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Neuronal oscillations and synchronicity associated with gamma-hydroxybutyrate during resting-state in healthy, male volunteers

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Abstract

Rationale. Gamma-hydroxybutyrate (GHB) is a putative neurotransmitter, a drug of abuse, an anesthetic agent and a treatment for neuropsychiatric disorders. In previous electroencephalography (EEG) studies, GHB was shown to induce an electrophysiological pattern of "paradoxical EEG-behavioral dissociation" characterized by increased delta and theta oscillations usually associated with sleep during awake states. However, no detailed source localization of these alterations and no connectivity analyses have been performed yet.

Objectives and Methods. We tested the effects of GHB (20 and 35 mg/kg, p.o.) on current source density (CSD), lagged phase synchronization (LPS) and global omega complexity (GOC) of neuronal oscillations in a randomized, double-blind, placebo-controlled, balanced cross-over study in 19 healthy, male participants using exact Low Resolution Electromagnetic Tomography (eLORETA) of resting-state high-density EEG recordings.

Results. Compared to placebo, GHB increased CSD of theta oscillations (5-7 Hz) in the posterior cingulate cortex (PCC) and alpha1 (8-10 Hz) oscillations in the anterior cingulate cortex. Higher blood plasma values were associated with higher LPS values of delta (2-4 Hz) oscillations between the PCC and the right inferior parietal lobulus. Additionally, GHB decreased GOC of alpha1 oscillations.

Conclusion. These findings indicate that alterations in neuronal oscillations in the PCC mediate the psychotropic effects of GHB. Theta oscillations emerging from the PCC in combination with stability of functional connectivity within the default mode network might explain the GHB-related "paradoxical EEG-behavioral dissociation". Our findings related to GOC suggest a reduced number of relatively independent neuronal processes, an effect that has also been demonstrated for other anesthetic agents.

Keywords: Sodium oxybate, gamma-hydroxybutyric acid, source localization, lagged phase synchronization, global omega-complexity, EEG-behavioral dissociation, sedation

Introduction

Due to the depressant pharmacological effect of GHB on the central nervous system, early work focused on its use as an anesthetic agent (e.g., Appleton and Burn 1968; Laborit 1964). The clinical use of GHB as general anesthetic is approved in Germany, Italy and France, even though its actual use is declining (Fuller et al. 2003). Moreover, sodium oxybate, a non-proprietary synonym for GHB, gained attention in the psychiatric context because of its indication for the treatment of cataplexy in narcoleptic patients and its anxiolytic and antidepressant properties (Rinaldi et al. 1967; Bosch et al. 2012). Galloway and colleagues (2000) investigated illicit GHB use and reported its subjective central effects to be comparable to both alcohol and 3,4-methylenedioxymethamphetamine. The central effects range from drowsiness, euphoria and heightened sexuality to disinhibition and empathogenesis, which were recently addressed in an experimentally controlled study (e.g. Bosch et al. 2015).

It has been argued that GHB fulfils the criteria for being categorized as a bona fide neurotransmitter (e.g., Vayer, Mandel, and Maitre 1987). Despite the fact that GHB has a high affinity for the recently discovered GHB receptor (Snead 2000), most of the behavioral effects of exogenously applied GHB seem to be mediated through the $GABA_B$ receptor, given that most effects of GHB were mimicked by a $GABA_B$ agonist (baclofen) or reduced by a $GABA_B$ antagonist (CGP 35348) (Nissbrandt and Engberg 1993). GHB is commonly administered orally and is absorbed rapidly within the gastro-intestinal tract. For dosages ranging from 12.5mg/kg to 50mg/kg, peak plasma concentrations were reached after 20-60 minutes (Borgen et al. 2004; Ferrara et al. 1992; Liechti et al. 2016; Palatini et al. 1993).

Because of the strong sedative properties of GHB, the similarity of GHB-induced brain states and sleep-induced states was recognized early on. According to Mamelak and colleagues (1977), sleep induced by GHB is not distinguishable from natural sleep in regard to subjectively reported effects as well as to its characteristic electroencephalography (EEG) pattern. In addition, the order of natural sleep stages, as measured by polysomnographic recordings, does not seem to be affected by GHB. One of the most replicated findings across studies is an increase of slow-wave sleep (SWS) and a decrease of non-rapid eye-movement sleep after the intake of GHB (Entholzner et al. 1995; Lapierre et al. 1990; Mamelak et al. 1977).

Few studies, however, have explicitly examined the effect of GHB on electrophysiology of *awake* human subjects (Jimenez et al. 1982; Metcalf et al. 1966), although several studies have investigated the transition of the waking state to sleep or coma (Ducloux et al. 1989; Jenney et al. 1962; Orioli et al. 1966; Schneider et al. 1963). These studies consistently found a pattern referred to as “paradoxical EEG-behavioral dissociation”, characterized by an abrupt

shift from alpha to delta and theta rhythms during awake state, which is similarly observed during SWS. Thus, GHB seems to mimic the electrophysiological pattern of falling asleep, which is characterized by a shift of neuronal oscillations towards low frequencies, without being accompanied by actual loss of consciousness. The observed electrophysiological similarities of falling asleep with the effects of GHB could potentially explain the common sedating properties of the substance. However, the studies in which this “paradoxical EEG-behavioral dissociation” was found often lacked a placebo control group or an adequate randomization and blinding procedure.

Here, we aimed to fill this knowledge gap using a double-blind placebo controlled design to investigate the effects of GHB on neuronal oscillations in awake humans. Furthermore, it is unclear in which brain areas these patterns of "paradoxical EEG-behavioral dissociation" emerge. Using state-of-the-art methods for source reconstruction, we quantified the effect of GHB on the current source density, lagged phase synchronization of neuronal oscillations and global omega complexity across brain areas. By identifying a neuronal correlate of the sedative effects of GHB, based on electrophysiological measurements, we contribute a valuable supplement to the knowledge about the sometimes contradictory pharmacological profile of the substance regarding its anesthetic properties. Based on the existing literature, we expected to replicate the dissociation between electrophysiology and behavior characterized by the emergence of low frequency waves without losing consciousness and to elucidate information about the localization and synchronization of the underlying neuronal generators.

Methods

Participants. Twenty healthy, male participants (mean age 25.8±5.1 years) completed the study. In order to be included they had to fulfil the following criteria: (i.) male sex, (ii.) age within the range of 18 to 40 years, (iii.) absence of any somatic or psychiatric disorders, (iv.) no first-degree relatives with a history of psychiatric disorders, (v.) non-smoking, (vi.) without a history of drug abuse (lifetime use > 5 occasions, with exception of occasional cannabis use). None of the participants reported previous experiences with GHB in their life. Participants were instructed to abstain from alcohol for 24 hours before the session. Because the bioavailability of GHB is markedly reduced when taken together with food (Borgen et al. 2004), participants were asked to neither have breakfast nor drink caffeinated beverages on the morning of a testing session. Urine samples were collected on the two experimental days in order to ensure that all participants abstained from illegal substance use. All participants were instructed about potential risks concerning the administered substance and were compensated for the completion of the study. One subject had to be excluded prior to analysis because a majority of the EEG data was contaminated by strong artifacts. Thus, a remaining total of 19 subjects were included in the statistical analysis.

General Procedure

Permission. The study was approved by the Swissmedic and the Cantonal Ethics Committee of Zurich. Additionally the study was registered at ClinicalTrials.gov (NCT02342366) and, according to the declaration of Helsinki, all participants provided written informed consent.

Study design. In order to investigate the pharmacological effects of GHB, a placebo-controlled, randomized, balanced cross-over design was chosen. Every subject attended 4 sessions: Screening, experimental day I, experimental day II and a follow-up session. Experimental days were separated by a washout phase of exactly seven days.

Drug administration. Previous studies used dosages of GHB ranging from 10mg/kg (Grove-White and Kelman 1971) up to 72mg/kg (Abanades et al. 2006). For the present study every subject received one out of two different dosage levels, which comprised of 20mg/kg and 35mg/kg GHB dissolved in 3dl of orange juice. These doses were chosen in line with existing literature indicating this range to produce subjective and cognitive alterations without causing unwanted side effects (e.g. Brenneisen et al. 2004; Palatini et al. 1993).

Blood samples. The quantification of GHB in human plasma was performed according to the procedure of Meyer and colleagues (2011). Because blood samples were drawn 20

minutes before and 30 minutes after the EEG recording, the GHB plasma levels for a given time must be estimated. We used a straightforward approach, calculating the mean of the two surrounding measurements in order to create an appropriate estimation of each individual's GHB plasma levels during the EEG recording.

Subjective measurement. Acute subjective effects of the GHB administration were measured by a Visual Analog Scale (VAS). The dimensions *general drug effect*, *sedation*, *stimulation* and *dizziness* were assessed on a scale ranging from zero ("no effect") to ten ("strong effect"). The VAS was issued at time points t – 15, + 40, + 60, + 100, + 120 and + 180 minutes.

Electroencephalogram recordings

A BioSemi ActiveTwo electrode system (BioSemi, Netherlands) including 64 scalp electrodes was used to record EEG data during the period of plasma peak drug concentration. Horizontal and vertical eye movements were monitored by four additional electrooccculogram electrodes. Electrophysiological signals were sampled at a rate of 2048 Hz. For detailed information about the preprocessing procedure see supplement.

Current Source Density. To test drug effects on the electrophysiology of the brain, it is of specific interest to determine not only the topographic scalp map of the electrical currents, but also have an estimate of the underlying neuronal sources. Exact low-resolution brain electromagnetic topographic (eLORETA) (http://www.uzh.ch/keyinst/loreta.htm) was used to compute a three-dimensional EEG current source density map by applying a three-spherical shell model that restricts the solution space to grey matter and hippocampus, resulting in 6239 different voxels (each 5mm x 5mm x 5mm). The head model used to compute the intracerebral current source density values was registered to the digitized MRI brain average of the Talairach and Tournoux atlas (Brain Imaging Centre, Montreal Neurological Institute). It must be noted that the assumption of synchronized discharge results in a high correlation of neighbouring voxels (Pascual-Marqui et al. 1994).

The LORETA approach has been widely applied and has received numerous cross-modal validations when combined with different encephalic activity localizing methods, such as functional, structural and diffusion spectrum magnetic resonance imaging, or positron emission tomography (Babiloni et al. 2011; Horacek et al. 2007; Mulert et al. 2005; Olbrich et al. 2009; Pizzagalli et al. 2004; Thatcher et al. 2012; Vecchio et al. 2015). eLORETA exerts several advantages over older versions because of its unbiased localization properties and

independence of the reference-choice (Jatoi et al. 2014; Pascual-Marqui 2007; Pascual-Marqui et al. 2011).

In this study, a cross-registration between spherical and realistic head geometry was used in order to calculate the electrode coordinates (Towle et al. 1993). To compute spectral density ($\mu A/mm^2$) the signal was split into six separate frequency bands: delta (2-4 Hz), theta (5-7 Hz), alpha1 (8-10 Hz), alpha2 (10-12 Hz), beta1 (13-20 Hz) and beta2 (20-30 Hz).

Lagged Phase Synchronization. In addition to the examination of local neuronal activity, it is of specific interest to investigate how different regions integrate information in order to generate neural assemblies. Phase synchronization of neuronal oscillations is known to be a fundamental mechanism for enabling coordinated activity between spatially distinct brain regions (Uhlhaas and Singer 2010). Thus, numerous authors argue that the favorable and most studied approach for investigating large-scale integration of neuronal networks is phase synchronization (including Varela et al. 2001). Oscillatory synchronization represents a fast and efficient mechanism for communication between spatially distinct brain regions (Buzsáki and Draguhn 2004). Therefore, quantifying phase synchronization allows us to describe the physiological processes underlying non-linear functional connections between these distinct neuronal assemblies (Varela et al. 2001).

In the present study, lagged phase synchronization as implemented in the eLORETA software was used to characterize altered functional connectivity between brain areas following the administration of GHB. This method has received cross-modal validation from diffusion tensor imaging (Vecchio et al. 2015) and fMRI (de Ridder et al. 2011). Accordingly, this method has been employed increasingly in recent studies (Canuet et al. 2011; Hilty et al. 2011; Kometer et al. 2015; Kühnis et al. 2014; Ramyead et al. 2015).

Lagged phase synchronization allows us to calculate the functional connectivity between two regions of interest (ROI) for all previously defined frequency bands separately. In order to obviate a selection bias regarding the ROIs used for the further analysis, a whole-brain parcellation approach based on Brodmann areas was chosen (Pascual-Marqui et al. 2011). Accordingly, centroid voxels of all 42 Brodmann areas in both hemispheres were defined as ROIs, before computing all possible combinations of lagged phase synchronization between the ROIs (Canuet et al. 2011).

Global Omega Complexity. In addition to analysis of the phase-synchronization between specific areas, analysis of coordinated activity across the entire scalp was quantified by the so-called global omega complexity index. Wackermann (1996) proposed a strategy for describing entire field-potential distributions by incorporating interpolated potential maps,

resulting in a single space-domain descriptor (see also Lehmann et al. 1987). The global omega complexity index can be used to assess the spatial synchronization over the entire scalp (Toth et al. 2009). Thus, it depicts the complexity of the trajectory in the K-dimensional state space (where K denotes the number of electrodes) by decomposing the signal into spatial principal components (Saito et al. 1998). High values of omega complexity are associated with high numbers of independent (neuronal) processes underlying the field-potential maps (Szelenberger et al. 1995). In other words, global omega complexity measures the degree of differentiation of a highly dynamic system and thereby reflects functional integration and specialization (Tononi and Edelman 1998). Global omega complexity has been shown to reflect mental states such as sleep or drug effects (Toth et al. 2012; Huber et al., 2006; Tononi and Edelman 1998; Szelenberger et al. 1995; Kondakor et al. 1999; Mölle et al. 1997).

In order to compute global omega complexity the software eLORETA was used. All artifact-free segments derived from the 64 scalp electrodes were included, resulting in a single value for each previously defined frequency band for every condition (eyes-open placebo, eyes-closed placebo, eyes-open GHB, eyes-closed GHB). The index was estimated against an average reference (Saito et al. 1998).

Statistical analysis

In order to analyze subjective evaluations of the drug effects as measured by the VAS and the GHB plasma values, a repeated measures (2*6) ANOVA was applied using the *R project for statistical computing* (Version 3.2.3) software. The time points ("time") of the questionnaires and the conditions GHB vs. placebo ("drug") were taken as factors. The regressors were calculated by subtracting the value of the placebo condition from the corresponding value of the GHB condition. If the main effect for the factor "drug" turned out to be significant, post hoc paired t-tests were applied for every time point. All *p*-values were corrected using the sequential rejective procedure from Holm (1979), which has been proven to be a powerful advancement of the Bonferroni algorithm (Hochberg and Benjamini 1990).

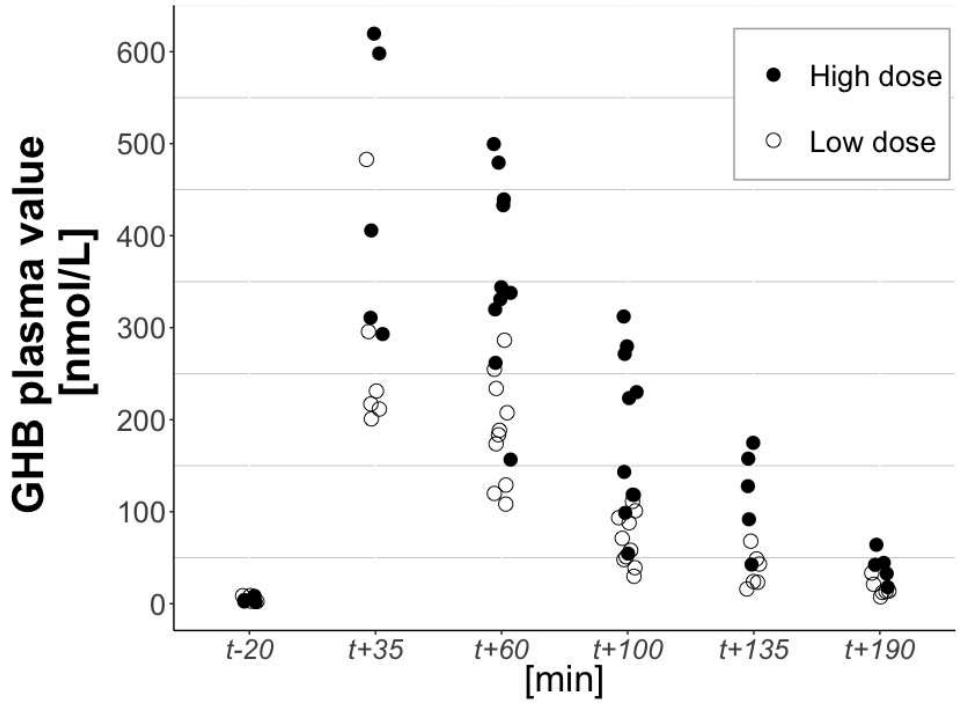

Fig. 1 Scatterplot displaying GHB plasma values [nmol/mL] of each participant for the two dosage levels 25mg/kg (low dose) and 35mg/kg (high dose) for all time points, when blood withdrawal took place.

For calculating the statistical differences in electrophysiology between the conditions, a nonparametric mapping approach as implemented in the eLORETA software was used (Pascual-Marqui et al. 2011). Using a voxel-by-voxel-dependent t-test it was possible to calculate the contrast between the conditions for all frequency bands separately, while correcting for multiple comparisons across voxels and frequencies. Appropriate correction for multiple comparisons was achieved by applying a randomization strategy with 5000 permutations. The possibility of detecting a dose-dependent effect by calculating placebo vs. high-dose and placebo vs. low-dose was not pursued for two reasons.

First, as seen in Fig. 1 there was a considerable overlap in the GHB plasma levels between the two conditions. Therefore, it was not feasible to calculate contrasts based on different dosages of the drug. Second, when compared to other studies using a similar approach (e.g. Kometer et al. 2015) the sample size of the present study was moderate. Thus, in order to increase the statistical power for detecting a potential drug effect, the benefits of combining the two dosage levels were clear.

Additionally, the difference between placebo and GHB was correlated with the items of the VAS questionnaire and the estimated GHB plasma values at the given time point. That is, the difference between the GHB and the placebo condition was calculated and a voxel-wise product moment correlation was computed for each variable. We estimated the empirical probability distribution for the highest dependent sample t-values under the null hypothesis, when permutated 5000 times. Thus, it was not necessary to assume a Gaussian distribution for the dependent variable (Nichols and Holmes 2002) and an adequate correction for multiple comparisons across voxels and frequencies could be achieved. When computing the statistical significance for cortical voxels, the omnibus null hypothesis was rejected, as soon as at least one t value (two-tailed) reached the critical threshold for a significance level of $p < .05$.

Results

GHB reached highest concentration in plasma at t + 35 min and went back to baseline levels at t + 190 min (see Figure 2). Repeated measures ANOVA (2*6) revealed significant main effects regarding the factor “drug” for GHB plasma values and all VAS items except for “stimulation” (see Table 1). The course of mean scores from all VAS items peaked at t + 35 min, in close correspondence with GHB plasma concentrations (see Figure 3).

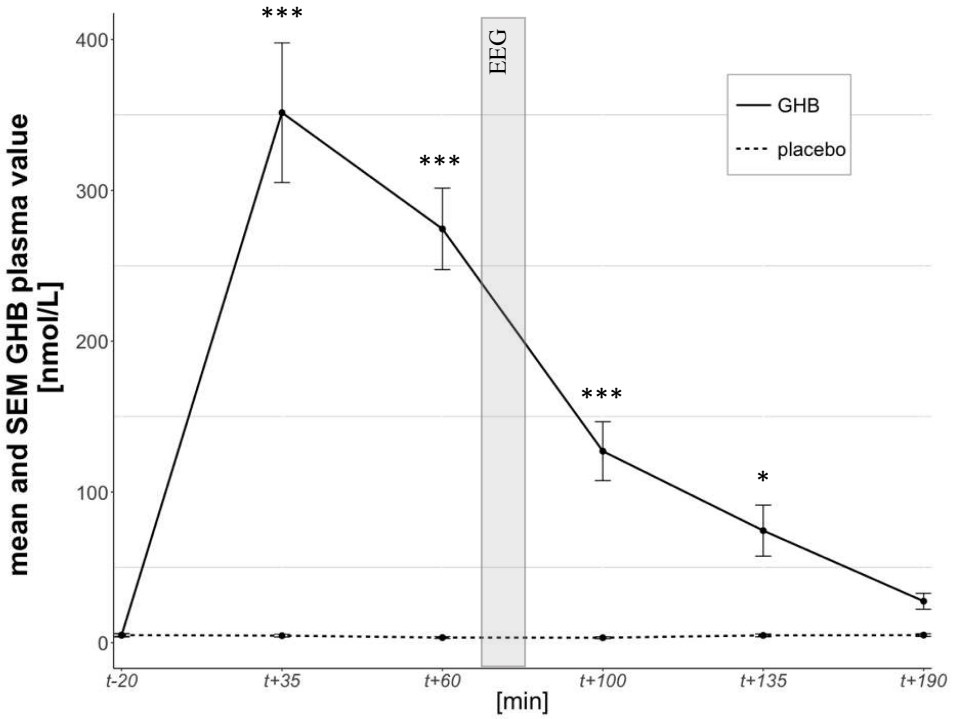

Fig. 2 Mean (and standard error of mean) GHB plasma values in nmol/mL for all three dosage levels. Paired t-tests used for comparison of GHB with placebo condition: * $p < .05$, ** $p < .01$, *** $p < .001$. All p-values are corrected for multiple comparisons by Holm’s sequential rejective procedure. Time point of EEG recording is depicted in *grey*.

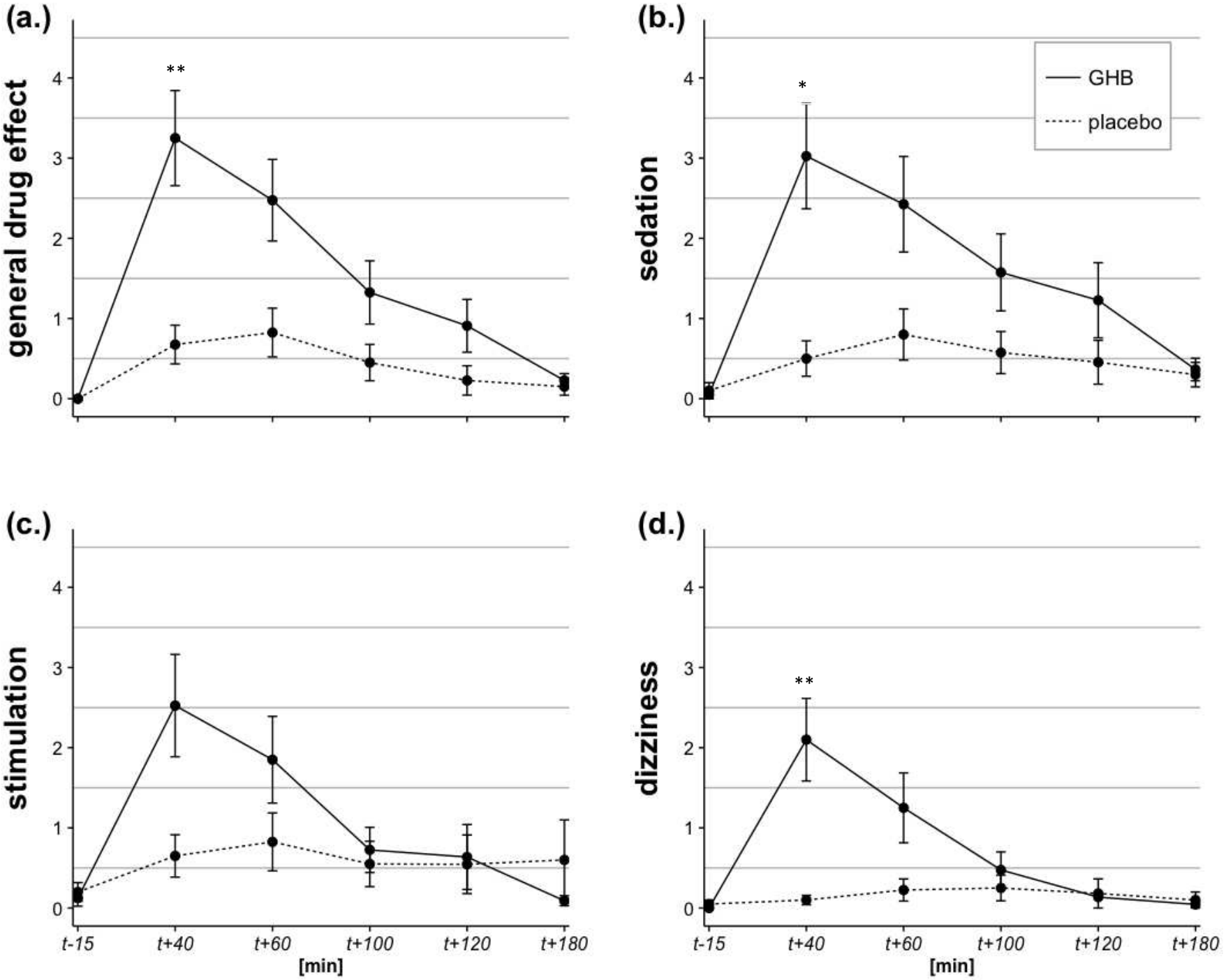

Fig. 3 Time course of mean (and SEM) from VAS scores for the conditions GHB and placebo. (a.) general drug effect, (b.) sedation, (c.) stimulation, (d.) dizziness. Paired t-tests: * $p < .05$, ** $p < .01$, *** $p < .001$. All p-values are corrected for multiple comparisons by Holm's sequential rejective procedure.

Current Source Density. When comparing placebo with GHB during eyes-closed condition, the current source density analysis for neuronal oscillations revealed statistically significant differences in the theta (5-7 Hz) and alpha1 (8-10 Hz) bands (see Fig. 4). In the theta band GHB significantly increased current source density values in 33 voxels, located in the posterior cingulate cortex (PCC) (22 voxels in BAs 23, 29, 30, 31), cingulate gyrus (10 voxels in BAs 23, 31) and precuneus (1 voxel in BA 31). The maximal increase in theta oscillations was found in the PCC (X = 5, Y = -45, Z = 25, t = -6.26, p = .006). In the alpha1 band GHB significantly increased current source density in 24 voxels, mainly located in the anterior part of the cingulate cortex (22 voxels in BAs 23, 24), as well as one voxel in the PCC (BA 23) and the insula (BA 13), respectively. The global maximum increase within the alpha1 band was located in the cingulate gyrus (X = -5, Y = -15, Z = 30, t = -6.11, p = .008). The differences of current source density between placebo and GHB were distributed almost symmetrically between the two hemispheres. Surprisingly, no statistically significant changes in any frequency band were found when contrasting placebo with GHB in the eyes-open condition, even though the strongest effects were consistently found in the theta (t = -4.12, p = .188) and the alpha1 band (t = -4.30, p = .143). Additionally, the spatial distribution of the altered activity strongly overlapped with the eyes-closed condition (see Supplement). When correlating the GHB plasma values with the current source density difference of neuronal oscillations between placebo and GHB, no significant effects were found for the eyes-closed and the eyes-open condition.

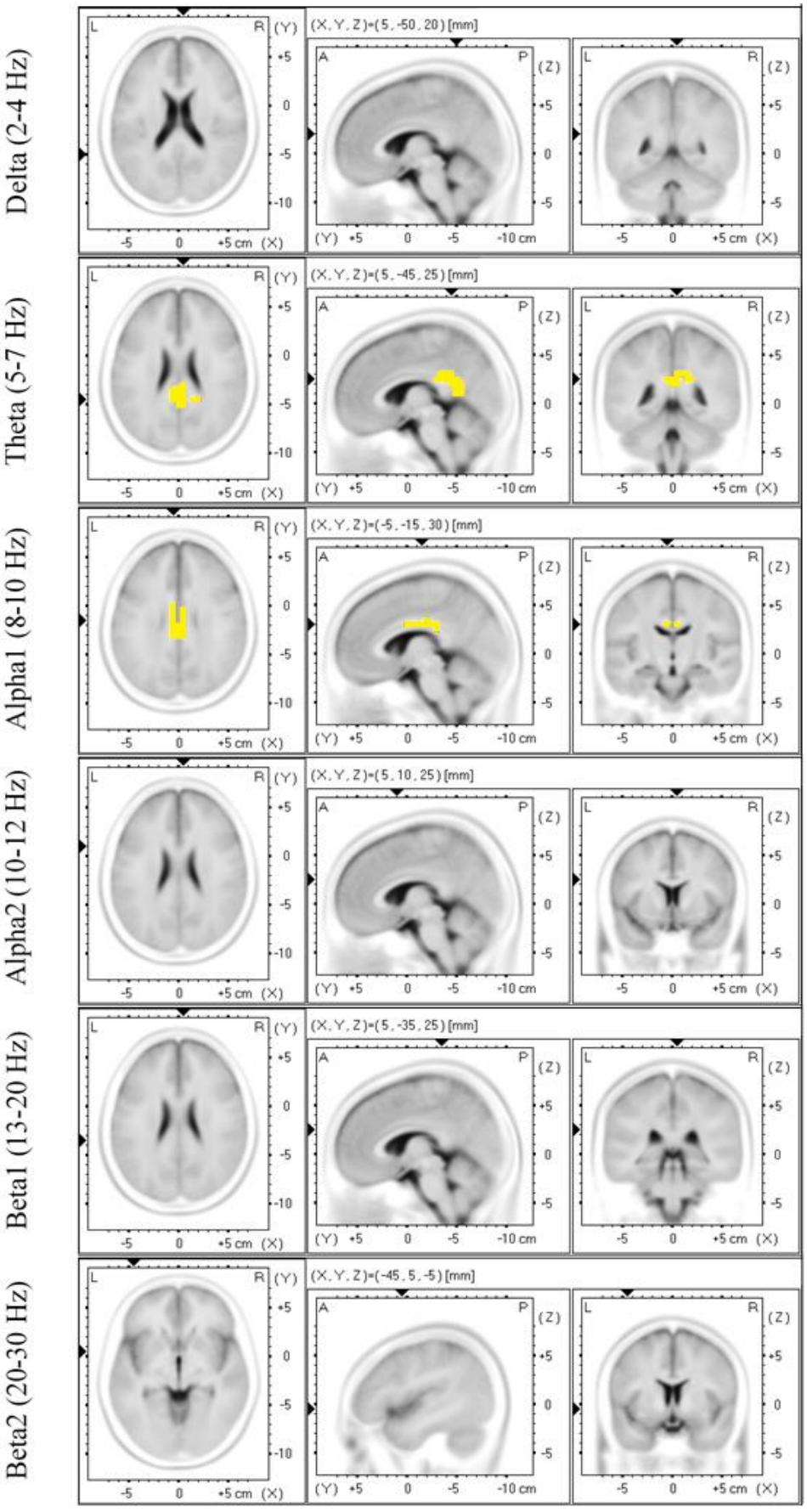

Fig. 5 Statistical map of voxel-wise comparisons of current source density for all frequency bands in the eyes closed condition. Significantly increased voxels by GHB are depicted in *yellow* ($p < .05$).

Lagged Phase Synchronization. Analysis of lagged phase synchronization, when contrasting placebo with GHB, revealed no significant differences in any frequency band for both eyes-closed (p_{max} = .1608) and eyes-open (p_{max} = .9812) conditions. When correlating GHB plasma values with the lagged phase synchronization contrast between placebo vs. GHB, one connection was significantly increased in the eyes-open condition. As depicted in Fig. 5, the increased lagged phase synchronization value in the delta band (r > 0, p = .040) was found in the right hemisphere between the PCC (BA 29, X = 5, Y = -50, Z = 5) and the inferior parietal lobulus (BA 40, X = 50, Y = -30, Z = 45). During eyes-closed no significant difference was found (r > 0, p_{max} = .106).

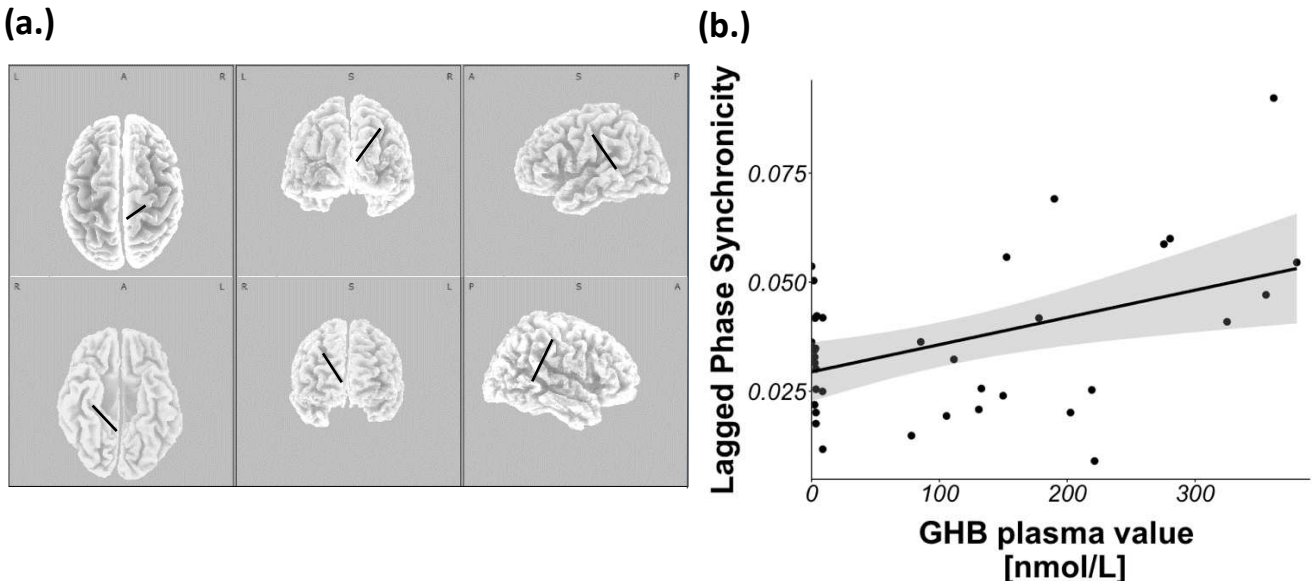

Fig. 5 (a.) Significantly increased connection of the GHB plasma regression affecting delta oscillations between the posterior cingulate cortex and inferior parietal lobulus from six different angles is depicted in *black*. (b.) Scatterplot of the relation between the LPS values for both conditions per participant of the connection depicted in (a.) and the GHB plasma values [nmol/L] in the eyes-open condition with a regression line with 95% confidence interval bar embedded.

The regression analysis with subjective measures revealed one significant correlation. When correlating the lagged phase synchronization values with the VAS item "dizziness" a negative correlation (r < 0, p = .033) was found in the delta band between the precentral (BA 4, X = -35, Y = -25, Z = 55) and the postcentral gyrus (BA 2, X = -55, Y = -25, Z = 50). Furthermore, at a trend level the score of the VAS item "stimulation" was negatively correlated with delta oscillations of lagged phase synchronization (r < 0, p = .058) between the PCC (BA 29, X = -55, Y = -50, Z = 5) and the fusiform gyrus (BA37, X = 45, Y = -55, Z = -15).

Global Omega Complexity. The administration of GHB compared to placebo during the eyes-open condition significantly decreased global omega complexity in alpha1 oscillations (p

= .0014). Furthermore, at a trend level global omega complexity in theta oscillations was found to be decreased as well (p = .061). All other frequency bands were not significantly affected by the effect of the drug (all p < .155). During eyes-closed none of the analyzed contrasts for each frequency band reached statistical significance. However, global omega complexity in theta oscillations showed a trend-level decrease for GHB compared to placebo (p = .066).

Regression analysis of global omega complexity was performed, correlating all items of the VAS. Additionally, we computed the correlation of the drug-induced change of global omega complexity with GHB plasma values. Global omega complexity in both eyes-open and eyes-closed conditions correlated neither with subjective measures (r < 0, p_{max} = .388), nor with GHB plasma values (r < 0, p_{max} = .303).

Discussion

In the present study, we assessed the pharmacological effect of GHB on current source density, lagged phase synchronization and global omega complexity of neuronal oscillations in comparison to placebo by means of electrophysiological neuroimaging. Signs of a "paradoxical EEG-behavioral dissociation", which is characterized by slowing of oscillations during the awake state, could be partially replicated, as we found a concordant increase of theta oscillations after GHB administration. Additionally, we were able to observe a trend-level increase in delta oscillations induced by GHB. Previous studies used doses ranging from 35 mg/kg up to 63 mg/kg, which could potentially have resulted in a stronger shift towards low frequencies, similar to the induction of sleep (Jenney et al. 1962; Metcalf et al. 1966). Additionally, we observed an increase in alpha1 oscillations, which is in line with the findings from Jimenez et al. (1982) but stands in contrast to the results from Metcalf et al. (1966), where the alpha frequency band mainly diminished during wakefulness. Given that Jimenez et al. (1982) used dosages up to 20 mg/kg and Metcalf et al. (1966) doses from 35 to 63 mg/kg, it is likely that the observed difference in alteration of alpha oscillations between these studies arises from the different dosage levels used. At the behavioral level, GHB induced subjective effects of sedation, stimulation, and dizziness, peaking at 40 minutes after the administration of the drug. In addition, the course of the "general drug effect" scale strongly corresponded to the course of GHB plasma levels, well reflecting the drug effect on a subjective level.

We were able to complement the existing literature by adding information about the localization of the underlying neuronal sources generating the topographic map of electrical currents on the scalp. Our data suggest that GHB induced alterations of current source density are mainly located bilaterally in the cingulate cortex. Specifically, drug-induced changes of alpha oscillations were found in the ACC, which – among other functions – is a key region in the maintenance of an alert state (Posner & Rothbart, 1998). Interestingly, an increase of spatially coherent frontal alpha power has also been observed during the process of losing consciousness in healthy individuals anesthetized with propofol (Purdon et al. 2013). Thus, our findings may confirm the sedative properties of GHB and its use as an anesthetic agent by demonstrating electrophysiological similarities to another well-established anesthetic compound.

The GHB-induced increase in current density of theta oscillations was most pronounced in the PCC, a major node within the default mode network (DMN) (Raichle 2015) with numerous structural connections to widespread regions of the brain (Leech et al. 2012). The

activity of the PCC has been proposed as a neuronal correlate of the level of consciousness. Vogt and Laureys (2005) summarized evidence for this assumption by comparing several states characterized by reduced levels of consciousness, including sleep, vegetative state, epilepsy, and anesthesia. They found lowered PCC activity in terms of decreased glucose metabolism to be a common feature of reduced levels of consciousness. Delta and theta oscillations are well known - amongst other things - for enacting inhibitory functions on cognitive and sensory processes (see also Harmony 2013; Knyazev 2007) by rhythmically decreasing the excitability of local groups of neuronal networks (Lakatos 2008). Moreover, a study in rats showed GHB to induce theta-range oscillatory hyperpolarizations (Williams 1995). Our data suggest that these slow oscillations emerge from the PCC. Theta oscillations located at the PCC are thought to have their origin in the dorsal hippocampus, mostly because of the phase reversal of theta occurring only in the hippocampus and are subsequently volume-conducted to the PCC (Talk 2004). As maximal high-affinity binding on $GABA_B$ receptors was found in hippocampus (Maitre 1997) and this region has been shown to produce theta rhythms depending on GABAergic neurotransmission (Konopacki et al. 1997, Leung & Shen 2007), it is not surprising to find the modulatory effect of GHB on neuronal oscillations to be most pronounced at this region, which is closely linked by multiple direct and indirect routes (Myashita & Rockland 2007). The absence of altered oscillatory activity regarding the hippocampus could probably be best explained by the complex cyto-architecture of this region, possibly resulting in a cancellation of opposing neuronal structures. Moreover, the thalamus is known to generate low frequency oscillations via GABAergic inhibition (Kim et al. 1997). The solution space of eLORETA does not include thalamic structures. Therefore, it is not possible to exclude the possibility of an additional involvement of the thalamus in generating the observed patterns in the present study (Pascual-Marqui et al. 1994).

Although we found a trend in the direction of increased strength of functional connections after the intake of GHB, significance was not reached when contrasting the drug effect with placebo. When considering the trade-off between exhibiting sufficient statistical power to detect a plausible effect and the possibility of identifying a wide range of altered connections across the brain, we opted for a whole-brain approach that reduced the chances of observing small to moderate effects. From our perspective, the evidence in the literature was not strong enough to justify focusing solely on a subset of regions, or using one-tailed test statistics. An additional limitation for detecting supposedly small changes in functional connectivity could have been induced by the similarity in blood plasma measures between the administered dosage levels. Consequently, statistical significance was reached when the drug

effect on lagged phase synchronization was correlated with GHB plasma values. Most interestingly, this effect was found on connectivity between the inferior parietal lobule and the PCC, two major nodes within the DMN. When monitoring DMN connectivity during the different stages from wakefulness to sleep, previous research found it to be decreased drastically after losing consciousness (Sämann et al. 2011; Horovitz et al. 2009). Additionally, De Havas (2011) found DMN connectivity to be decreased after 24 hours of sleep deprivation and Bosch et al. (2013) found decreased PCC to ACC connectivity after partial sleep deprivation. When considering the role of lowered PCC glucose metabolism as well as decreased DMN connectivity as a neuronal correlate for reduced levels of consciousness, an interesting explanation for the pattern of "paradoxical EEG-behavioral dissociation" can be proposed: it is possible that the inhibitory function of slow waves on cognitive and sensory functions (see also Harmony 2013; Knyazev 2007) does not necessarily result in a loss of consciousness, as long as the requisite level of functional connectivity between the PCC and other nodes within the DMN can be maintained. Therefore, the interplay between slow, inhibitory oscillations generated at the PCC and the stable (or even slightly increased) connectivity of the DMN could be responsible for producing the unique electrophysiological profile of GHB at low to moderate doses, characterized by states that resemble loss of consciousness (reflected in the emergence of low frequency oscillations), without being reflected on a behavioral level.

Additionally, Stamatakis and colleagues (2010) expected changes in connectivity between nodes of the default mode network (DMN) when sedating participants with different dosages of propofol, but found increased connectivity between the PCC and areas that are not widely reported to be synchronized with the DMN. This effect was correlated with increasing dosages of the anesthetic agent. Our data showed – at a trend level – that the score on the subscale "stimulation" was negatively correlated with the strength of the connection between the PCC and the fusiform gyrus regarding delta oscillations. Thus, we were able to add further evidence to support the assertion that heightened functional connectivity of the PCC with regions normally not implicated in the DMN could serve as a neuronal correlate of sedation.

The global omega complexity index significantly differed between GHB and placebo when focusing on alpha oscillations. Remarkably, theta oscillations also seem to be affected at a trend level. In general, our finding of decreased omega complexity after the administration of GHB reflects a decreased number of relatively independent neural processes. Accordingly, a low number of independent processes indicate a high degree of neuronal synchronization and are therefore predictive for functional connectivity indices (Kondakor et al. 1999). However, the global omega complexity index can serve as a single, global marker for information

processing complexity and therefore adds further degrees of freedom for the interpretation of the results. Moreover, when correlating the drug effect on global omega complexity with GHB plasma values, significance was not reached. One explanation for the absence of the expected correlation could be that minor changes in blood plasma are sufficient to produce an effect of GHB on global omega complexity, such that it does not alter in a dose-dependent fashion. However, a more plausible explanation implies a highly localized effect of GHB, which is not necessarily reflected in a global index of omega complexity. This hypothesis receives further support from analysis of current source density, where we found changes limited to the cingulate cortex.

Our results concerning global omega complexity correspond somewhat with the theory of the entropic brain (Carhart-Harris et al. 2014). The global omega complexity index can be associated to the concept of entropy. High complexity values reflect high entropy in numerous localised areas across the brain (Friston et al. 1995). Therefore, the observed decrease in complexity could be associated with a low entropic state of the brain. Carhart-Harris et al. (2014) proposed the theory of the entropic brain in order to explain qualities of different states of consciousness in terms of varying degrees of entropy. High entropic states, as seen for example after administration of the hallucinogen psilocybin, are opposed to low entropic states that can be observed in strongly sedated or anesthetized humans. Accordingly, during the last decade, entropy monitoring became a standard method for evaluating the depth of anesthesia (Jordan et al. 2008; Olofson et al. 2008). Moreover, this theory further characterizes high entropic states in terms of a decrease in alpha power of signals located at the PCC. The observed increase in alpha power after the administration of GHB (though only partially located at the PCC) suggests additional relevance for lowered states of consciousness, characterized by low entropy.

After oral doses of 25 mg/kg and 35 mg/kg the VAS scores showed a strong general drug effect and significantly increased sedation. We were able to replicate the “paradoxical EEG-behavioral dissociation” reported in earlier studies after the administration of GHB in awake human subjects. Moreover, changes in current source density suggested theta oscillations emerging from the PCC to play a crucial role in producing the unique pharmacological profile of GHB. Our results give rise to a possible explanation regarding the paradoxical absence of convergence between electrophysiology and behavior. Namely, the increased slow oscillatory activity of the PCC seems to be a primary mechanism by which GHB alters electrophysiological patterns at the scalp surface, in a manner usually observed in states of drastically reduced levels of consciousness. Because of the stable or even slightly increased DMN connectivity induced

by GHB, such inhibitory functions could be compensated for and thereby may not be observable at a behavioral level, as would otherwise be expected when considering the shift towards lower frequencies after the administration of GHB. Additionally, analysis of global omega complexity following the administration of GHB was used to characterize common neuronal correlates of induced sedation, similar to other anesthetic compounds and akin to low entropic states.

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Tables

Table 1

Table of F-values (degrees of freedom) derived from repeated measures ANOVA for all items of the VAS and GHB blood plasma levels with "drug" and "time" as factors.

	F value(df) "drug"	*F value(df) "time"*	*F value(df) "drug * time"*	*Residual sum squared*
VAS				
general drug effect	30.39(1)***	13.29(5)***	4.73(5)**	387.83
sedation	23.08(1)***	7.47(5)***	3.45(5)*	518.20
stimulation	6.00(1)	5.83(5)***	2.92(5)	468.79
dizziness	17.37(1)***	6.90(5)***	5.97(5)***	218.56
GHB				
Blood plasma	237.92(2)***	47.75(5)***	27.32(10)***	37956

Note. * $p < .05$, ** $p < .01$, *** $p < .001$. All p-values are corrected for multiple comparisons.

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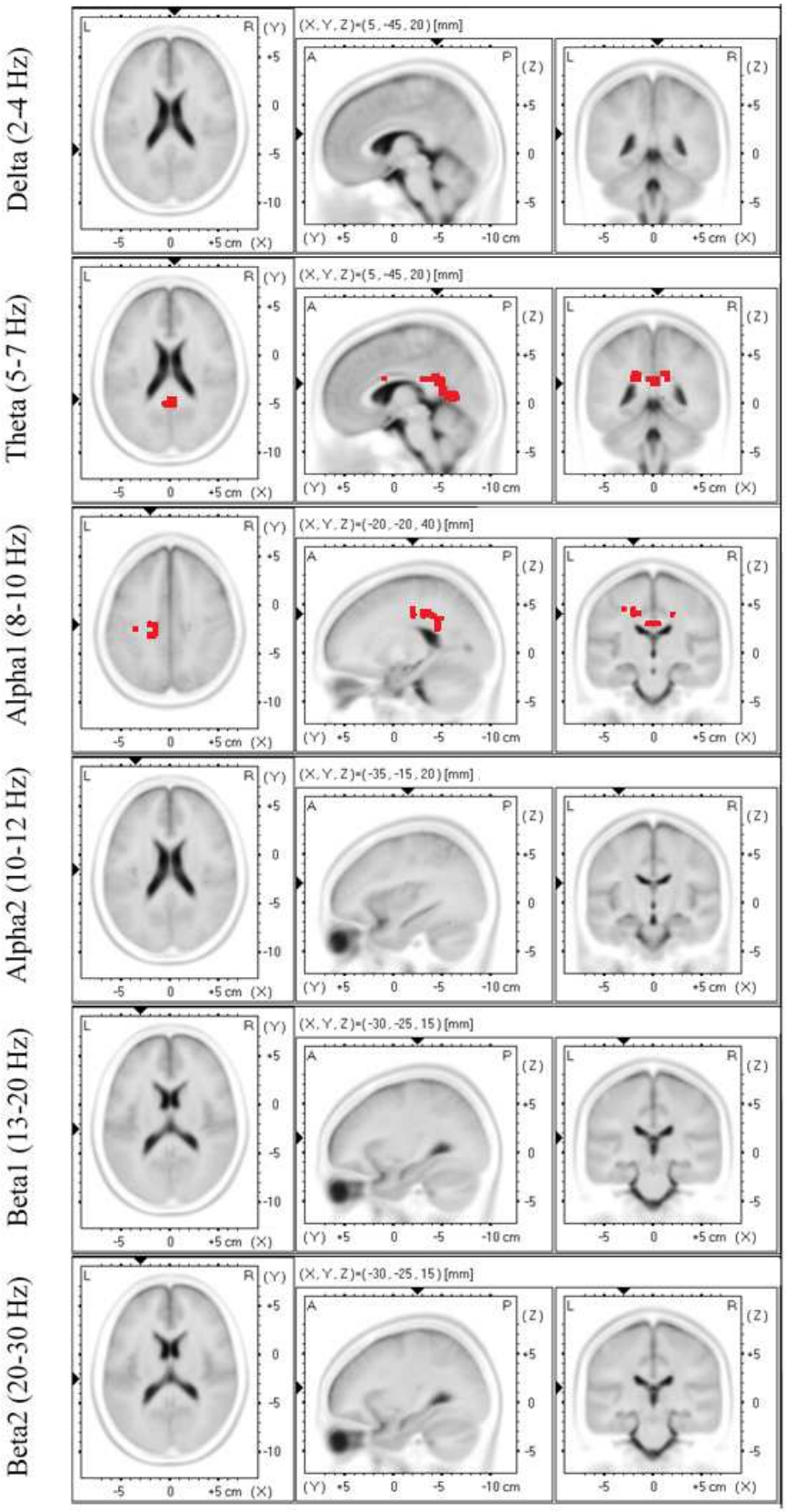
Supplement

Preprocessing. EEG data were bandpass-filtered from 0.5-48 Hz in order to attenuate channel drifts and satisfying the stationary assumption necessary for computing independent component analysis (ICA) (Onton and Makeig 2006). The whole artefact-rejection procedure was performed using the Brain Vision Analyzer 2 software (Brain Products GmBH). First, a trained researcher manually inspected all raw datasets in order to remove epochs that were obviously contaminated by sweat, technical, or muscle artefacts. Second, an infomax ICA was performed removing eye blinks as well as vertical and horizontal eye movements (Bell and Sejnowski 1995; Lee and Seung 1999). Third, channels with bad data quality were interpolated using spherical splines (Perrin, Pernier, Bertrand, and Echallier 1989). Forth, the complete data was re-referenced to the average of all electrodes. Fifth, the artefact-free parts were segmented in epochs with a length of two seconds each. Last, a repeated measure ANOVA with the factors placebo vs. GHB and eyes-open vs eyes-closed was applied in order to test whether the number of artefact free segments differ between the conditions. All contrasts remained non-significant ($p > .05$).



Suppl. Fig. 1 Statistical map of voxel-wise comparisons of current source density for all frequency bands in the eyes open condition. The threshold for this condition was adjusted, therefore *red* depicts highest voxel values that remained non-significant ($p > .05$, corrected).