unchanged in patients with major depression (Yildiz-Yesiloglu and Ankerst, 2006).

Finally, ^{1}H-MRS detects the overlapping CH_2 resonance peaks of the neurotransmitters glutamate (Glu), glutamine (Gln), and gamma-aminobutyric acid (GABA) usually subsumed as Glx. In depression, a strikingly consistent finding is a decrease of Glx (Yildiz-Yesiloglu and Ankerst, 2006). Some studies have further reported an increase of Glx after treatment with ECT (Michael et al., 2003; Pfleiderer et al., 2003).

Overall, several open questions regarding ^{1}H-MRS findings in major depression remain. In particular, the effects of antidepressant medication on brain metabolites have only rarely been investigated. The small number of treatment-monitoring studies focusing on the hippocampus has thus far only investigated the effects of ECT. To the best of our knowledge, there are currently no published studies, which report biochemical effects of pharmacological treatment within the hippocampus of patients with major depression.

Technically, the published studies to date are frequently limited by using ratios instead of absolute measures of the respective metabolic compounds. In addition, most studies employed 1.5 T MR systems, where the separate quantification of the Glx components could be less reliably performed than at higher field strengths.

Here, we report a cross-sectional comparison of hippocampal metabolite levels in untreated patients with major depressive disorder and in healthy volunteers. Furthermore, we studied the metabolic changes related to 8-wk treatment with citalopram or nortriptyline to identify correlates and predictors of treatment response. We performed the study on a clinical 3 T MR scanner with absolute quantification of the metabolites NAA, Cho, Cr, MI, and separation of the C4-CH_2 resonance of glutamine (Gln) from the other components of the Glx signal.

Patients and methods

Subjects

Eighteen patients with DSM-IV unipolar major depressive disorder (mean age 36 ± 10 yr; 10 male, 8 female) were recruited from the Department of Psychiatry, University of Bonn. Diagnosis was based on the Structured Clinical Interview for DSM disorder (SCID). Fourteen subjects had first depressive episodes and four suffered from recurrent depression. Depression severity was determined by the Beck Depression Inventory (BDI) Rating Scale. All participants were in-patients. Baseline ^{1}H-MRS examination was performed after admission. All patients had not been taking antidepressants for at least 8 wk prior to the examination. None of the patients were suffering from any other significant medical disease or comorbid psychiatric disorder. The subjects were participants of a large project on predictors of treatment response in major depression and were randomized to either citalopram or nortriptyline open-label monotherapy.

Thirteen patients (age 38 ± 8 yr; 8 male, 5 female) underwent a second ^{1}H-MRS examination after 8 wk monotherapy with citalopram ($n = 7$, average dose 20 mg/d) or nortriptyline ($n = 6$, average dose 105 mg/d) in standard dose ranges. ^{1}H-MRS datasets from two of these subjects had to be omitted due to poor data quality, leaving 11 datasets (citalopram, $n = 5$; nortriptyline, $n = 6$). Seven patients did not undergo follow-up examination due to various reasons (side-effects in both treatment arms, non-compliance with

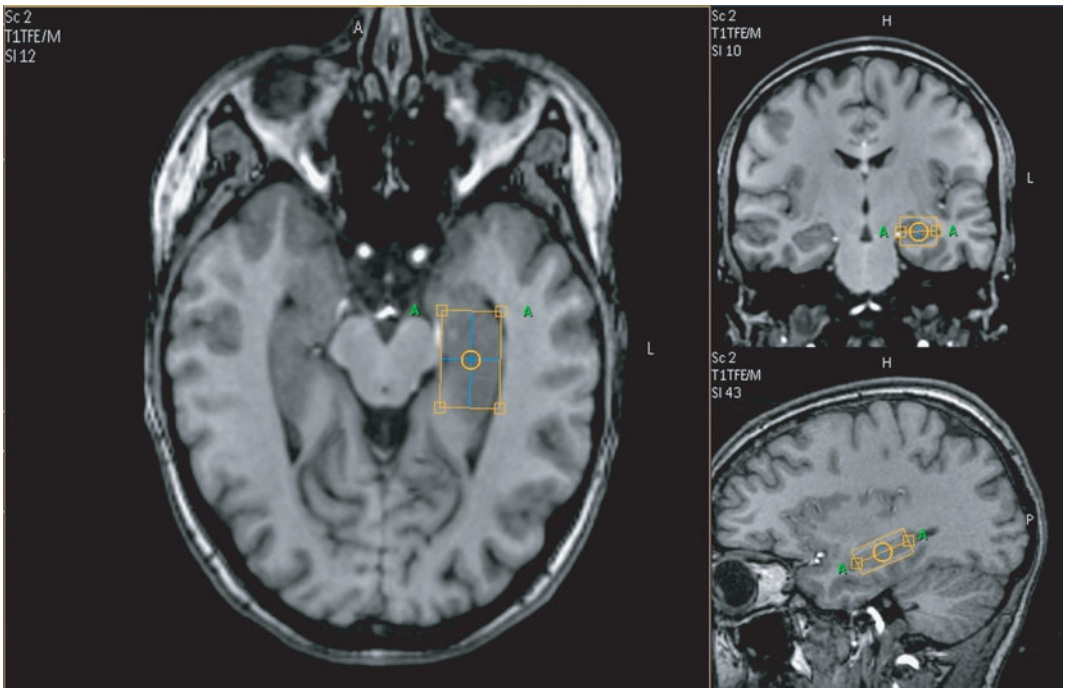

Figure 1. Display of image-guided volume of interest (VOI) selection on axial (angulated parallel to temporal lobe), coronal and sagittal T1w MRI. VOI size in anterior–posterior dimension 28 mm, left–right 17 mm and cranial–caudal 13 mm.

the treatment protocol, need of antipsychotic treatment, refusal of second MRI examination). Depression severity at follow-up was also determined by the BDI rating scale.

For cross-sectional comparison at baseline, ten volunteers were recruited (age 36 ± 19 yr; 6 male, 4 female). All were free of medical, psychiatric or neurological disorders and were not taking medication.

After a full description of the protocol, all patients and control subjects gave informed consent. The study was approved by the local ethics committee.

Magnetic resonance examination

Examinations were performed with a clinical 3 T whole-body MR system (Gyroscan Achieva 3.0 T, Philips Medical Systems, Best, The Netherlands) using a transmit/receive head coil designed for imaging and proton spectroscopy.

After completion of a standard MRI protocol for image-guided volume of interest (VOI) selection, a 6 ml VOI was placed in parallel angulation to the medial temporal lobe centred on the left hippocampus (Figure 1). The VOI included most of the hippocampal body and parts of its head, of the amygdala and of the parahippocampal gyrus. Left and right borders in the axial slice were determined by the brainstem and the inferior horn of the lateral ventricle. Single-volume ^{1}H-MRS was performed including acquisition of water-suppressed, point-resolved spectroscopy (PRESS) localized spectra with TE/TR 140/2000 ms and 30/2000 ms (Figure 2). Applying 128 signal averages with 1024 samples at 2 kHz bandwidth (non-interpolated frequency resolution 2 Hz), measurement time was 4 min for each spectrum. Metabolite signals were time-domain quantified applying the AMARES algorithm of the jMRUI software package (Naressi et al., 2001; Vanhamme et al., 1997), and signal ratios of NAA and Cho relative to Cr were determined from the spectra with TE 140 ms. Metabolite ratios of MI, Glx and Gln were taken from the short-TE spectra at TE 30 ms, with Glx defined as the summation of four overlapping resonance components of Glu-, Gln-, and GABA-CH_2 in the frequency range 2.1–2.4 ppm; Gln ratios were additionally derived from the isolated CH_2 (C-4) resonance of Gln at 2.47 ppm (partly coinciding with NAA-CH_2 signals). Absolute metabolite concentrations of NAA, Cr, Cho, and MI (mmol/l brain tissue) in the hippocampus were calculated using the water signal of the VOI from an unsuppressed acquisition with TE/TR 140/3500 ms and 32 signal averages as an internal reference. Partial CSF volume in the VOI was corrected for by unsuppressed bi-exponential T2 relaxometry (Traber et al., 2006).

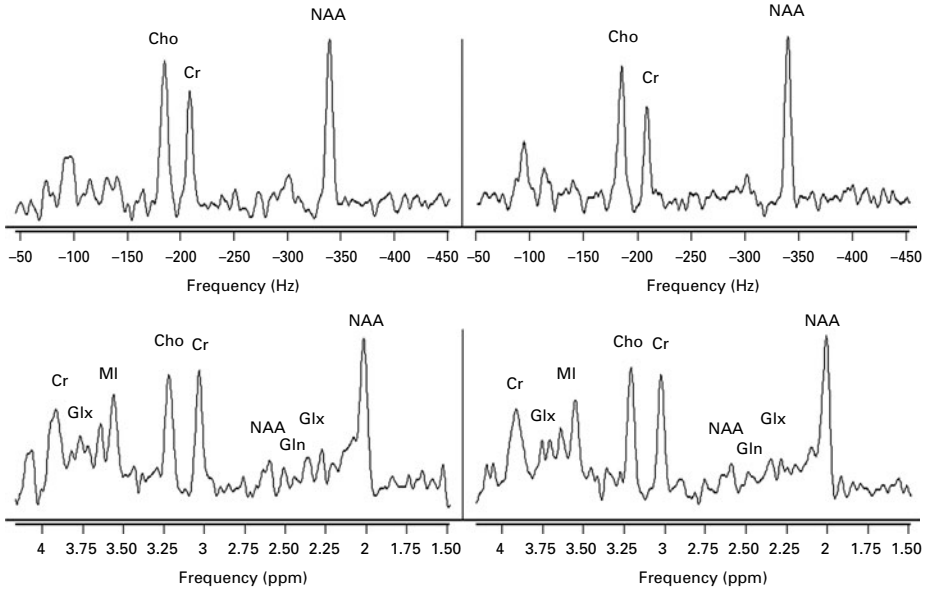

Figure 2. ^{1}H-MRS hippocampal spectra of a 37-yr-old male patient at baseline (left) and after 8 wk of treatment (right). PRESS acquisitions at 3 T with TR 2000 ms/TE 140 ms (above) and 30 ms (below). Cho, Choline; Cr, creatine; Gln, glutamine; Glx, glutamine/glutamate/GABA; MI, myo-inositol; NAA, *N*-acetylaspartate.

Statistical analysis

There were no significant differences between the patient and the control groups with regard to age ($t=0.58$, $p=0.32$) and gender ($\chi^2=0.52$, $p=0.82$). Thus, all metabolic ratios and absolute measures were compared with a Student's t test between patients and controls at baseline. Correlation analyses (Pearson) were used to test for associations of severity of depression (BDI) with ratios or metabolic concentrations within the group of patients. To assess treatment response, depression ratings (BDI) and ^{1}H-MRS measures were compared between baseline and 8-wk follow-up by paired t tests. Furthermore, the differences between depression ratings (BDI) at baseline and at the 8-wk follow-up were calculated. These were correlated with the respective differences in ^{1}H-MRS measures. Further, the change of BDI was correlated with baseline ^{1}H-MRS measures to identify metabolic predictors of treatment response. Furthermore, all demographic, clinical and metabolic measures at baseline and follow-up and the respective differences were compared between the two treatment groups (citalopram/nortriptyline) by non-parametric Mann–Whitney U tests.

Results

The cross-sectional comparison at baseline revealed significantly lower Glx/Cr ($t=3.28$; $p=0.003$) and Gln/Cr ($t=2.64$, $p=0.015$) in patients. On a trend level, we also found a reduced Gln/Glx ratio ($t=1.80$, $p=0.085$) and reduced Cho ($t=1.83$, $p=0.079$). All other ratios and concentrations (of NAA, Cho, Cr and MI) did not differ between the groups. Of the determined ratios and metabolite concentrations, none correlated with the BDI at baseline ($t1$). The data of the cross-sectional comparison are given in Table 1a.

After 8 wk treatment ($t2$), the BDI had significantly decreased in the patients ($BDI_{t1}=23.5$, S.D. $=8.5$; $BDI_{t2}=12.2$, S.D. $=8.8$; $t=5.3$, $p<0.001$), indicating positive treatment response. There was, however, no significant group difference between time-points $t1$ and $t2$ in any ^{1}H-MRS ratio or metabolic measure. Results of the longitudinal comparison are summarized in Table 1b.

The difference in BDI between $t1$ and $t2$ ($dBDI=BDI_{t2}-BDI_{t1}$) is an indicator for individual treatment response. We observed a highly significant negative correlation between dBDI and change in Cho level ($n=11$, $r=-0.762$, $p=0.006$) (Figure 3a) and a significant negative correlation of dBDI and change in NAA level ($n=11$, $r=-0.615$, $p=0.044$) (Figure 3b). This reflects an increase of Cho and NAA concentrations with positive treatment response. None of the other differences in metabolic measures or metabolic ratios between $t1$ and $t2$ correlated with dBDI.

Finally, we correlated dBDI with baseline ^{1}H-MRS measures to identify predictors of treatment response. We found a positive correlation of dBDI with baseline

Table 1. Spectroscopic data of unmedicated patients with major depressive disorders at baseline compared to (a) data from healthy comparison subjects, and (b) to the respective data after 8 wk treatment

	NAA (mmol/l)	Cho (mmol/l)	Cr (mmol/l)	NAA/ Cr	MI (mmol/l)	Glx/ Cr	Gln/ Cr	Gln/ Glx
(a) Baseline comparison								
Patients ($n=18$)	9.42	2.49	8.89	1.53	6.32	1.87	0.25	0.13
Mean (S.D.)	(1.35)	(0.37)	(1.43)	(0.20)	(0.93)	(0.38)	(0.11)	(0.04)
Controls ($n=10$)	9.87	2.74	9.81	1.48	6.16	2.38	0.41	0.17
Mean (S.D.)	(1.25)	(0.28)	(1.28)	(0.21)	(1.56)	(0.36)	(0.19)	(0.06)
t test[a]								
t value	0.85	1.83	1.68	0.64	0.32	3.28	2.64	1.80
p value	0.405	0.079	0.105	0.526	0.755	**0.003**	**0.015**	0.085
(b) Follow-up comparison								
Patients at $t1$ ($n=11$)	9.38	2.53	8.72	1.54	6.22	1.91	0.25	0.12
Mean (S.D.)	(1.46)	(0.38)	(1.60)	(0.20)	(1.04)	(0.31)	(0.11)	(0.05)
Patients at $t2$ ($n=11$)	9.66	2.52	8.96	1.57	5.71	1.81	0.28	0.13
Mean (S.D.)	(1.19)	(0.39)	(2.10)	(0.26)	(1.54)	(0.33)	(0.15)	(0.04)
t test[b]								
t value	0.60	0.17	0.39	0.54	1.53	0.57	0.51	0.34
p value	0.561	0.871	0.707	0.601	0.159	0.585	0.626	0.743

Cho, Choline; Cr, creatine; Gln, glutamine; Glx, glutamine/glutamate/GABA; MI, myo-inositol; NAA, *N*-acetylaspartate. The table summarizes means and S.D. for absolute (mmol/l brain tissue) and relative metabolite concentrations in the hippocampal volume of interest.

[a] t and p values for patients at baseline vs. controls; significant p values in boldface.

[b] t and p values for the comparison of baseline ($t1$) with 8 wk treatment ($t2$).

Cho ($n=13$, $r=0.564$, $p=0.044$) and of dBDI with baseline NAA ($n=13$, $r=0.565$, $p=0.044$). This indicates that low Cho and NAA at baseline are associated with positive treatment response. None of the other baseline metabolic measures or metabolic ratios correlated with dBDI.

There was no significant difference in any MRS measure between the two treatment groups (citalopram/nortriptyline).

Discussion

In the cross-sectional comparison of untreated subjects with unipolar major depressive episode and healthy volunteers, we found a reduced Glx/Cr ratio. Even though the multiplet structure of Glx is not as prominent as other singlet peaks in the ^{1}H-MRS spectra complicating reliable quantification of its components and is subject to variations over time (Schirmer and Auer, 2000), there is a striking consistency over a number of studies showing reduced Glx levels in major depression (Capizzano et al., 2007; Yildiz-Yesiloglu and Ankerst, 2006). Our finding is in particular agreement with an earlier report showing a reduction of Glx/Cr in a combined voxel of the amygdala and the anterior part of the hippocampus in patients with major depression (Michael et al., 2003).

We quantified an additional low-intensity peak, which at higher magnetic fields (>2 T) can almost solely be attributed to Gln. We found a significantly reduced Gln/Cr ratio and a decreased Gln/Glx ratio at a statistical trend level ($p=0.085$) in patients compared with controls. This suggests that Gln is reduced in total and also in relation to other components of the Glx signal (Glu and GABA). Low Gln has not yet been reported in major depression. However, increased Glu in depressed subjects was shown previously (Sanacora et al., 2004). Glu as the major excitatory neurotransmitter is metabolized to Gln after uptake in astrocytes. Novel micro-array post-mortem studies provide evidence for increased Glu and decreased Gln as a consequence of reduced expression of L-glutamate-ammonia ligase in major depression (Choudary et al., 2005; Sanacora et al., 2004), which might explain our ^{1}H-MRS finding of reduced Gln in the hippocampus.

Due to the low intensity of Gln resonance and its partial overlap with NAA-CH_2 signals even at 3 T, the inter-individual variations in the Gln/Cr and Gln/Glx ratios are considerably large and therefore interpretation of our findings, regarding reduced Gln, needs to

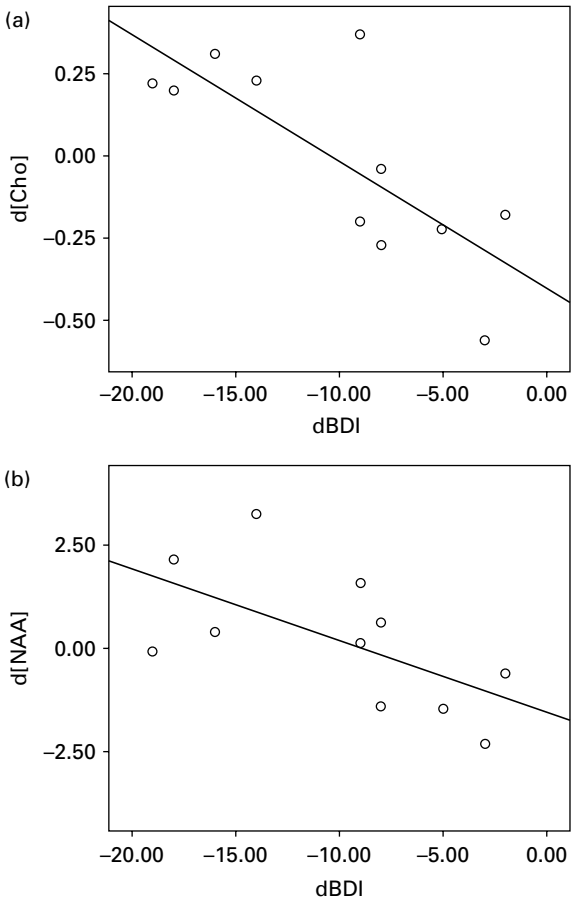

Figure 3. Plot of changes in BDI ($dBDI = BDI_{t2} - BDI_{t1}$) vs. changes in (a) absolute Cho ($d[Cho] = [Cho]_{t2} - [Cho]_{t1}$) concentrations, and (b) NAA ($d[NAA] = [NAA]_{t2} - [NAA]_{t1}$) concentrations (mmol/l brain tissue) between baseline ($t1$) and follow-up investigation ($t2$). Statistics are given in the text.

be treated with caution. Nevertheless, the observed reduction exceeds 40% for Gln/Cr and 20% for Gln/Glx at baseline and cannot be attributed to the spectral overlap with the low intensity NAA-CH_2 multiplet, as this would require concentration changes of similar magnitude for NAA which we did not observe.

The second finding of the present study is the correlation of NAA and Cho increases with treatment response. In the cross-sectional group comparison, NAA was not reduced in depressed subjects, which is in agreement with the majority of publications (Capizzano et al., 2007; Yildiz-Yesiloglu and Ankerst, 2006). Cho was reduced in depressed subjects at baseline at a statistical trend level ($p = 0.079$). Both metabolite concentrations did not significantly increase in the pairwise comparison between baseline and 8-wk treatment. However, there was a correlation of $r = -0.615$ for NAA change and of $r = -0.762$ for Cho change with the individual treatment response. This indicates that independent of the baseline severity of symptoms, response to treatment is associated with an increase of NAA and Cho.

NAA is a marker for density and functional status of neurons with roles in energy metabolism and myelinization. The protective effect of antidepressants on hippocampal neuronal density evidenced by NAA concentrations has been demonstrated in tree shrews (Czeh et al., 2001). The association of NAA increase with clinical improvement in the patients of our study supports the model of neuronal restoration as a prerequisite for clinical response in humans, which has been derived from animal studies (e.g. Henn and Vollmayr, 2004; Santarelli et al., 2003). To the best of our knowledge, these are the first data to show evidence for neuronal restoration in the hippocampus in association with clinical response to pharmacological treatment in patients. Gonul et al. (2006) found an increase of the metabolic ratio NAA/Cr in the frontal lobe in patients with depression after drug treatment. Michael et al. (2003) reported increased NAA in the amygdala and anterior hippocampus after successful ECT. However, Ende et al. (2000), did not find an effect on hippocampal NAA after ECT.

The association of Cho increase with treatment response in the present study is similar to the effect observed by Ende et al. (2000) in the hippocampus after ECT. Sonawalla et al. (1999) reported an increase of Cho/Cr in the basal ganglia of depressed subjects responding to fluoxetine. Czeh et al. (2001) reported a decline of Cho and neuronal density in the hippocampus of stressed tree shrews, while the decrease of both was not observed in these animals, when the serotonin reuptake inhibitor tianeptine was given. These findings suggest that Cho might be a sensitive marker for neuronal restoration in depression.

Low baseline NAA and Cho levels were correlated with response to treatment, irrespective of the severity of baseline symptoms. This is in contradiction of Kado et al. (2006), who described an association of clinical response with high baseline NAA/Cr ratio in the frontal lobe of patients with geriatric depression. In that study, however, the participants were older, the ^{1}H-MRS target region was different from ours and only metabolic ratios, rather than absolute metabolic measures were reported.

Low NAA and Cho as positive response predictors point to a functional condition, which may facilitate treatment response. This functional condition might be characterized by a deprived neuronal state with the potential for restoration. The deprived state is

reflected by low NAA and Cho levels. The potential for restoration is evidenced by the increase of NAA and Cho.

It needs to be stressed, however, that not all patients with the clinical syndrome of major depression reveal this state. Some individuals show baseline NAA and Cho levels in the range of those seen in healthy subjects, which might explain the failure to observe cross-sectional group differences of NAA and Cho levels in this and in other studies. Furthermore, these subjects with initially high NAA and Cho show no increase of NAA and Cho during treatment, and do not respond well clinically. Thus, different neuronal conditions within the hippocampus can be associated with clinical syndromes which all fulfil the criteria for major depressive episodes.

However, the deprived neuronal state with the potential for restoration, expressed by low NAA and Cho, and increase of both by treatment, which was observed in responders, needs to be further investigated and might be a key to the understanding of the variability of treatment response in major depression.

In the present study, the subjects received two different antidepressants acting through either serotonin or mainly norepinephrine. We did not observe differences in any measures between both groups. This supports the concept that neurogenesis is a common mechanism in all types of effective antidepressive treatment, as demonstrated in animal studies (Henn and Vollmayr, 2004; Santarelli et al., 2003).

Our study has limitations. The sample size is small and the results are not corrected for multiple comparisons. Thus, these data serve as hypothesis-generating data, which require confirmation. Depression was rated with the BDI, which is a self-rating scale. Observer-rating scales are frequently used in clinical trials with antidepressant agents. However, novel analysis in a large patient cohort found that the BDI has high internal validity and detects and rates depression equally well as observer-based scales (Uher et al., 2008).

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Statement of Interest

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