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## Hippocampal glutamate concentration predicts cerebral theta oscillations during cognitive processing

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**Abstract** *Rationale:* Brain waves reflect collective behavior of neurons and provide insight into distributed network processing. Frontal and hippocampal theta oscillations (4–7 Hz) were linked to cognitive tasks and animal studies have suggested an involvement of glutamatergic neurotransmission in integrative frontal-hippocampal processing. Human evidence for such relationships is lacking. *Methods:* Here, we studied the associations between glutamate concentrations in the hippocampal region, measured by a 3-T proton magnetic resonance spectroscopy (1H-MRS), and EEG theta activity during an auditory target detection paradigm. *Results:* A robust relationship between hippocampal glutamate and frontal theta activity during stimulus processing was found. Moreover, frontal theta oscillations were related to response speed. *Conclusion:* The results suggest a functional coupling between the frontal cortex and hippocampal region during stimulus processing and support the idea of the hippocampus as a neural rhythm generator driven by glutamatergic neurotransmission. These preliminary data show, for the first time, a relationship between in vivo measured glutamate and basic cerebral information processing in humans.

**Keywords** EEG · Theta · Oscillator · Oscillation · Hippocampus · Glutamate

### Introduction

Synchronous oscillations at distinct frequency ranges are viewed as an important mechanism linking single-neuron activity to behavior and mental disorders (Basar-Eroglu et al. 1992; Gallinat et al. 2004). The crucial role of hippocampal theta oscillations (4–12 Hz) in mnemonic processes (Miller 1989; Givens 1996) is increasingly targeted in the accumulating body of literature. Synchronous activity of hippocampal theta activity was closely linked to hippocampal key features, including long-term potentiation (LTP) and synaptic plasticity (Maren et al. 1994). In the hippocampus, theta activity was suggested to be a major operational mode of grouping and segregating neuronal assemblies; the activity assigns computational tasks to these assemblies (Buzsaki 2002). In addition, theta oscillations or phase-locked neuronal discharges were also observed in hippocampal afferent and efferent structures, i.e., the entorhinal cortex, the perirhinal cortex, and the amygdala (Alonso and Garcia-Austt 1987; Pare and Collins 2000), although these structures are not capable of independently generating theta activity.

In human investigations, theta activity was recorded during resting conditions and was shown to dramatically increase during mental tasks like memory paradigms (Kahana et al. 1999; Klimesch 1999), working memory (Onton et al. 2005; Gevins et al. 1997), and in experiments using the oddball paradigm (Yordanova et al. 2003; Basar-Eroglu et al. 1992; Röschke and Fell 1997). The most consistent increase in oscillatory theta activity was found at frontal and central midline electrodes (Gevins et al. 1997). In line with this, EEG- and MEG-based source modeling of theta oscillations suggests a current generator in the anterior cingulate cortex (ACC) (Gevins et al. 1997; Reischies et al. 2005).

Task-related theta responses in both the scalp EEG and hippocampal recordings were suggested to reflect temporal

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cooperation between the hippocampus and its immediate efferent and afferent structures (Kirk and Mackay 2003; Klimesch 1999; Buzsaki 2002). It was argued that theta oscillations represent a basic resonance phenomenon mediating the information flow through the hippocampus, thalamus, and frontal/temporal neocortex (Kirk and Mackay 2003). Accordingly, paradigms eliciting hippocampal theta oscillations (e.g., oddball and visual maze) in humans (Fell et al. 2004, 2005) were shown to induce cortical theta oscillations (Kahana et al. 1999; Röschke and Fell 1997). It was therefore hypothesized that scalp recorded theta oscillations may serve as a window to hippocampal activity (Kirk and Mackay 2003; Klimesch 1999), which is not directly volume-conducted to the scalp, *per se* (Halgren et al. 1995).

However, there is also strong evidence that the glutamate system is involved in mediating hippocampal theta activity. For instance, the removal or isolation of glutamate-containing pathways eliminates hippocampal theta activity (Vanderwolf and Leung 1983; Buzsaki 2002). Similar results were observed after urethane application (Ylinen et al. 1995), which attenuates glutamate release from presynaptic vesicles. At the microlevel, the NMDA receptor is likely to mediate slow wave responses in the hippocampus because antagonists (e.g., ketamine) were observed to alter the frequency and power of theta oscillations (Vorob'ev et al. 1997; Dimpfel and Spuler 1990). This follows the theory that glutamate is the major excitatory neurotransmitter in the hippocampus, mediating excitatory postsynaptic potentials (Kandel and Siegelbaum 2000) that are essential for EEG oscillations and evoked potentials (Javitt et al. 1995). In contrast to the current understanding of the neurochemical processes that presumably mediate oscillatory theta activity in animals, very little is known about the neurotransmitter mechanisms of the hippocampal-cortical interplay in humans at this point.

Here, we present a study examining the relationships between the glutamate level in the hippocampal region and scalp-recorded cerebral theta oscillations during auditory stimulus processing. The concentrations of glutamate in the left hippocampal region and in the ACC were measured in 38 subjects using a dedicated proton magnetic resonance spectroscopy (1H-MRS) method at 3 T (Schubert et al. 2004; Gallinat et al. 2005; Elster et al. 2005). EEG was recorded for each subject in a separate experimental session. The auditory oddball paradigm was chosen because it elicits event-related theta activity occurring in frontal lobe structures (Röschke and Fell 1997) and in the hippocampus (Fell et al. 2005), and reflects different memory-based processing stages of auditory target detection (Yordanova et al. 2003). Therefore, the hypothesis was tested that glutamate concentration in the hippocampal region is linked to theta activity. In an exploratory analysis, the association of other main frequency ranges with glutamate concentration was investigated.

## Materials and methods

### Subjects

The study was approved by the ethics committee of the Charité University Medicine, Campus Mitte (Berlin, Germany). The subjects were healthy individuals of German descent who were recruited through newspaper advertisements. All subjects gave written informed consent. Somatic and psychiatric health status was evaluated by a structured psychiatric interview (Mini-International Neuropsychiatric Interview) performed by a psychiatrist. Subjects who met the criteria for an axis I or II disorder according to Diagnostic and Statistical Manual of Mental Disorders-IV criteria were excluded. Further reasons for exclusion were neurological and general medical disorders or clinically relevant abnormalities. A total of 19 male (age 35.8±9.6 years) and 19 female (age 35.5±11.3 years) volunteers participated in the study. The magnetic resonance spectroscopy session was carried out 24 h after the recording of the EEG to ensure that the time of day was kept constant across both measurements.

### Magnetic resonance spectroscopy

For each subject, magnetic resonance measurements were carried out on a 3-T scanner (MEDSPEC 30/100, Bruker Biospin, Ettlingen, Germany) using a circularly polarized head coil. After automated global shim of the linear,  $xz$ ,  $z^2$ , and  $x^2-y^2$  field components,  $T_1$ -weighted images were acquired using MDEFT [ $T_E=5.5$  ms,  $T_R=23.4$  ms; 64 contiguous slices, 2-mm-thick; 1-mm inplane ( $x-y$ ) resolution]. Magnetic resonance spectra were acquired from  $2 \times 3 \times 2$  cm<sup>3</sup> voxels, including the left hippocampus, and from  $2.5 \times 4 \times 2$  cm<sup>3</sup> voxels, including the anterior cingulate (Fig. 1). For metabolite quantification (see below), spectra were also acquired from equal voxels in the center of metabolite phantoms (0.1 M metabolite, pH 7.2, and 37°C). After manual shimming to water, line widths (full width at half maximum) of 7–9 Hz and 6–7 Hz for the two brain voxels and radiofrequency power needed for a 90° excitation pulse was determined. Subsequently, calibration of water suppression (3 G chemical shift selective pulses of 25.6 ms duration) was carried out, followed by acquisition of spectra with the PRESS (point resolved spectroscopy) sequence using a Shinnar-LeRoux-optimized 90° pulse and Mao refocusing pulses (Mao et al. 1986). Eight subspectra of 16 phase-cycled scans were each recorded with  $T_R=3$  s, yielding 128 averages. To ensure maximum selectivity for the glutamate C4 resonance, an echo time of 80 ms was chosen as per a recent extensive study (Schubert et al. 2004). Before further processing, these eight individual metabolite subspectra were corrected for eddy currents using water-unsuppressed spectra ( $n=8$ ). Spectra quantification was carried out using a program package based on a time domain-frequency domain-fitting procedure (Elster et al. 2005; Schubert et al. 2004). This method involves automatic correction for frequency shifts caused by in-

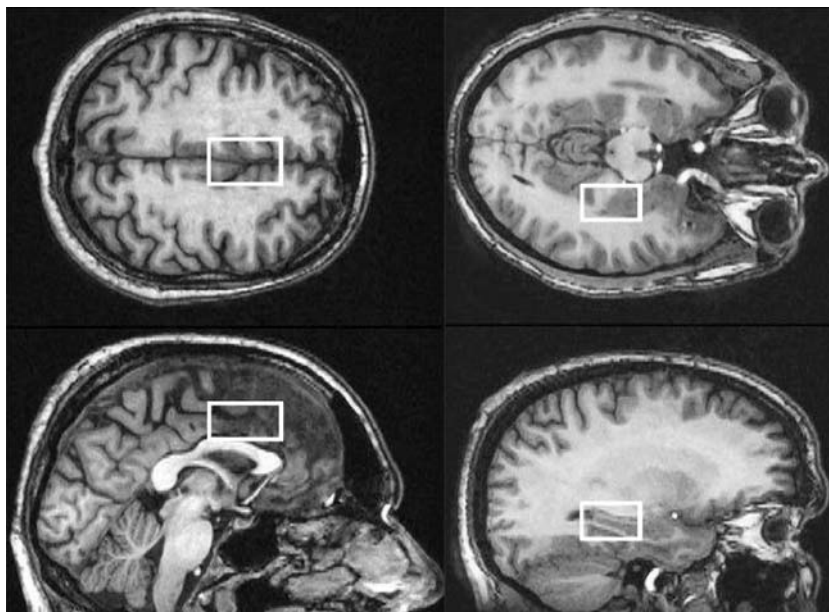
voluntary subject motion and system instabilities, inclusion of phantom basis spectra (see above) and prior knowledge into the fit, and background estimation. In the present spectra, the resonances of total choline, total creatine, *N*-acetylaspartate (NAA), and glutamate and glutamine resonances were fitted by inclusion of phantom spectra for the latter three, and imposing the following prior knowledge (Schubert et al. 2004): constant frequency differences for glutamate, glutamine, and NAA; equal line widths; and adjustment of signal line shape to purely Lorentzian. At the chosen echo time of 80 ms, the baseline was not entirely flat. To account for baseline features, the fitting procedure routinely includes estimation of the baseline nonparametrically by regularization (Elster et al. 2005). Extensive tests yielded mean uncertainties (corresponding to Cramér-Rao lower bounds with added uncertainties from baseline modeling) for the fitting of glutamate of 13.1% for the hippocampus voxel and 10.5% for the ACC voxel. Metabolite amplitudes returned by the fitting procedure were corrected for different coil loading by the phantoms and the individual subject's head (principle of reciprocity) and for relaxation effects. The effective transverse relaxation time of glutamate was determined using echo times of 50, 80, 135, 250, and 330 ms from three healthy volunteers to be 171 (22) ms for the hippocampus and 194 (37) ms for the anterior cingulate voxel (standard deviation in parentheses). No correction for longitudinal relaxation effects was carried out. The deviation caused by the  $T_1$  effect at  $T_R=3$  s and an assumed  $T_1$  of glutamate at 3 T of about 1.2 s (Mlynarik et al. 2001) was largely compensated by the deviation estimated for the aqueous, buffered glutamate phantom where  $T_R$  was set at 5 s and  $T_1$  was determined to be 1.47 s. Thus, glutamate might, at worst, be systematically underestimated by about 5%, owing to unaccounted  $T_1$  effects, which would lead to uniform scaling of the calculated concentrations. However, because we did not determine the true  $T_1$  values of glu-

tamate in the voxels of interest, the correction remains speculative and was therefore neglected.

It should be noted that GABA, a major candidate interferent in the quantification of the glutamate C4 resonance (and a neurotransmitter whose concentration might change as well), was not included in the spectrum analysis. However, extensive tests on both metabolite phantoms and in vivo brain spectra indicated our fitting procedure to be largely insensitive to GABA contribution, mainly because of the fair separation of the two resonance signals at the chosen echo time of 80 ms (Schubert et al. 2004). Likewise, aspartate is a compound likely to contribute to the fit in the chosen spectral region. We therefore tested the effect of including fixed aspartate amplitudes corresponding to concentrations at and above the physiological level (i.e., from 1 to 3 mmol/l) (Govindaraju et al. 2000) in the fit. Fitting of spectra from the hippocampus voxels of 11 subjects gave mean increases of the predicted glutamate level of at most 0.5% at 3 mmol/l aspartate, regardless of the GABA resonance being included in the fit or not. Therefore, the present method of glutamate fitting can be considered unaffected by aspartate contribution. For the sake of stability, both GABA and aspartate were omitted from the fit.

To correct the metabolite concentrations for the fraction of cerebrospinal fluid (csf) in the voxels studied, segmentation into gray matter, white matter, and csf of the  $T_1$ -weighted images was performed using spm99 (Ashburner and Friston 1997). Classification of pixels was based on which spm99 tissue classification had the greatest probability. It should be noted that the possible error introduced by erroneous estimation of csf fraction is marginal given the small absolute fractions. Therefore, errors in csf estimation caused by the small chemical displacement of the metabolites and the deviation of the radiofrequency pulses from perfect rectangularity were regarded as negligible.

**Fig. 1** Voxel positions shown on typical brain MDEFT images. *Left panel:* anterior cingulate cortex; *right panel:* left hippocampus



## EEG recording

Recording took place in a sound-attenuated and electrically shielded chamber adjacent to the recording apparatus. Subjects were seated in a slightly reclined chair with a headrest. EEG was recorded from 32 channels referenced to Cz during recording and off-line rereferenced to a common average reference. Electrodes were positioned according to the International 10/20 system with the additional electrodes FC1, FC2, FC5, FC6, CP1, CP2, CP5, CP6, PO9, PO10, TP9, and TP10. Fpz served as ground. Eye movements were recorded across an electrode, which is 1 cm lateral to the left eye (Lo1). Electrode impedance was less than 10 k $\Omega$ . Recording was performed (Brain Amp, MES, Munich; 500 Hz sampling rate) while the subjects' eyes were closed, using an auditory oddball paradigm with pseudorandomized presentation of rare targets (55 sinus-tones at 1,000 Hz, 83 dB sound pressure level; 40 ms duration including 10 ms rise and 10 ms fall time) among frequent nontargets [175 double-clicks with 500 ms ISI (international sensitivity index), 1 ms square waves at 83 dB]. Stimuli were presented binaurally using headphones with an ISI ranging between 1.5 and 4.6 s. Subjects were instructed to pay attention and make a speeded button response with their right thumb when a target stimulus was presented. Spectral frequency analysis was performed with the BrainVision Analyser, MES, Munich.

## Analysis of frequency band activities

Fast Fourier transformation (FFT) (256-point segmentation, frequency resolution=1.525 Hz) was performed for the analysis of frequency band activities. The focus of the analysis was the auditory target stimuli. The frequency band activities were separately computed for prestimulus (-1,500 to 0 ms) and poststimulus (0 to 1,500 ms) time intervals. Frequency band partition for theta activity was 4.5–8.0 Hz. In addition, delta (0.5–4.0 Hz), alpha (8.5–12.5 Hz), beta-1 (13.0–18.0 Hz), beta-2 (18.5–30.0 Hz), and gamma (35.0–45.0 Hz) frequency band activities were computed. For the statistical analysis, the power of each frequency band was separately computed relative to the total power of all frequency bands (0.5–45.0 Hz). This normalization procedure was successfully applied previously (Gasser et al. 1982; Herrmann et al. 1983). For every subject, at least 30 artifact-free (activity did not exceed  $\pm 100$   $\mu$ V) prestimulus and poststimulus EEG epochs were submitted to the FFT analysis. Due to previously observed task-related theta increase at frontal midline electrodes (Gevins et al. 1997), the frontal electrodes F3, F4, F7, F8, Fc1, Fc2, and Fz were selected and pooled for further analyses.

## Analysis of glutamate concentrations and frequency band activities

The association between glutamate concentrations and frequency band activities was investigated in a multiple regression using the glutamate concentration as dependent variable and the frequency band activities as predictor variables. In addition, separate regression analyses were performed using the hippocampal and ACC glutamate concentrations as dependent variables. Furthermore, to control whether the glutamate concentrations are specifically related to stimulus processing, separate analyses were conducted using either the prestimulus or the poststimulus frequency band activities as predictor variables. Thus, a total of four different multiple regression analyses were performed. The independent variables in these analyses were entered in one single step. In cases where a significant association was found between the predictor variables and the dependent variable, partial correlation analyses were conducted to control for the mediating effects of age and gender.

## Analysis of reaction times, frequency band activities, and glutamate concentrations

To study the relationships between reaction times (RTs), frequency band activities, and glutamate concentrations, multiple regression analyses with the independent factors, poststimulus frequency band activities and glutamate concentrations, were performed. Multiple regression analyses were separately computed for hippocampal and ACC glutamate concentration, and for the pre and poststimulus frequency power. In cases where a significant result was found, partial correlation analysis was conducted to control for mediating effects of age and gender.

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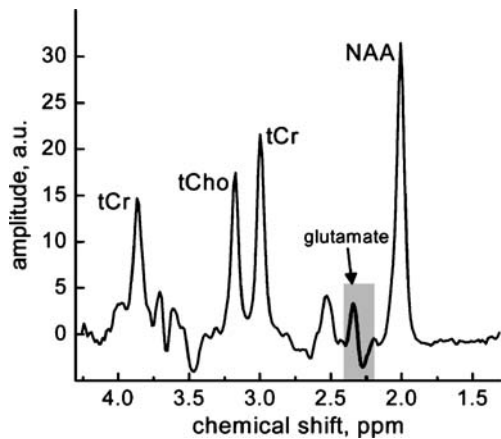
## Results

### Magnetic resonance spectroscopy

A typical PRESS spectrum acquired from a voxel containing the hippocampus (Fig. 2) shows, along with the flat baseline, good resolution in the glutamate C4 region where glutamine and GABA make only minor contributions (see also Schubert et al. 2004). Figure 3 demonstrates spectra of metabolites as they were predicted from the fit along with the baseline and residual. The glutamate concentration was determined to be  $10.9 \pm 1.4$  (range 8.4–14.6) mmol/l in the left hippocampus and  $11.7 \pm 1.2$  (range 8.5–14.0) mmol/l in the ACC.

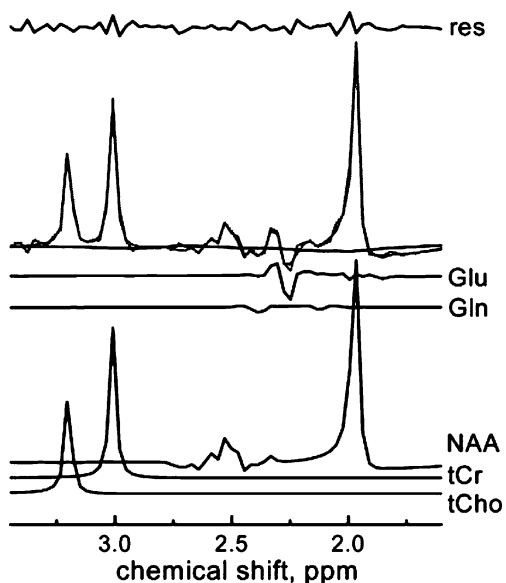
### Frequency band activities

FFT of frequency band activity revealed a significant increase in poststimulus (0 to 1,500 ms poststimulus) in comparison to prestimulus (-1,500 to 0 ms) power in the



**Fig. 2** Typical PRESS spectrum ( $T_R=3$  s and  $T_E=80$  ms) acquired from a voxel containing the hippocampus. The C4 proton multiplet resonance of glutamate used for quantification is *highlighted*. *tCr* total creatine, *tCho* total choline, and *NAA* *N*-acetylaspartate

delta, theta, and beta-1 frequency bands (Fig. 4). No significant amplitude increase was found for either alpha or gamma power, while the beta-2 power significantly decreased ( $t=-2.676$  and  $p=0.011$ ). Because no significant poststimulus change in alpha and gamma power was observed, we excluded these frequency bands from further examination of the relationships between frequency band power and glutamate concentration in hippocampus and ACC.



**Fig. 3** Typical fitting results for an in vivo spectrum. Predicted components (*shifted* for better visibility): *res* residual, *Glu* glutamate, *Gln* glutamine, *NAA* *N*-acetylaspartate, *tCr* total creatine, and *tCho* total choline. The original spectrum is *dotted* and the spectrum and baseline fits not shifted

## Glutamate concentrations and frequency band activities

The multiple regression analyses for glutamate concentration in the hippocampal region using poststimulus frequency power as predictor variables revealed a significant effect of the predictor theta frequency band ( $T=2.324$  and  $p=0.026$ ). No other frequency band (i.e., delta, beta-1, and beta-2) showed a significant relationship to the hippocampal glutamate concentration. The partial correlation analysis performed to control for age and gender effects revealed a significant correlation between frontal poststimulus theta activity and hippocampal glutamate ( $r=0.416$  and  $p=0.012$ ), demonstrating that age and gender do not account for the association between the two variables (see Fig. 5 for a scatterplot of the age- and gender-corrected theta activity and hippocampal glutamate data). The multiple regression analysis of hippocampal glutamate concentration using the prestimulus frequency bands as predictor variables, however, did not reveal any significant effects. Likewise, the multiple regression analyses of ACC glutamate concentrations using the prestimulus or the poststimulus frequency bands as predictor variables did not show any significant effects.

## Reaction times, frequency band activities, and glutamate concentrations

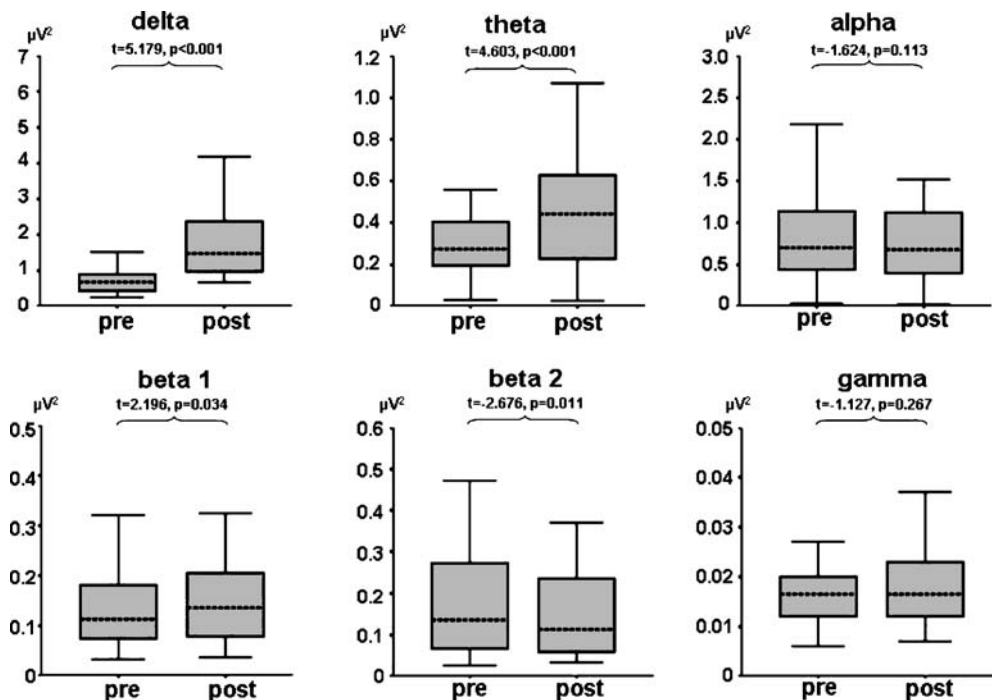
The relationships between RTs with poststimulus frequency band activities (theta, delta, beta-1, and beta-2) and glutamate concentrations (hippocampus and ACC) were examined in multiple regression analysis using RTs as dependent variable and poststimulus frequency band activities and glutamate concentrations as independent variables. The analysis revealed a significant relationship between RTs and the predictor variable theta band activity ( $T=-2.401$  and  $p=0.022$ ). Partial correlations between the control variables age and gender also showed a significant relationship between theta activity and RTs ( $r=-0.361$  and  $p=0.031$ ; Fig. 6), indicating that high theta activity is linked to short RTs. No other significant associations between RTs and frequency band activity or glutamate concentrations were found in the multiple regression analysis.

## Discussion

### Hippocampal glutamate concentration and oscillatory theta activity

Our study revealed evidence demonstrating an association between the glutamate concentration of the hippocampal region and theta oscillations over frontal scalp areas during auditory signal processing in humans. This association was observed for oscillations in the theta frequency range but not for other frequency ranges, suggesting that theta activity mediates neuronal coupling between the

**Fig. 4** Boxplot of the absolute power in the different frequency bands for a prestimulus and a poststimulus interval. Significant poststimulus increases in power were observed in the delta, theta, and beta-1 frequency band

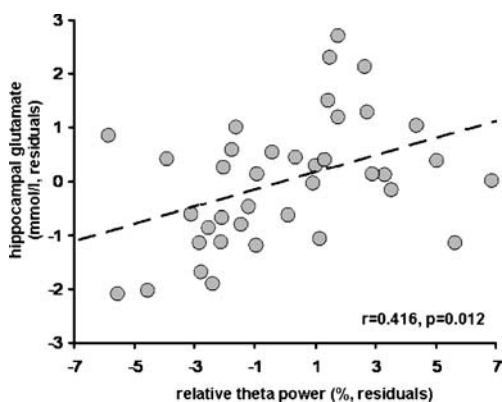


(frontal) cortex and the hippocampus via glutamatergic neurotransmission.

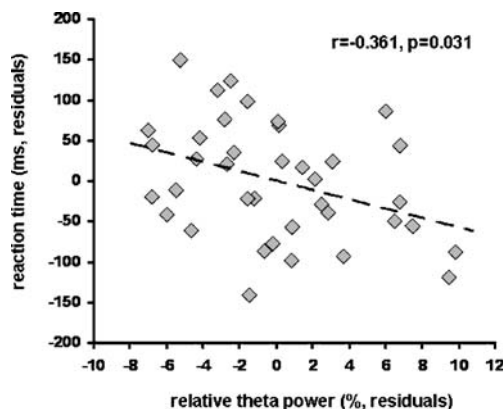
The increase in theta power corresponds to previous studies on auditory target detection (Basar-Eroglu et al. 1992; Röschke and Fell 1997), selective attention (Basar-Eroglu et al. 1992), working memory (Gevins et al. 1997), and memory encoding (Klimesch 1999). Theta activity, which was shown to be functionally relevant for cognitive processing in humans (Klimesch 1999), is the most stable task-related component in animal recordings (Basar et al. 2001). Further studies have also observed theta band activity across sensory modalities (Basar et al. 2001; Gevins et al. 1997). This suggests that theta plays a fundamental role in stimulus evaluation and memory processing, which corresponds to our finding of negative

correlations between theta activity and RTs. Further, a negative correlation between the RTs and the p300 amplitude elicited in an oddball paradigm was previously reported (Ford 1999). Recently, theta activation was observed in or near the hippocampus as revealed by MEG recordings and source reconstruction of working memory responses in humans (Tesche and Karhu 2000). This supports earlier findings of intracranially recorded hippocampal theta activity modulated by behavioral and perceptive conditions in epileptic patients (Meador et al. 1991).

Brain imaging studies and intracerebral recordings showed a coactivation of the hippocampus and cerebral cortex during episodic memory (Maguire et al. 2000), working memory (Gazzaley et al. 2004), and oddball paradigms (Halgren et al. 1995). However, the body of



**Fig. 5** Scatterplot of hippocampal glutamate concentrations and frontal theta activity during poststimulus auditory processing. A significant positive correlation between hippocampal glutamate and frontal theta oscillations was observed ( $p<0.02$ ). Residual values after linear correction of age and gender effects are plotted and can therefore consist of negative values



**Fig. 6** Scatterplot of reaction times and frontal poststimulus theta band activity during task performance. A significant negative correlation between RTs and frontal theta oscillations was observed ( $p<0.04$ ). High theta band activity predicted short RTs. Residual values after linear correction of age and gender effects are plotted

literature still lacks direct evidence demonstrating parallel theta measures in hippocampal and cortical areas (Kahana et al. 1999). Principally, the temporal integration in long distance (interareal) neuronal networks was shown to involve slow frequency theta activity (Sarnthein et al. 1998). Because the hippocampus has widespread anatomical and functional connections to all parts of the cortex (Sirota et al. 2003) and possesses intrinsic oscillatory properties (Buzsaki 2002), this structure is in a key position to modulate large-scale network oscillations in the theta frequency range. Accordingly, the encoding of new information, which is related to hippocampal activity, synchronizes theta in a small frequency window in the human scalp recordings (Klimesch 1999). In turn, lesions or agents that block theta oscillations, impair performance in learning and working memory paradigms (Kirk and Mackay 2003).

#### Neurotransmitter systems that account for the generation of theta oscillations

Different neurotransmitter systems are suggested to play a role in the genesis of theta oscillations (Vanderwolf 1988). The association of frontal theta with hippocampal glutamate concentration observed here corresponds to the strong glutamatergic influence upon hippocampal theta activity found in animal studies (Leung and Shen 2004; Vanderwolf and Leung 1983). The results of the present study suggest that the function of the hippocampus as a rhythm generator is also mediated by glutamatergic neurotransmission in humans. Further, this is compatible with the key role of glutamatergic neurotransmission in memory processes and hippocampal LTP (Bliss and Collingridge 1993), which in turn, is predicted by hippocampal theta oscillations (Maren et al. 1994). Evidence of the influence of glutamatergic neurotransmission on theta activity was also shown in scalp-recorded human EEG, specifically in a decrease in theta activity after administration of caroverine, an agent with glutamate-antagonistic effects (Saletu et al. 1995). Theta modulation after exposure to different NMDA receptor antagonist was also observed in animal experiments in the frontal cortex, along with the hippocampus and other areas (Dimpfel and Spuler 1990). In this study, however, we did not find a significant correlation between the glutamate level measured in the ACC and frontal theta activity. This may indicate that oscillatory activity generated in the ACC is not related to the local glutamate concentration. The result further underlines the role of the hippocampus as a rhythm generator acting on current generators in cortical areas, which in turn may be related to other neurotransmitter mechanisms, e.g., GABA (Drexler et al. 2005).

#### General considerations about in vivo glutamate measurement

It is well known that the extracellular and vesicle pool of neurotransmitter-related glutamate comprise only a fraction

of total cerebral glutamate, the rest being involved in the cells' energy metabolism (Hamberger and Nystrom 1984; Pouwels et al. 1999). This raises the question of a true association of our EEG results to neurotransmission. The following considerations, however, support a close relationship between neurotransmitter-related glutamate and metabolic glutamate. First, human and animal studies using in vivo brain microdialysis during epileptic seizures showed abnormally increased extracellular glutamate concentrations (Ding et al. 1998), confirming pathophysiological epilepsy models of abnormal excitability. Increased concentrations of glutamate and/or glutamine were also found in homogenized brain samples or single voxel 1H-MRS measurements containing the epileptic focus, reflecting multiple physiological compartments of brain tissue (Petroff et al. 1995; Savic et al. 2000). Although the extracellular glutamate concentration is tightly regulated, these findings indicate a distinct relationship between whole-tissue glutamate (as detected by a single voxel MRS) and transmitter glutamate (released into the extracellular space). However, findings in epilepsy may not be relevant in physiological conditions and it cannot be ruled out that the glutamate measured in the present study largely reflects neurons' metabolic glutamate pool, which might be independent of glutamatergic neurotransmission. Therefore, the correlation of this signal with frontal theta activity may reflect a more general function of glutamate for cellular mechanisms and metabolism within the hippocampal area. Future studies must clarify which part of the cellular glutamate fraction is responsible for neuronal oscillations.

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#### Conclusion

The current data show, for the first time, a functional relationship between in vivo measured brain glutamate concentrations and cerebral theta activity during auditory stimulus processing in humans. Although the present evidence is preliminary, we suggest that glutamatergic cells may collectively modulate oscillatory theta activity, thereby promoting coherent processing and plasticity across distributed cortical networks during behavioral and cognitive processing. Thus, the determination of hippocampal glutamate concentration, together with functional brain measures, opens the door for future research on neurotransmitter-driven hippocampal network processing in humans.

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**Duality of interest** J. Gallinat and D. Kunz contributed equally to this work.

## References

- Alonso A, Garcia-Aust E (1987) Neuronal sources of theta rhythm in the entorhinal cortex of the rat. I. Laminar distribution of theta field potentials. *Exp Brain Res* 67:493–501
- Ashburner J, Friston K (1997) Multimodal image coregistration and partitioning—a unified framework. *Neuroimage* 6:209–217
- Basar E, Basar-Eroglu C, Karakas S, Schurmann M (2001) Gamma, alpha, delta, and theta oscillations govern cognitive processes. *Int J Psychophysiol* 39:241–248
- Basar-Eroglu C, Basar E, Demiralp T, Schurmann M (1992) P300-response: possible psychophysiological correlates in delta and theta frequency channels. A review. *Int J Psychophysiol* 13:161–179
- Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361:31–39
- Buzsaki G (2002) Theta oscillations in the hippocampus. *Neuron* 33:325–340
- Dimpfel W, Spuler M (1990) Dizocilpine (MK-801), ketamine and phencyclidine: low doses affect brain field potentials in the freely moving rat in the same way as activation of dopaminergic transmission. *Psychopharmacology (Berl)* 101:317–323
- Ding R, Asada H, Obata K (1998) Changes in extracellular glutamate and GABA levels in the hippocampal CA3 and CA1 areas and the induction of glutamate acid decarboxylase-67 in dentate granule cells of rats treated with kainic acid. *Brain Res* 800:105–113
- Drexler B, Roether CL, Jurd R, Rudolph U, Antkowiak B (2005) Opposing actions of etomidate on cortical theta oscillations are mediated by different gamma-aminobutyric acid type A receptor subtypes. *Anesthesiology* 102:346–352
- Elster C, Schubert F, Link A, Walzel M, Seifert F, Rinneberg H (2005) Quantitative magnetic resonance spectroscopy: semi-parametric modeling and determination of uncertainties. *Magn Reson Med* 53:1288–1296
- Fell J, Dietl T, Grunwald T, Kurthen M, Klaver P, Trautner P, Schaller C, Elger CE, Fernandez G (2004) Neural bases of cognitive ERPs: more than phase reset. *J Cogn Neurosci* 16:1595–1604
- Fell J, Kohling R, Grunwald T, Klaver P, Dietl T, Schaller C, Becker A, Elger CE, Fernandez G (2005) Phase-locking characteristics of limbic P3 responses in hippocampal sclerosis. *Neuroimage* 24:980–989
- Ford JM (1999) Schizophrenia: the broken P300 and beyond. *Psychophysiology* 36:667–682
- Gallinat J, Winterer G, Herrmann CS, Senkowski D (2004) Reduced oscillatory gamma-band responses in unmedicated schizophrenic patients indicate impaired frontal network processing. *Clin Neurophysiol* 115:1863–1874
- Gallinat J, Strohle A, Lang UE, Bajbouj M, Kalus P, Montag C, Seifert F, Wernicke C, Rommelspacher H, Rinneberg H, Schubert F (2005) Association of human hippocampal neurochemistry, serotonin transporter genetic variation, and anxiety. *Neuroimage* 26:123–131
- Gasser T, Bacher P, Mocks J (1982) Transformations towards the normal distribution of broad band spectral parameters of the EEG. *Electroencephalogr Clin Neurophysiol* 53:119–124
- Gazzaley A, Rissman J, Desposito M (2004) Functional connectivity during working memory maintenance. *Cogn Affect Behav Neurosci* 4:580–599
- Gevens A, Smith ME, McEvoy L, Yu D (1997) High-resolution EEG mapping of cortical activation related to working memory: effects of task difficulty, type of processing, and practice. *Cereb Cortex* 7:374–385
- Givens B (1996) Stimulus-evoked resetting of the dentate theta rhythm: relation to working memory. *Neuroreport* 8:159–163
- Govindaraju V, Young K, Maudsley AA (2000) Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR Biomed* 13:129–153
- Halgren E, Baudena P, Clarke JM, Heit G, Marinkovic K, Devaux B, Vignal JP, Biraben A (1995) Intracerebral potentials to rare target and distractor auditory and visual stimuli. II. Medial, lateral and posterior temporal lobe. *Electroencephalogr Clin Neurophysiol* 94:229–250
- Hamberger A, Nystrom B (1984) Extra- and intracellular amino acids in the hippocampus during development of hepatic encephalopathy. *Neurochem Res* 9:1181–1192
- Herrmann WM, Rohmel J, Streitberg B, Willmann J (1983) Example for applying the COMSTAT multimodal factor analysis algorithm to EEG data to describe variance sources. *Neuropsychobiology* 10:164–172
- Javitt DC, Schroeder CE, Steinschneider M, Arezzo JC, Ritter W, Vaughan HGJ (1995) Cognitive event-related potentials in human and non-human primates: implications for the PCP/NMDA model of schizophrenia. *Electroencephalogr Clin Neurophysiol Suppl* 44:161–175
- Kahana MJ, Sekuler R, Caplan JB, Kirschen M, Madsen JR (1999) Human theta oscillations exhibit task dependence during virtual maze navigation. *Nature* 399:781–784
- Kandel ER, Siegelbaum SA (2000) Synaptic integration. In: Kandel ER, Schwartz JH, Jessel TM (eds) *Principles of neural science*. McGraw-Hill, New York, pp 207–228
- Kirk IJ, Mackay JC (2003) The role of theta-range oscillations in synchronising and integrating activity in distributed mnemonic networks. *Cortex* 39:993–1008
- Klimesch W (1999) EEG alpha and theta oscillations reflect cognitive and memory performance: a review and analysis. *Brain Res Brain Res Rev* 29:169–195
- Leung LS, Shen B (2004) Glutamatergic synaptic transmission participates in generating the hippocampal EEG. *Hippocampus* 14:510–525
- Maguire EA, Mummery CJ, Buchel C (2000) Patterns of hippocampal-cortical interaction dissociate temporal lobe memory subsystems. *Hippocampus* 10:475–482
- Mao J, Mareci TH, Scott KN, Andrew ER (1986) Selective inversion radiofrequency pulses by optimal control. *J Magn Reson* 70:310–318
- Maren S, DeCola JP, Swain RA, Fanselow MS, Thompson RF (1994) Parallel augmentation of hippocampal long-term potentiation, theta rhythm, and contextual fear conditioning in water-deprived rats. *Behav Neurosci* 108:44–56
- Meador KJ, Thompson JL, Loring DW, Murro AM, King DW, Gallagher BB, Lee GP, Smith JR, Flanigin HF (1991) Behavioral state-specific changes in human hippocampal theta activity. *Neurology* 41:869–872
- Miller R (1989) Cortico-hippocampal interplay: self organizing phase-locked loops for indexing memory. *Psychobiology* 17:115–128
- Mlynarik V, Gruber S, Moser E (2001) Proton T (1) and T (2) relaxation times of human brain metabolites at 3 tesla. *NMR Biomed* 14:325–331
- Onton J, Delorme A, Makeig S (2005) Frontal midline EEG dynamics during working memory. *Neuroimage* 27(2):341–356
- Pare D, Collins DR (2000) Neuronal correlates of fear in the lateral amygdala: multiple extracellular recordings in conscious cats. *J Neurosci* 20:2701–2710
- Petroff OA, Pleban LA, Spencer DD (1995) Symbiosis between in vivo and in vitro NMR spectroscopy: the creatine, N-acetylaspartate, glutamate, and GABA content of the epileptic human brain. *Magn Reson Imaging* 13:1197–1211
- Pouwels PJ, Brockmann K, Kruse B, Wilken B, Wick M, Hanefeld F, Frahm J (1999) Regional age dependence of human brain metabolites from infancy to adulthood as detected by quantitative localized proton MRS. *Pediatr Res* 46:474–485
- Reischies FM, Neuhaus AH, Hansen ML, Mientus S, Mulert C, Gallinat J (2005) Electrophysiological and neuropsychological analysis of a delirious state: the role of the anterior cingulate gyrus. *Psychiatry Res* 138:171–181
- Rösche J, Fell J (1997) Spectral analysis of P300 generation in depression and schizophrenia. *Neuropsychobiology* 35:108–114

- Saletu B, Grunberger J, Anderer P, Linzmayer L, Konig P (1995) Acute central effects of the calcium channel blocker and antilutamatergic drug caroverine. Double-blind, placebo-controlled, EEG mapping and psychometric studies after intravenous and oral administration. *Arzneimittelforschung* 45: 217–229
- Sarnthein J, Petsche H, Rappelsberger P, Shaw GL, von Stein A (1998) Synchronization between prefrontal and posterior association cortex during human working memory. *Proc Natl Acad Sci USA* 95:7092–7096
- Savic I, Thomas AM, Ke Y, Curran J, Fried I, Engel JJ (2000) In vivo measurements of glutamine + glutamate (Glx) and N-acetyl aspartate (NAA) levels in human partial epilepsy. *Acta Neurol Scand* 102:179–188
- Schubert F, Gallinat J, Seifert F, Rinneberg H (2004) Glutamate concentrations in human brain using single voxel proton magnetic resonance spectroscopy at 3 tesla. *Neuroimage* 21: 1762–1771
- Sirota A, Csicsvari J, Buhl D, Buzsaki G (2003) Communication between neocortex and hippocampus during sleep in rodents. *Proc Natl Acad Sci USA* 100:2065–2069
- Tesche CD, Karhu J (2000) Theta oscillations index human hippocampal activation during a working memory task. *Proc Natl Acad Sci USA* 97:919–924
- Vanderwolf CH (1988) Cerebral activity and behavior: control by central cholinergic and serotonergic systems. *Int Rev Neurobiol* 30:225–340
- Vanderwolf CH, Leung LS (1983) Hippocampal rhythmical slow activity: a brief history and effects of entorhinal lesions and phencyclidine. In: Seifert W (ed) *The neurobiology of the hippocampus*. Academic, London, pp 275–302
- Vorob'ev VV, Akhmetova ER, Kovalev GI (1997) Participation of N-methyl-D-aspartate receptors in modification of the frequency composition in rat electroencephalogram. *Eksp Klin Farmakol* 60:11–14
- Ylinen A, Soltesz I, Bragin A, Penttonen M, Sik A, Buzsaki G (1995) Intracellular correlates of hippocampal theta rhythm in identified pyramidal cells, granule cells, and basket cells. *Hippocampus* 5:78–90
- Yordanova J, Rosso OA, Kolev V (2003) A transient dominance of theta event-related brain potential component characterizes stimulus processing in an auditory oddball task. *Clin Neurophysiol* 114:529–540