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Intracranial electroencephalography power and phase synchronization changes during monaural and binaural beat stimulation

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Abstract

Auditory stimulation with monaural or binaural auditory beats (i.e. sine waves with nearby frequencies presented either to both ears or to each ear separately) represents a non-invasive approach to influence electrical brain activity. It is still unclear exactly which brain sites are affected by beat stimulation. In particular, an impact of beat stimulation on mediotemporal brain areas could possibly provide new options for memory enhancement or seizure control. Therefore, we examined how electroencephalography (EEG) power and phase synchronization are modulated by auditory stimulation with beat frequencies corresponding to dominant EEG rhythms based on intracranial recordings in presurgical epilepsy patients. Monaural and binaural beat stimuli with beat frequencies of 5, 10, 40 and 80 Hz and non-superposed control signals were administered with low amplitudes (60 dB SPL) and for short durations (5 s). EEG power was intracranially recorded from mediotemporal, temporo-basal and temporo-lateral and surface sites. Evoked and total EEG power and phase synchronization during beat vs. control stimulation were compared by the use of Bonferroni-corrected non-parametric label-permutation tests. We found that power and phase synchronization were significantly modulated by beat stimulation not only at temporo-basal, temporo-lateral and surface sites, but also at mediotemporal sites. Generally, more significant decreases than increases were observed. The most prominent power increases were seen after stimulation with monaural 40-Hz beats. The most pronounced power and synchronization decreases resulted from stimulation with monaural 5-Hz and binaural 80-Hz beats. Our results suggest that beat stimulation offers a non-invasive approach for the modulation of intracranial EEG characteristics.

Introduction

Human electroencephalography (EEG) activity can be entrained by rhythmic sensory stimulation. For instance, a train of rhythmic flicker stimuli may cause photic driving, i.e. an increase in EEG power around the driving frequency. Such rhythmic stimulation yields maximal responses within modality-specific brain regions and at modality-specific resonance frequencies. Visual flicker stimulation, for instance, has been shown to cause maximal responses at EEG frequencies around 10, 20, 40 and 80 Hz, and at posterior loci (e.g. Herrmann, 2001). In the auditory domain, such so-called steady-state responses can be induced in a similar way, e.g. by using repetitive click stimuli. In addition, amplitude-modulated tones or sounds can be employed for steady-state stimulation. Thereby, the steady-state response occurs at the frequency of the amplitude modulation, and not at the carrier frequency (e.g. Picton *et al.*, 2003).

A number of previous scalp EEG studies have investigated EEG responses to amplitude-modulated tones. Stimulus-related responses were observed at modulation rates up to 200 Hz, with the largest responses being recorded at approximately 40 Hz (e.g. Picton *et al.*, 2003). For 40-Hz beats, the EEG amplitude enhancement caused by binaural beat stimulation (see Materials and methods for an explanation of monaural and binaural beats) was shown to be approximately a factor of three to four times smaller than for stimulation with monaural beats of identical loudness, at least for scalp EEG positions (Schwarz & Taylor, 2005; Draganova *et al.*, 2008). Source localization of the responses to monaural and binaural beats reveals the most prominent neural activation at temporal and central sites, in particular in the vicinity of the Sylvian fissure (Karino *et al.*, 2006; Draganova *et al.*, 2008), as well as in temporo-lateral and temporo-basal areas (Pratt *et al.*, 2009).

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Moreover, preliminary findings have suggested that auditory beats may induce phase synchronization, i.e. an increased stability of phase relationships between different brain regions (e.g. Lachaux *et al.*, 1999), although this has never been directly tested. Oscillatory responses to monaural and binaural beats were reported to show specific interareal phase delays. For instance, for unilateral stimulation with 40-Hz beats, these phase delays are often in the order of only several degrees for nearby scalp positions (Schwarz & Taylor, 2005). On the basis of these findings, one may assume that stimulation with auditory beats may result in increased phase synchronization, in particular in temporal regions (which show the strongest amplitude enhancements).

Although several studies have tried to assess the behavioral effects, particularly of binaural beat stimulation (e.g. Lane *et al.*, 1998; Padmanabhan *et al.*, 2005; Reedijk *et al.*, 2013), there are very few systematic studies addressing the electrophysiological changes related to monaural and binaural stimulation with different beat frequencies. We examined how EEG power and phase synchronization are affected by auditory beat stimulation, based on intracranial EEG recordings in 10 presurgical epilepsy patients. Recordings were acquired from temporo-lateral, temporo-basal and mediotemporal electrode contacts. Monaural and binaural beat stimulation was applied in the theta (5 Hz), alpha (10 Hz), low gamma (40 Hz) and high gamma (80 Hz) range.

Materials and methods

Generation of monaural and binaural beats

Auditory beat stimuli are amplitude-modulated signals that can be produced by a superposition of sine waves with nearby frequencies. Auditory beats have been mainly investigated with two approaches (Fig. 1). So-called monaural beats result from the physical superposition of two acoustic waves. For instance, the superposition of two sine waves with frequencies of 440 and 480 Hz yields a composite sine wave with a carrier frequency of 460 Hz and an amplitude that is modulated at a rate of 40 Hz. In the case of binaural beats, one sine wave (e.g. 440 Hz) is presented to the left ear and the other sine wave (e.g. 480 Hz) is presented to the right ear. In spite of the absence of an actual (i.e. physical) superposition, this approach also gives rise to the subjective perception of an amplitude-modulated tone (e.g. a 40-Hz modulation). The binaural beat effect originates from the activity of brainstem neurons in the superior olives that are sensitive to phase shifts between both ears, a mechanism enabling sound location (Kuwada *et al.*, 1979). These neurons fire action potentials in response to the frequency difference between both ears, and cause a subjective perception of the binaural beat frequency.

In the present study, auditory stimulation was applied with monaural and binaural beats in the theta (5 Hz), alpha (10 Hz), low gamma (40 Hz) and high gamma (80 Hz) range. These frequencies appear to be most relevant for possible applications, because of the putative facilitating role of theta and gamma oscillations in cognitive processes (e.g. Nyhus & Curran, 2010; Lisman & Jensen, 2013) and the potentially inhibitory effects of alpha oscillations (e.g. Klimesch *et al.*, 2007; Jensen & Mazaheri, 2010; Jensen *et al.*, 2012; Klimesch, 2012). The non-superposed sine waves, which the beat signals were composed of, served as control stimuli. Power and phase synchronization results were statistically evaluated with Bonferroni-corrected non-parametric label-permutation tests (Maris & Oostenveld, 2007).

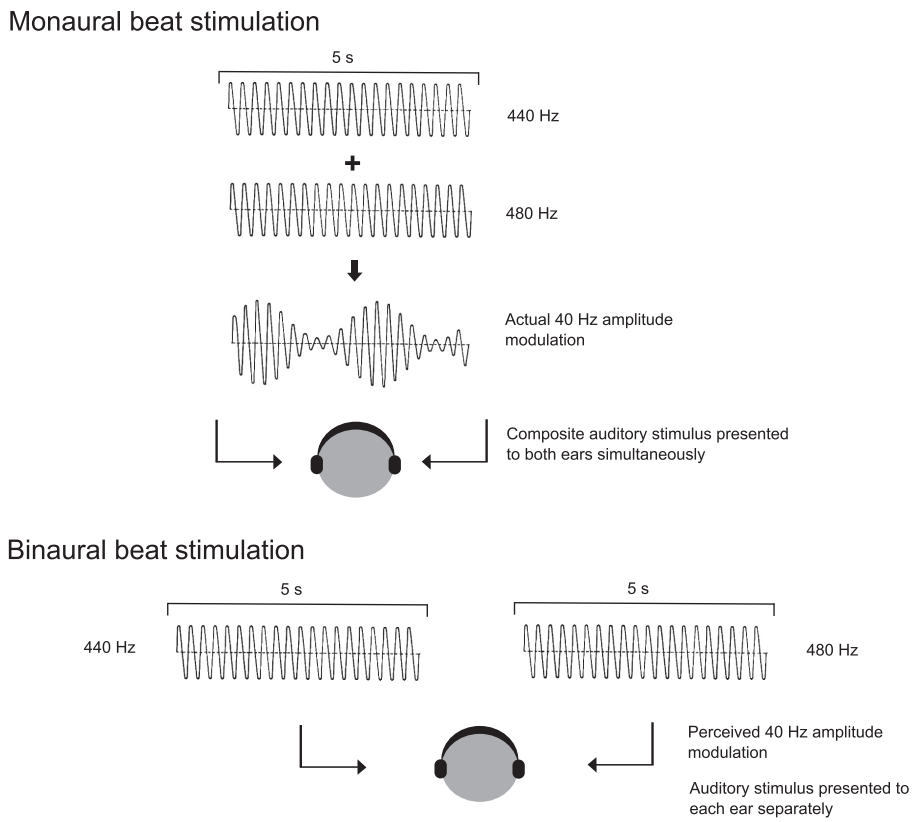

FIG. 1. Schematic illustration of the composition and experimental application of monaural and binaural beats (40-Hz beats are depicted here as an example).

Auditory stimulation

In the case of monaural beats, the audio signal consisted of two superposed sine waves with nearby frequencies, and the same signal was presented to both ears. In the case of binaural beat stimulation, one sine wave was presented to the left ear and the other sine wave was presented to the right ear. Stimulation trials lasted for 5 s and were separated by stimulation-free intervals with randomized lengths ranging between 2.5 and 5 s. Stimulations were randomly selected from 13 conditions: four monaural, four binaural and five control conditions. In other words, the sequence of conditions was randomized across single trials for each subject. Monaural and binaural beats were constructed by using sine waves of the following frequencies: (i) 440 and 445 Hz (5-Hz beats); (ii) 440 and 450 Hz (10-Hz beats); (iii) 440 and 480 Hz (40-Hz beats); and (iv) 440 and 520 Hz (80-Hz beats). The individual non-superposed sine waves (440, 445, 450, 480, and 520 Hz) served as within-subject control stimuli. For instance, for stimulation conditions with either monaural or binaural 5-Hz beats, the corresponding sine waves at 440 and 445 Hz would be used as control stimuli. More precisely, control stimuli were always presented separately (according to the random trial sequence), but the EEG responses for pairs of condition-specific control stimuli (in the above example, 440 and 445 Hz) were later combined for statistical evaluation (see below). All auditory stimuli were applied with a 60-dB sound pressure level.

To ensure that patients paid attention to the auditory stimuli, they were instructed to indicate, via button presses, whether they recognized (i.e. subjectively perceived) a beat stimulus or not. Behavioral data from two patients were unreliable, as they confused response keys during the experiment, and thus could not be evaluated. Prior to the main experiment, several training stimuli were presented to the patients to allow them to practice this task. Every 6–8 min, stimulus presentation was interrupted by a short break of approximately 1 min, and at the end of the breaks patients were asked whether they were willing to continue. Accordingly, the duration of the main experiment varied between 15 and 40 min, and the total number of auditory stimuli varied between 87 and 214.

Patients and electrodes

Ten temporal lobe epilepsy patients (six females; mean age, 37.2 ± 12.9 years) undergoing presurgical evaluation participated in the study. The study was approved by the Ethics Committee of the Medical Faculty of the University of Bonn and conformed to the 2013 Declaration of Helsinki of the World Medical Association. All patients gave written informed consent. Seven patients were implanted with bilateral hippocampal depth electrodes from a posterior direction (Van Roost *et al.*, 1998). Depth electrodes consisted of 10 cylindrical platinum contacts with a diameter of 1.3 mm and a length of 1.6 mm, which were located along the longitudinal axes of the hippocampi, with the anterior contacts reaching into the rhinal cortex. Five of those patients were additionally implanted with temporo-lateral and temporo-basal strip electrodes (stainless steel; diameter, 4 mm; center-to-center distance between electrodes, 1 cm). Three patients were implanted only with temporo-lateral and temporo-basal strip electrodes. In all patients, the placement of intracerebral electrode contacts was ascertained from magnetic resonance images. Moreover, surface recordings were acquired from positions Cz, C3, C4, T5, T6 and Oz (10–20 system) in seven patients (Table 1). In the remaining three patients, surface EEG electrodes could not be mounted, owing to complications with scalp wounds resulting from electrode implantation.

Presurgical evaluation revealed hippocampal seizure foci in six patients, temporal foci in three patients, and a frontal focus in one patient (Table 1). In six patients the seizure focus was in the right hemisphere, in three patients it was in the left hemisphere, and in one patient there were bilateral foci. In the case of a hippocampal focus, only subdural and depth electrodes from the contralateral hemisphere were included in the analysis. In the case of a temporal or frontal focus, subdural electrodes from the contralateral hemisphere and bilateral depth electrodes were included in the analysis. Electrodes with permanently low signal quality and electrodes that showed epileptiform activity or other artefacts in > 40% of all trials were excluded. In total, recordings from 65 mediotemporal, 67 temporo-lateral, 59 temporo-basal and 42 surface electrode contacts were analysed (Table 1; Fig. 2).

EEG recording and artefact rejection

Intracranial electroencephalograms were referenced to linked mastoids, bandpass-filtered [0.01 Hz (6 dB/octave) to 300 Hz (12 dB/octave)], and recorded with a sampling rate of 1000 Hz. All EEG trials were visually inspected for epileptiform, movement or technical artefacts, and 8.4% of all trials were discarded. Channels with permanently low signal quality or with artefacts present in > 40% of trials were completely excluded from further analysis.

EEG analysis

Electroencephalography data were analysed for the following channel groups: mediotemporal (depth), temporo-lateral (subdural),

TABLE 1. Location of seizure foci and number of electrodes within the different electrode groups that were entered into the analysis after artefact rejection

		Number of selected electrodes				
Patient	Seizure focus	Mediotemporal	Temporo-basal anterior	Temporo-basal posterior	Temporo-lateral	Surface
P1	Hippocampus left	10	4	4	6	–
P2	Hippocampus right	10	4	4	6	6
P3	Temporal right	–	4	–	16	–
P4	Temporal right	–	4	3	15	6
P5	Hippocampus left	1	4	4	6	6
P6	Hippocampus right	–	4	4	6	–
P7	Hippocampus left	10	4	4	6	6
P8	Temporal right + left	10	–	–	–	6
P9	Frontal right	16	–	–	–	6
P10	Hippocampus right	8	4	4	6	6

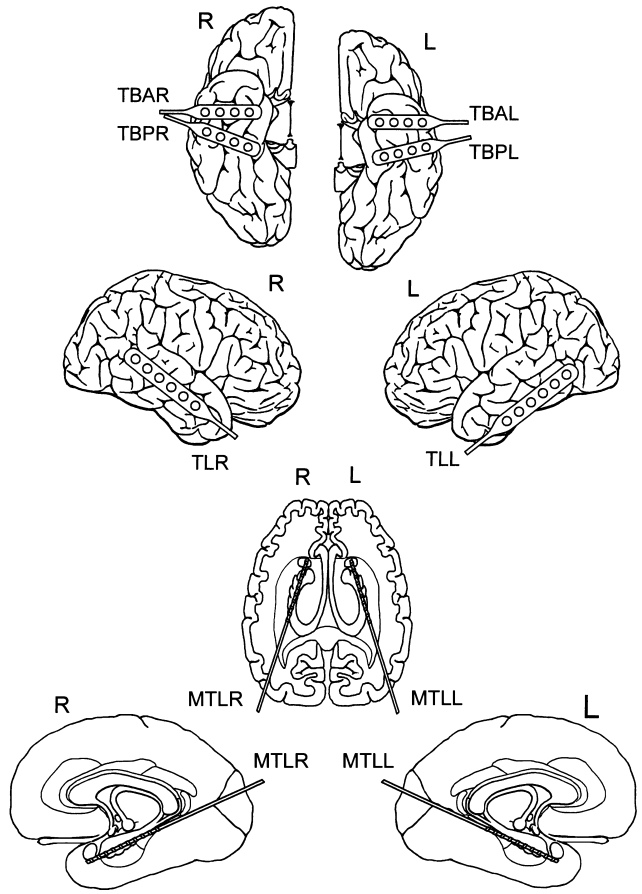

FIG. 2. Electrode sites for one patient as an example. Subdural electrodes – temporo-lateral right (TLR), temporo-lateral left (TLL), temporo-basal anterior right (TBAR), temporo-basal anterior left (TBAL), temporo-basal posterior right (TBPR), and temporo-basal posterior left (TBPL). Depth electrodes – mediotemporal lobe right (MTLR) and mediotemporal lobe left (MTLL).

anterior temporo-basal (subdural), posterior temporo-basal (subdural), and surface. To quantify EEG responses resulting from auditory stimulation, EEG trials were segmented for the different conditions by use of the time intervals between –1 and 7 s with regard to stimulus onset. Raw EEG data were then filtered with second-order zero-phase Butterworth filters (MATLAB; Mathworks) at 5 Hz (edge frequencies, 4.5–5.5 Hz), 10 Hz (9.5–10.5 Hz), 40 Hz (39.5–40.5 Hz), and 80 Hz (79.5–80.5 Hz). Then, filtered EEG trials were trimmed to the time intervals between 1 and 5 s after stimulus presentation in order to avoid influences of edge effects caused by the filtering and presence of event-related components. The filtered EEG trials were then Hilbert-transformed, and power was calculated from the squared absolute values of the complex time series. Power was quantified on the basis of both single trials and averaged trials. Single-trial power was extracted for individual trials, and then averaged across all trials for each condition (total activity). Power values for averaged trials were extracted from the averaged time series of each condition (evoked activity). Both single-trial power changes and the phase stability of single-trial responses contribute to the power results for averaged trials.

Phase synchronization effects were estimated for all possible channel pairs within the five channel groups. Phases for each time point within each trial (between 1 and 5 s after stimulus presentation) were calculated on the basis of the complex Hilbert-transformed time series. Then, phase synchronization was quantified for each trial on the basis of the circular variance of phase differences between the channel pairs across all time points of the trial (Lachaux *et al.*, 1999). Finally, for each condition, phase synchronization values were averaged across all trials. Both power and synchronization values were normalized by dividing by the average values taken across all conditions for each channel group and patient.

Statistical analysis

Statistical evaluation was based on non-parametric label-permutation tests (Maris & Oostenveld, 2007). In a first step, single-trial power, averaged-trial power and phase synchronization values were collected across all channels or channel pairs of each channel group (mediotemporal, anterior temporo-basal, posterior temporo-basal, temporo-lateral, and surface) and across all patients for each condition, i.e. beat stimulation and control conditions. Beat stimulation and control conditions were then compared by the use of paired *t*-tests. Control conditions were individually adjusted to the beat conditions (see above), and results for the two control signals were averaged. In a second step, those cases with significant *t*-test results ($P < 0.05$) were subjected to a label-permutation test. For this purpose, condition labels (beat and control) for all channels or channel pairs were randomly permuted 10 000 times, and *t*-values were again calculated on the basis of paired *t*-tests for each permutation. Then, the *t*-value for the original data was ranked among the *t*-values resulting from random label permutation, which yielded the final significance value. To correct for multiple comparisons (eight beat conditions × five channel groups) only *P*-values of < 0.00125 ($= 0.05/40$) were regarded as statistically significant.

Control analyses

In order to test whether the observed effects could also be produced by pure tones, we calculated single-trial power, average-trial power and phase synchronization changes related to presentation of the control stimuli (see above) for those frequencies and locations for which statistically significant effects were detected for beat vs. control stimulation. For the power analyses, the poststimulus interval (1–5 s) was compared with the prestimulus interval (−0.5 to 0 s), which served as the baseline. As the amount of data points biases phase synchronization results, the poststimulus interval (1–1.5 s) was compared with the prestimulus interval (−0.5 to 0 s) for the phase synchronization analyses. Statistical evaluation of the poststimulus vs. prestimulus contrast for control stimulation was performed in the same way as for the beat vs. control stimulation contrast.

We also evaluated which changes in total EEG power (single trial) at subharmonic and harmonic frequencies were caused by beat stimulation. Responses at the first subharmonic frequency, in addition to the first, second and third harmonic frequencies, were analysed. For instance, for 5-Hz monaural (or binaural) stimulation, total power changes at 2.5 Hz (first subharmonic), 10 Hz (first harmonic), 15 Hz (second harmonic) and 20 Hz (third harmonic) were evaluated; and for 10-Hz monaural (or binaural) stimulation, total power changes at 5 Hz (first subharmonic), 20 Hz (first harmonic), 30 Hz (second harmonic) and 40 Hz (third harmonic) were evaluated. Responses at the harmonic and subharmonic frequencies were calculated by applying the same EEG analyses and statistical procedures as for the original response frequencies.

Results

Behavioral responses

On average, monaural beat stimuli were correctly recognized in 78.6% of all cases (range, 50.8–97.2%) and binaural beat stimuli were correctly recognized in 57.2% of all cases (range, 5.9–88.5%). Control stimuli were wrongly classified as beats in 3.6% of all cases (range, 0–24.2%). A two-way repeated measures ANOVA with the factors beat frequency (5, 10, 40, and 80 Hz) and beat type (monaural and binaural) revealed no significant main effect for beat type and no beat frequency × beat type interaction, but a significant main effect for beat frequency ($F_{3,27} = 6.808$; $P = 0.013$, Huynh–Feldt-corrected). The main effect was attributable to a significant linear contrast for beat frequency ($F_{1,9} = 8.540$; $P = 0.017$; no significant quadratic or cubic contrast), which indicated higher recognition rates for low-frequency beats than for high-frequency beats with the following sequence: 5-Hz beats (correct recognition, 80.6%) > 10-Hz beats (77.0%) > 40-Hz beats (60.7%) > 80-Hz beats (50.4%).

EEG power

In general, beat-related power decreases were found more frequently than power increases (12 vs. five significant effects). Power increases (Table 2) were mainly found for stimulation with monaural 40-Hz beats at the surface (total and evoked responses) and at temporo-lateral sites (evoked responses). Furthermore, increases in total power were found for binaural 40-Hz beats at mediotemporal sites and for binaural 10-Hz beats at temporo-lateral sites. Beat-related power decreases (Table 3) were mainly found for monaural 5-Hz beats, both in total power (mediotemporal, temporo-basal, and temporo-lateral) and in evoked power (mediotemporal and temporo-basal posterior). In addition, decreases in total power were found for binaural 5-Hz beats (temporo-basal) and binaural 80-Hz beats (mediotemporal, temporo-basal, and temporo-lateral).

Phase synchronization

Beat-related decreases were found more frequently than increases (five vs. two significant effects). Synchronization increases (Table 2) were found for stimulation with monaural 10-Hz beats at mediotemporal sites and for stimulation with binaural 5-Hz beats at temporo-lateral sites. Synchronization decreases (Table 3) were found for monaural 5- and 40-Hz beats (both mediotemporal), for binaural 5-Hz beats (temporo-basal anterior), and for binaural 80-Hz beats (mediotemporal and temporo-lateral). Overall, the stimulation conditions with the largest numbers of statistically significant effects were monaural 5-Hz beats and binaural 80-Hz beats, which were both associated with power and synchronization decreases. Figures 3 and 4 show the significant differences in EEG power and phase synchronization during beat vs. control stimulation for monaural 5-Hz beats and binaural 80-Hz beats.

TABLE 2. Statistically significant ($P < 0.00125$) increases in EEG power (single-trial power = total power; power from averaged trials = evoked power) and phase synchronization resulting from monaural and binaural beat stimulation (based on non-parametric permutation tests; see Materials and methods)

Electrode group	EEG power (single trial)	EEG power (averaged trials)	Phase synchronization
Mediotemporal	40 Hz binaural	–	10 Hz monaural*
Temporo-basal anterior	–	–	–
Temporo-basal posterior	–	–	–
Temporo-lateral	10 Hz binaural*	40 Hz monaural*	5 Hz binaural*
Surface	40 Hz monaural	40 Hz monaural*	–

*Effects at the lowest possible significance level of $P < 0.0001$.

TABLE 3. Statistically significant ($P < 0.00125$) decreases in EEG power (single-trial power = total power; power from averaged trials = evoked power) and phase synchronization resulting from monaural and binaural beat stimulation (based on non-parametric permutation tests; see Materials and methods)

Electrode group	EEG power (single trial)	EEG power (averaged trials)	Phase synchronization
Mediotemporal	5 Hz monaural* 80 Hz binaural*	5 Hz monaural*	5 Hz monaural* 40 Hz monaural 80 Hz binaural
Temporo-basal anterior	5 Hz monaural 5 Hz binaural 80 Hz binaural*	–	5 Hz binaural
Temporo-basal posterior	5 Hz monaural 5 Hz binaural 80 Hz binaural	5 Hz monaural	–
Temporo-lateral	5 Hz monaural* 80 Hz binaural	–	80 Hz binaural
Surface	–	–	–

*Effects at the lowest possible significance level of $P < 0.0001$.

Control analyses

We additionally tested whether the control stimuli (poststimulus vs. prestimulus) could produce similar effects as the beat stimuli (beat vs. control). Applying the same statistical procedures and threshold ($P < 0.00125$), we found that only the increase in evoked 40-Hz power at surface sites (single trial and average trial) and temporo-lateral sites (average trial) occurred similarly after presentation of the control stimuli (Fig. 5). However, the power increases for the beat stimuli seem to surpass the increases for the control stimuli (if this was not the case, we would have detected no effects for the contrast beat vs. control stimuli). On the other hand, four effects were also statistically significant for the control stimuli, but are not in line with the effects reported for the beat stimuli. We observed increases in total 80-Hz power (single trial) at anterior and posterior temporo-basal sites and at mediotemporal sites for the control stimuli. Furthermore, we found an increase in 80-Hz phase synchronization at temporo-lateral sites for the control stimuli.

We also analysed the changes in total EEG power related to beat stimulation at subharmonic and harmonic response frequencies. Similarly to the responses at the original frequency, we found power increases mainly for 10-Hz monaural and binaural beat stimulation and for 40-Hz binaural beat stimulation at the first subharmonic frequency and the first harmonic frequency (Table 4). Additionally, we observed power increases for 80-Hz monaural beat stimulation at the second harmonic frequency (i.e. at 240 Hz). Power decreases were mainly found for 5-Hz monaural and binaural stimulation and 80-Hz binaural stimulation (at the first subharmonic frequency and the first and second harmonic frequencies), i.e. for the same stimulation conditions for which we observed decreases at the original frequency (Table 5).

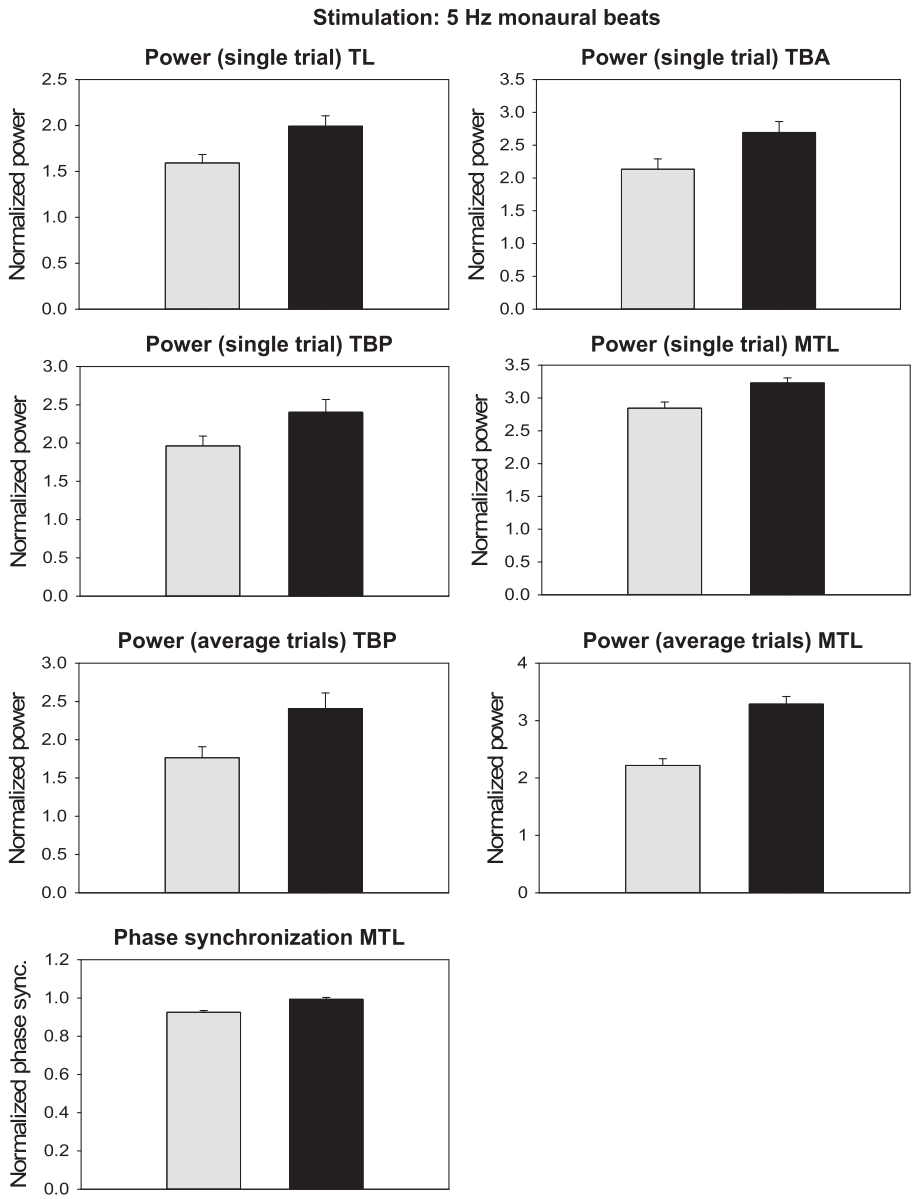

FIG. 3. Statistically significant ($P < 0.00125$) differences in EEG power and phase synchronization during stimulation with monaural 5-Hz beats (gray bars, mean and standard error of the mean) vs. control stimuli (black bars). MTL, mediotemporal lobe; TBA, temporo-basal anterior; TBP, temporo-basal posterior; TL, temporo-lateral.

Discussion

This is the first study, to our knowledge, to address the electrophysiological effects of monaural and binaural beat stimulation by means of intracranial recordings in humans. Furthermore, not only EEG power, but also phase synchronization changes resulting from auditory beat stimulation are reported here for the first time. Most importantly, our findings show that EEG power and phase synchronization can be significantly modulated by weak (60 dB SPL) and short-duration (5 s) stimulation with auditory beats not only at temporo-basal, temporo-lateral and surface sites, but also at mediotemporal sites.

As hypothesized, monaural 40-Hz stimulation yielded power increases at surface and temporo-lateral sites, which is in accordance with previous studies (e.g. Picton *et al.*, 2003; Schwarz & Taylor, 2005; Draganova *et al.*, 2008). These increases were found for total power on the basis of single trials (surface), and for evoked power on the basis of averaged trials (surface and temporo-lateral). As the magnitude of evoked responses depends on the degree of inter-trial phase-locking of single-trial responses, this finding indicates high phase stability of single-trial responses to monaural 40-Hz stimulation. However, for most stimulation conditions, EEG power and synchronization decreases were found, which may have resulted from disturbance and irritation of ongoing oscillatory activity.

From a theoretical viewpoint, cortical networks of inhibitory interneurons that generate network oscillations with a peak at 40 Hz have been detected (e.g. Whittington *et al.*, 1995). These oscillatory characteristics result from the net excitation and the kinetics of inhibitory postsynaptic potentials, and from resonant delay-induced network synchronization (Whittington *et al.*, 1995; Maex & De Schutter, 2003; Bosman *et al.*, 2014). Such interneuron networks, which are able to control pyramidal cell activity, may

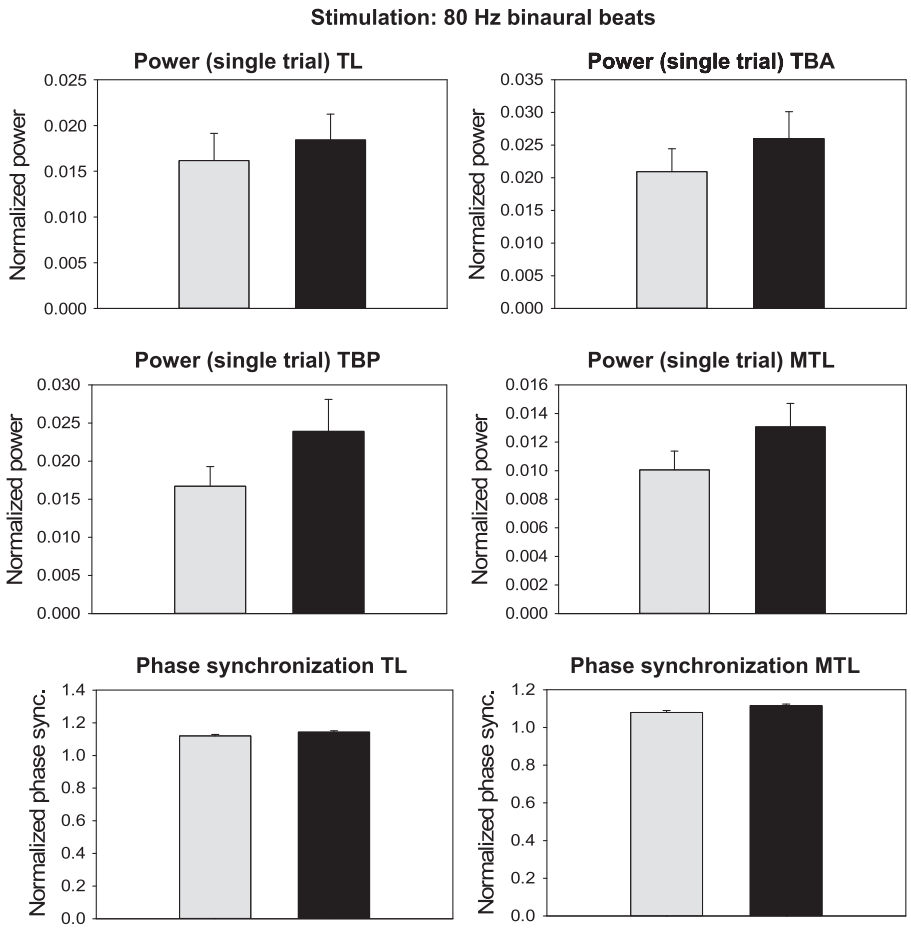

FIG. 4. Statistically significant ($P < 0.00125$) differences in EEG power and phase synchronization during stimulation with binaural 80-Hz beats (gray bars, mean and standard error of the mean) vs. control stimuli (black bars). MTL, mediotemporal lobe; TBA, temporo-basal anterior; TBP, temporo-basal posterior; TL, temporo-lateral.

play a particular role in sound processing (e.g. Moore & Wehr, 2013). The idea that these auditory interneuron networks can be best entrained at 40 Hz is in accordance with our results and findings from other steady-state stimulation studies (e.g. Picton *et al.*, 2003).

Overall, the numbers of significant effects were equal for monaural and binaural stimulation. Nevertheless, similar effects were observed only for 5-Hz beats for both monaural and binaural stimulation at temporo-basal sites (power decreases). For binaural 80-Hz stimulation, on the other hand, pronounced EEG power and synchronization decreases were found, but for monaural 80-Hz stimulation no significant effects were found at the stimulating frequency. However, monaural and binaural stimulation never resulted in opposite effects at the stimulation frequencies.

Control analyses demonstrated that these effects are largely specific for beat stimulation. We tested whether the observed effects could also be produced by the control stimuli. Only the increases in evoked and total 40-Hz power at surface and temporo-lateral sites were found to occur similarly after presentation of the control stimuli (poststimulus vs. prestimulus). These effects suggest that increased 40-Hz power at surface and temporo-lateral sites is not a specific response pattern for beat stimulation, but rather a common response to prolonged (here 5 s) auditory stimulation. However, four other effects, which were observed at 80 Hz for beat stimulation (beats vs. control stimuli) for the control stimuli, were not in line with the effects detected for beat stimulation. For 5 and 10 Hz, no corresponding effects were found. These results suggest that the reported effects for 5, 10 and 80 Hz are specific for beat stimulation.

In addition, we tested whether there are beat-related changes in total EEG power (single trial) at subharmonic and harmonic frequencies. These analyses revealed increases or decreases in EEG power at subharmonic and harmonic frequencies largely for the same stimulation conditions for which we found increases or decreases at the original response frequencies. For instance, for 80-Hz binaural beats, we found decreases in total power not only at 80 Hz (mediotemporal, temporo-basal, and temporo-lateral), but also at 160 Hz (temporo-lateral) and 320 Hz (mediotemporal and temporo-lateral). On the other hand, results for subharmonic and harmonic frequencies were rather heterogeneous in terms of power increases or decreases at different response frequencies. For instance, at a response frequency of 80 Hz, either power decreases (80-Hz binaural beats) or power increases (40-Hz binaural beats, first harmonic frequency) were found, depending on the stimulation condition. These findings indicate that the beat-related EEG changes are rather specific for the stimulation conditions and are not just caused by passive resonance properties of cortical networks.

The behavioral data indicated that low-frequency beats can be more reliably detected than high-frequency beats (correct recognition – 5-Hz beats > 10-Hz beats > 40-Hz beats > 80-Hz beats). On the

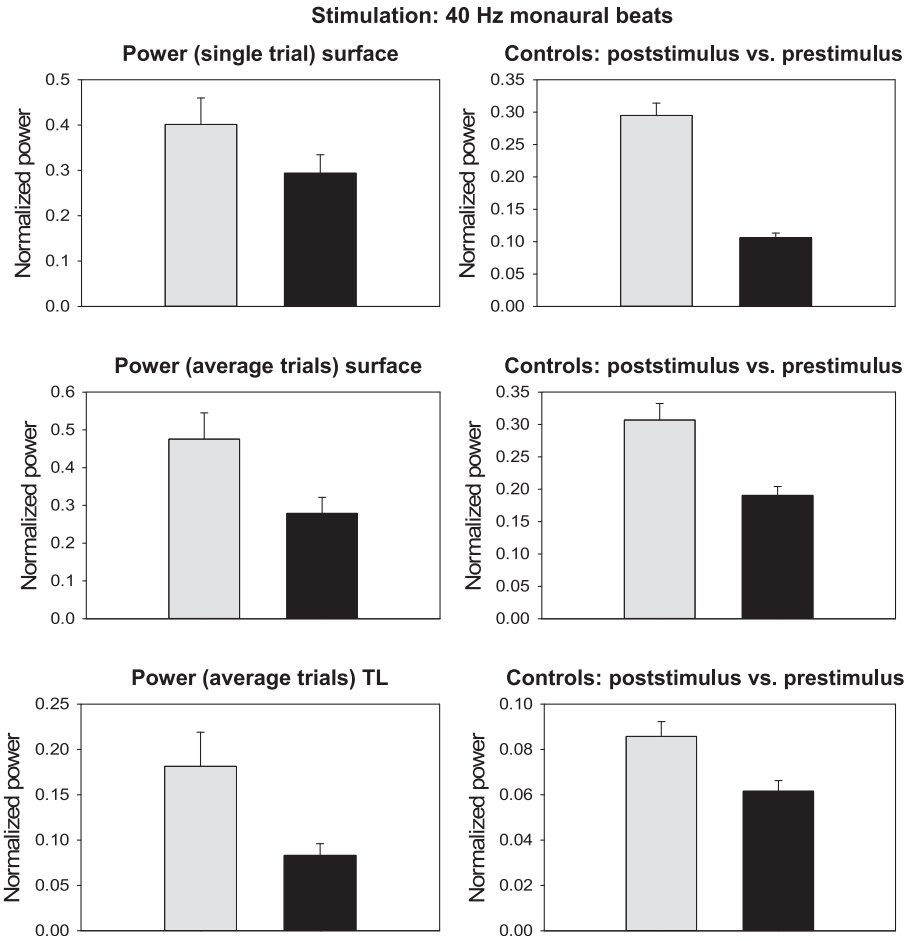

FIG. 5. Left column – statistically significant ($P < 0.00125$) differences in EEG power during stimulation with monaural 40-Hz beats (gray bars, mean and standard error of the mean) vs. control stimuli (black bars). Right column – statistically significant ($P < 0.00125$) differences in EEG power for control stimuli during poststimulus (gray bars, mean and standard error of the mean) vs. prestimulus (black bars) intervals. TL, temporo-lateral.

TABLE 4. Statistically significant ($P < 0.00125$) increases in total EEG power (single trial) at subharmonic and harmonic frequencies resulting from monaural and binaural beat stimulation (based on non-parametric permutation tests; see Materials and methods)

Electrode group	First subharmonic	First harmonic	Second harmonic	Third harmonic
Mediotemporal	10 Hz monaural* 10 Hz binaural*	10 Hz binaural* 40 Hz binaural*	80 Hz monaural*	–
Temporo-basal anterior	–	40 Hz binaural*	80 Hz monaural	–
Temporo-basal posterior	10 Hz binaural	40 Hz binaural	80 Hz monaural	–
Temporo-lateral	–	10 Hz binaural* 40 Hz binaural*	–	–
Surface	–	10 Hz monaural*	–	10 Hz monaural*

*Effects at the lowest possible significance level of $P < 0.0001$.

TABLE 5. Statistically significant ($P < 0.00125$) decreases in total EEG power (single trial) at subharmonic and harmonic frequencies resulting from monaural and binaural beat stimulation (based on non-parametric permutation tests; see Materials and methods)

Electrode group	First subharmonic	First harmonic	Second harmonic	Third harmonic
Mediotemporal	–	80 Hz monaural	–	80 Hz monaural* 80 Hz binaural*
Temporo-basal anterior	–	–	–	–
Temporo-basal posterior	5 Hz binaural*	–	–	–
Temporo-lateral	5 Hz monaural* 5 Hz binaural	5 Hz monaural* 80 Hz binaural	–	80 Hz binaural*
Surface	–	–	–	–

*Effects at the lowest possible significance level of $P < 0.0001$.

other hand, the highest numbers of electrophysiological effects (at the stimulation frequency and the subharmonic and harmonic frequencies) were found for 5-Hz and 80-Hz beats. This suggests that a lower incidence of subjective detectability of beat modulation at high frequencies does not preclude significant electrophysiological effects.

It is still an open question as to why certain beat stimulus conditions produce significant modulations whereas others do not (or even show effects in the opposite direction). Although the present data are in accordance with the previously reported entrainment properties of monaural and binaural 40-Hz stimulation (e.g. Schwarz & Taylor, 2005), our findings for the other stimulation frequencies are rather diverse. For instance, binaural 80-Hz stimulation resulted in pronounced decreases in total power and phase synchronization at this stimulation frequency. On the other hand, monaural 80-Hz stimulation did not show any effects at the stimulation frequency, but resulted in an increase in total power at the second harmonic frequency. These findings suggest that responses to monaural beat stimulation are not just topographically similar, but are stronger than responses to binaural beats (e.g. Schwarz & Taylor, 2005), and that the two conditions show fundamentally different effects, which might be expected with regard to their different neurophysiological origins (e.g. Kuwada *et al.*, 1979; Picton *et al.*, 2003; Draganova *et al.*, 2008). In future studies, the neural mechanisms underlying such effects need to be investigated in greater detail, in particular for those beat stimulation conditions that are applied for the purpose of behavioral modulation.

Phase synchronization has been shown to play a major role in cognitive processes, in particular in memory operations (e.g. Fries, 2005; Jutras & Buffalo, 2010; Fell & Axmacher, 2011). Whereas synchronized mediotemporal and neocortical theta and gamma activity have been reported to facilitate working and long-term memory functions (e.g. Jutras & Buffalo, 2010; Fell & Axmacher, 2011), synchronized mediotemporal alpha activity has been found to be negatively related to long-term memory performance (Staresina *et al.*, 2012). As a practical application of these findings, it has been demonstrated, for instance, that long-term memory performance can be modulated by weak electric in-phase vs. anti-phase stimulation of the rhinal cortex and hippocampus (e.g. Fell *et al.*, 2013) [see, for example, Laxton *et al.* (2010) and Suthana *et al.* (2012) for other deep brain stimulation approaches to memory enhancement]. In the present study, we found significant synchronization increases during application of 10-Hz monaural stimulation at mediotemporal sites and during 5-Hz binaural stimulation at temporo-lateral sites. Moreover, we found decreases in mediotemporal phase synchronization during stimulation with monaural 5- and 40-Hz beats. These findings suggest a potential role for 5-Hz binaural beats for the purpose of memory enhancement, but not for the other beat stimulation conditions applied in this study. On the basis of these results, future research may, for instance, address the influence of 5-Hz binaural beats on behavioral measures related to working and long-term memory performance.

In addition to these putative functional roles of increases in phase synchronization and power, decreases in power and phase synchronization – which were predominant in our study – could also be relevant for cognitive processes. Both effects, being decreases in both power and phase synchronization, probably reflect a reduction in neural synchronization, either at a local level (power decreases; so-called 'event-related desynchronization') or at a more global level (phase synchronization). If neurons or smaller networks behave more independently of one another, the possible amount of information represented by their activity might be higher than in a synchronized state (Hanslmayr *et al.*, 2012). It has, for instance, been shown that successful memory encoding is associated with transient broad-band power decreases in mediotemporal regions (Fell *et al.*, 2008). Reductions in widespread synchronization may also reflect the formation of smaller assemblies that are more sharply tuned to specific representations and have more specific effects on target areas, a process that is probably controlled by the medial temporal lobe (Axmacher *et al.*, 2008; Ranganath & Ritchey, 2012). Indeed, it has been found that memory formation at a global cortical scale is related to decreases rather than increases in phase synchronization (Burke *et al.*, 2013).

Furthermore, a non-invasive approach for the modulation of phase synchronization may also be clinically relevant in order to mitigate pathological synchronization. Several brain malfunctions, such as epilepsy, schizophrenia, autism, Alzheimer's disease, and Parkinson's disease, have been connected to abnormal phase synchronization (e.g. Uhlhaas & Singer, 2006). In particular, the development of epileptic seizures is supposed to be closely related to abnormal synchronization mechanisms (e.g. Wong *et al.*, 1986). Recent data suggest that decreases in phase synchronization often occur shortly before or during the early stages of epileptic seizures, whereas high levels of synchronization are observed during later stages (e.g. Mormann *et al.*, 2003; Wendling *et al.*, 2003), although this scenario is still controversial (e.g. Jiruska *et al.*, 2013). In any case, our findings show that intracranial phase synchronization can be modulated by beat stimulation in both directions, even at mediotemporal sites. Whereas monaural 10-Hz stimulation, for instance, resulted in increased mediotemporal phase synchronization, monaural 5- and 40-Hz stimulation had the opposite effect. In this sense, our findings may offer a non-invasive approach for the modulation of intracranial EEG synchronization that could have therapeutic applications.

Conflict of interests

None of the authors has a financial interest or a potential conflict of interest to declare.

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Abbreviations

EEG, electroencephalography.

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