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Evoked γ oscillations in human scalp EEG are test–retest reliable

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Abstract

Objective: Evoked, phase-locked γ oscillations of the electroencephalogram (EEG) have been demonstrated to be modulated by both bottom-up as well as top-down factors. However, to date the test–retest reliability of these oscillations has not been studied systematically.
Methods: We recorded EEG activity of 12 healthy volunteers in response to stimuli of different sizes. Each participant took part in two sessions separated by two weeks in time. To obtain an estimate of the reliability of evoked γ band responses (GBRs), we compared frequency and magnitude of phase-locked EEG oscillations between sessions.
Results: In response to large stimuli magnitude and frequency of the evoked GBR yielded significant reliability. However, this was not the case for stimuli which were too small to evoke detectable GBRs.
Conclusions: The results are in accordance with studies demonstrating a dependence of γ oscillations on stimulus parameters.
Significance: The current findings suggest that using appropriate stimulation, the evoked γ response has sufficient test–retest reliability for use in assessing clinical changes in neurophysiological status.

Keywords: Reliability; Phase-locking; Electroencephalography; Oscillation

1. Introduction

Since the discovery of the electroencephalogram (EEG) by Berger (1929), EEG oscillations in the δ (0–4 Hz), θ (4–7 Hz), α (7–13 Hz) and β (13–28 Hz) ranges are now part of the basic training for physicians. Although discovered early (Adrian, 1942), γ oscillations (28–90 Hz) have only recently been related to pathological brain function (see Herrmann and Demiralp, 2005, for a review). γ Oscillations seem to be related to a variety of neuropsychiatric disorders such as schizophrenia (Lee et al., 2003, 2001; Basar-Eroglu et al., 2006), attention-deficit hyperactivity disorder (ADHD) Yordanova et al. (2001), or autism (Grice et al., 2001; Brown et al., 2005). It seems reasonable to also use oscillatory brain responses in the γ range as a diagnostic tool for neuropsychiatric disorders (Spencer et al., 2003; Ribary et al., 1991). A prerequisite for such clinical application would be that γ band responses (GBRs) can reliably be detected such that variations in the GBR could be related to specific longer lasting aspects of brain function rather than random or transient variations. Along these lines Debener and Engel (2005) have recently commented a review on γ activity in neuropsychiatric disorders (Herrmann and Demiralp, 2005) that the reliability of γ responses needs to be demonstrated before they can be used for clinical diagnosis.

GBRs can be measured from a wide variety of brain structures (Basar et al., 2001). Two types of GBRs are usually distinguished: Evoked GBRs (eGBRs) are phase-locked to the onset of a stimulus and do not necessarily correspond to amplitude modulations in the single trials, whereas induced GBRs (iGBRs) usually occur in a later time window and are not time locked to the onset of a stimulus (Basar-Eroglu

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et al., 1996). Induced GBRs are usually detected as amplitude modulations in single trials, and are now commonly related to a wide variety of cognitive processes (Engel et al., 2001; Tallon-Baudry and Bertrand, 1999; Keil et al., 2001) as well as certain types of learning and memory (Gruber and Müller, 2005, 2006). Most researchers agree in that the earlier eGBRs depend on stimulus factors such as size and eccentricity (Busch et al., 2004). However, they also seem to be modulated by basic cognitive processes such as memory matching (Herrmann et al., 2004b) or attention (Busch et al., 2006; Tiitinen et al., 1993; Debener et al., 2003; Fell et al., 2003). Whereas the reliability of iGBRs has been shown before (Keil et al., 2003; Hoogenboom et al., 2006), such demonstrations lack for the eGBR.

In the current study, we investigated the test–retest reliability of eGBRs in response to sinusoidal gratings. Since a recent study revealed that size matters for evoking γ activity (Busch et al., 2004), we used stimuli of different size for our test.

2. Methods

2.1. Participants

Twelve healthy volunteers aged between 20 and 44 years (mean age 27 ± 6.721, 5 males, 7 females) participated in the current study. We decided that 12 participants would be enough to get an idea of the reliability of eGBRs. However, due to the small sample size, the results remain preliminary. All participants had normal or corrected to normal vision and were free of current or past neurological or psychiatric disorders. Before the first recording session started, participants gave their informed consent. The experimental procedure was in accordance with the guidelines of the local ethics committee of the university of Magdeburg and the declaration of Helsinki.

2.2. Stimuli and experimental procedure

All participants took part in two recording sessions, temporally separated by two weeks. During both recording sessions the participants had to perform the same task.

The task of the participants was to detect the orientation of monochromatic gratings with a spatial frequency of 2 cycles per degree visual arc (cpd) and a Michelson contrast of 50%. These gratings were either rotated 45° clockwise from a vertical orientation or counterclockwise. Examples of the stimuli can be found in Fig. 1. Participants indicated the detected orientation by pressing a button with one hand or another button with the other hand. The gratings subtended either 10° visual arc (large stimuli) or 1.3° (small stimuli). This results in 4 different stimuli, each of which was presented 100 times. Thus, in total the participants perceived 400 grating patterns during the experiment.

Response hands were counterbalanced across participants but remained constant during the two sessions. The stimuli were presented on a TFT monitor (width = 34.5 cm, height = 25.9 cm) placed at a distance of 110 cm in front of the participants. Monitor refresh rate was 75 Hz. Participants were instructed to fixate a small white cross in the center of the screen during the whole experiment. Furthermore, electrooculographic (EOG) activity (see Section 2.3) was recorded in order to discard trials that were contaminated with eye movements. Stimuli were presented for 1000 ms with inter stimulus intervals varying randomly between 1000 and 2000 ms.

2.3. Data acquisition

During data recording, participants sat in an electrically shielded and sound attenuated room (IAC, Niederkrüchten, Germany). The stimulation monitor was placed outside the cabin behind an electrically shielded window. All devices inside the cabin were battery operated to avoid line frequency interference (50 Hz in Germany). EEG activity was measured from 31 scalp locations according to an extended 10–20 system. The nose served as reference. Electrooculographic activity was measured from an electrode placed below the orbital rim in order to detect artifacts due to eye movements. Activity was recorded using sintered Ag/AgCl electrodes mounted in an elastic cap (Easycap, Falk Minow Services, Munich, Germany) and amplified by means of a BrainAmp amplifier (Brain Products, Munich, Germany). Electrode impedances were kept

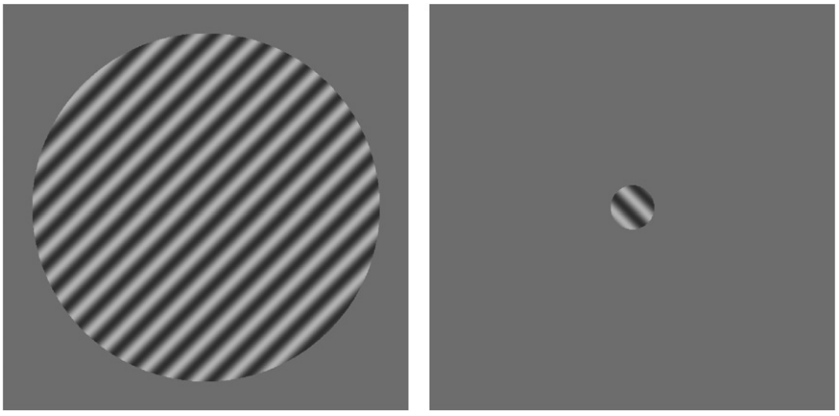

Fig. 1. Examples of the stimuli used in the experiment. Left: big stimulus, right: small stimulus.

below 5 kΩ. The EEG signals were analog filtered between 0.02 and 200 Hz, digitized at a rate of 500 Hz and stored on a computer hard disc for offline analysis. Digitized EEG data were transferred to a computer outside the recording cabin with a fiber optic cable. The data were digitally high-pass filtered offline with a cutoff frequency of 0.5 Hz in order to avoid slow shifts in the baseline. An automatic artifact rejection was computed which excluded trials from further analysis if the standard deviation within a moving 200 ms time window exceeded 40 μV in one channel. The automatic artifact rejection was supplemented by visual inspection to ensure that only trials without artifacts were included in the subsequent analysis. After artifact rejection on average 165 trials per session per subject went into the analysis.

2.4. Data analysis

In order to obtain a time–frequency representation of the EEG signals, a wavelet transform (WT, Herrmann et al., 2004a) was performed. The WT was computed by convolving the signal with a set of scaled and translated versions of a complex modulated gaussian. At 40 Hz the wavelet had a time resolution of $2\sigma_t = 50$ ms and a frequency resolution of $2\sigma_f = 13$ Hz. The exact time–frequency resolution of the wavelet depended on the analyzed frequency. The wavelets were normalized to have unit energy. From the wavelet transformed data three measures were derived: (i) the amount of evoked activity, (ii) the total γ activity irrespective of the phase, which roughly corresponds to induced activity and (iii) the strength of phase-locking. This phase-locking factor is bounded between 0 and 1, where 1 indicates perfect phase alignment across trials and 0 indicates a constellation in which the phases exactly cancel out each other, as it is the case for a uniform distribution of phases across trials. This resulted in a representation of the responses of every participant in the plane spanned by time and frequency, where the frequency of the participant's response could be analyzed as well as the magnitude of the response at that frequency. From the time–frequency planes for evoked and total activity, the average from a baseline 200–100 ms before stimulus onset was subtracted.

In a previous study, we reported strong eGBRs over posterior electrodes O1, O2, P3, Pz and P4 (Busch et al., 2006). This was confirmed by the current results. We therefore decided to average the time–frequency planes from these electrodes to quantify eGBRs. Response frequencies were defined for each condition in the time–frequency plane averaged across electrodes O1, O2, P3, Pz, and P4[1] as those frequencies between 28 and 90 Hz that showed the strongest increase in the time range between 50 and 130 ms after stimulation onset with respect to a baseline level (100–300 ms before stimulus onset). The time course of the γ band response was computed as the average of the time courses at these individually defined response frequencies. Thus, we acquired one time course for evoked, one for total activity and one for phase-locking. If a participant did not show a dominant response peak but rather multiple small peaks that could not be distinguished from noise, this response was excluded from the reliability analysis of response frequencies. Examples for this procedure are illustrated in Fig. 2. From the time courses we obtained the peak magnitude in the time window between 50 and 130 ms after stimulation onset. If for a particular condition no response frequency could be ascertained, the peak magnitude was extracted at the response frequency of the other condition. If a response frequency could not be ascertained for the other condition either, peak magnitude was extract-

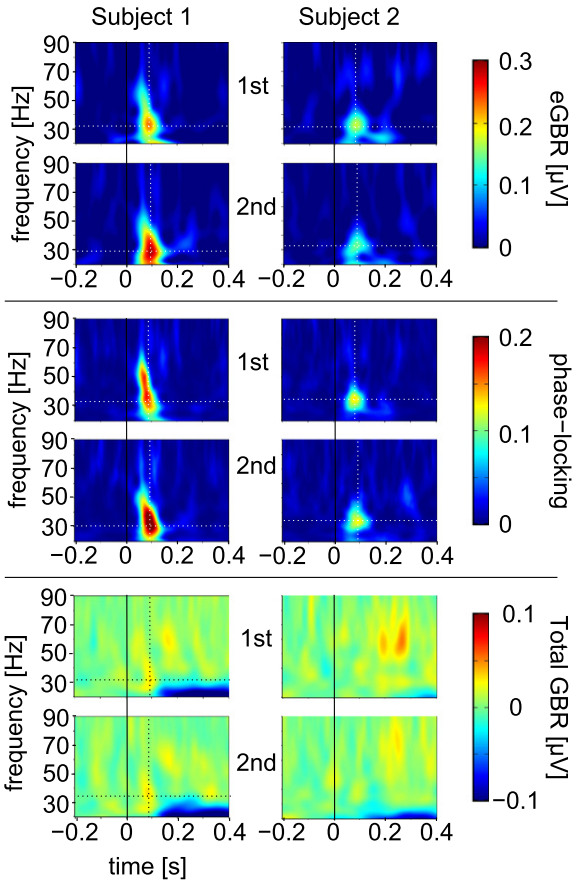

Fig. 2. Time–frequency representations of evoked activity (top), phase-locking (middle) and total activity (bottom) in response to the big grating stimulus from two single representative participants. In every display two time–frequency plots are shown. The first corresponds to the first session, the second to the second session. To obtain these time–frequency representations, the time–frequency representations from electrodes O1, O2, P3, Pz, P4 have been averaged. Dotted lines indicate the time–frequency position at which the values were extracted for further computation. Plots of eGBR and phase-locking show a high reliability across sessions, while this was less clear for total GBR. Note that for subject 2 no response frequency for total activity was ascertained in the early time window. Subject 2 was the only subject that displayed a late total GBR. This was, however, not test–retest reliable.

[1] Reliabilities of eGBR derived from single channels were generally smaller (by a value of approximately 0.2 for big stimuli) and less significant ($0.05 > p > 0.03$).

ed at 40 Hz. This procedure yields different analysis frequencies for eGBR, phase-locking and total GBR. However, the differences in analysis frequencies were well below the bandwidth of the employed wavelets.

Thus, for every session we analyzed 6 response frequencies and response magnitudes (two conditions: small, big stimulus; three characteristic values: evoked activity, total activity, phase-locking). In order to obtain a measure for test–retest reliability, the correlation for all these values was computed separately between the first and the second session (Keil et al., 2003). The correlations were tested by means of a t-test with $n-2$ degrees of freedom, where n denotes the number of participants that were included in the analysis. The statistics were performed using the function `cor.test()` from the statistical analysis software "R" (R Development Core Team, 2004).

To get an estimate of the reliability within a single session, we randomly split the trials from the first session into two subsets. These two subsets were compared in the same way as the data from the two sessions.

3. Results

For big stimuli a marked eGBR could be observed at posterior electrodes. This was not the case for small stimuli. Fig. 2 shows time–frequency representations of the evoked γ response from two participants. Fig. 3 displays topographies of the responses to big and small stimuli.

3.1. Behavioral data

Mean reaction times were 477 ± 65 ms. For both stimuli the mean reaction times were highly correlated between sessions (big stimuli: $r = .91$, $t(10) = 6.95$, $p < 0.0001$, small stimuli: $r = .89$, $t(10) = 6.02$, $p < 0.001$).

3.2. Evoked GBR

Response frequencies of evoked activity significantly correlated for the big stimulus ($r = .85$, $t(10) = 5.1$, $p < 0.001$). For the small stimulus, the number of responses for that a response frequency could be distinguished (5) was too small to calculate a meaningful correlation. Response magnitudes of evoked activity were significantly correlated only for the large stimulus ($r = .82$, $t(10) = 4.55$, $p < 0.01$), but not for the small stimulus ($r = .30$, $t(10) = 1.1$). Scatter plots of the response frequencies and magnitudes of the eGBR can be found in Fig. 4. A comparison between first and second session for big and small stimuli from two representative subjects is presented at the top of Fig. 2 (Table 1).

3.3. Phase-locking

Phase-locking revealed an even larger correlation across sessions, than did the evoked activity. Response frequencies as well as magnitudes for the big stimuli were highly correlated (response frequencies: $r = .95$, $t(8) = 8.95$, $p < 0.0001$, response magnitudes: $r = .88$, $t(10) = 5.98$, $p < 0.001$). For small stimuli response frequencies could only be distinguished in five participants which precluded the calculation of a meaningful correlation. Magnitudes of phase-locking in response to small stimuli were not significantly correlated ($r = .21$, $t(9) = 0.64$). Scatter plots of frequencies and magnitudes of phase-locking are presented in the right half of Fig. 4. Time–frequency representations of the responses from two representative subjects are compiled in the middle panel of Fig. 2.

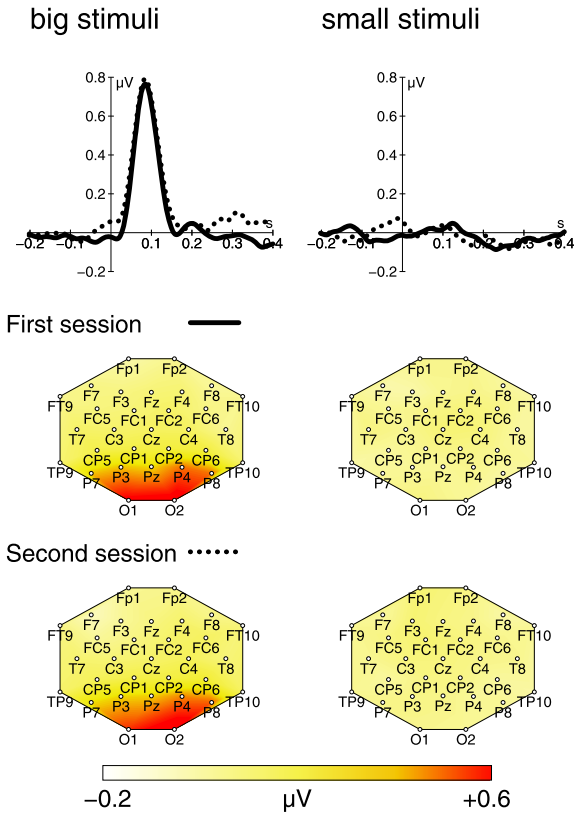

Fig. 3. Evoked GBR in response to big stimuli (left column) and small stimuli (right column). Top: Time courses at electrode O2, solid lines represent responses from the first session, dotted lines represent responses from the second session, middle: topographies from first session, bottom: topographies from second session. Data were averaged across all participants. Topographies correspond to the time window 60–115 ms after stimulus onset. Both time courses and topographies in response to big stimuli were highly reliable across sessions.

3.4. Total γ activity

Response frequencies of total γ activity could only be ascertained for three participants. Time–frequency planes from other participants did not show a clear peak at any frequency. This precluded the calculation of a meaningful correlation. The magnitudes of total γ activity were not significantly correlated ($r < .5$, $t(9) < 1.7$). Total γ activity was not significantly correlated in later time windows either. Time–frequency representations of the responses from

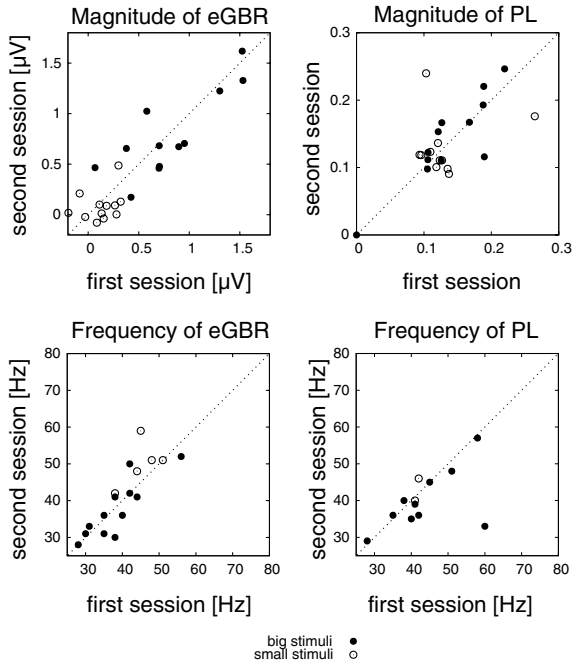

Fig. 4. Scatter plots of evoked responses. Top: response magnitudes, bottom: response frequencies. Left: Magnitude and frequency of evoked γ band response, right: magnitude and frequency of phase-locking. Solid circles represent responses to big stimuli, open circles represent responses to small stimuli. In all plots high reliability across sessions can be observed for big but not for small stimuli.

two representative subjects are displayed at the bottom of Fig. 2.

3.5. Event-related potentials

Event-related potentials (ERPs) are plotted in Fig. 5. Although we did not analyze ERP components statistically, two aspects can be noted about the ERP. First, the early responses to big as well as small stimuli are very similar across sessions. Second, in line with previous findings (Debener et al., 2002) the later responses to big stimuli are slightly more similar between sessions, than are those to small stimuli.

3.6. Split-half reliabilities

The results of the split-half analysis were comparable to those from the test–retest analysis. For big stimuli both, frequency as well as magnitude of the eGBR were significantly correlated (big stimuli: frequency: $r = .89$, $t(8) = 5.54$, $p < 0.001$, magnitude: $r = .80$, $t(10) = 4.29$, $p < 0.01$, small stimuli: frequency could only be ascertained in five participants, magnitude: $r = -.51$, $t(10) = -1.87$), whereas this was not the case for small stimuli (frequency could only be ascertained in five participants, magnitude: $r = -.51$, $t(10) = -1.87$). High correlations were obtained also for phase-locking (big stimuli: frequency: $r = .78$, $t(6) = 3.07$, $p < 0.05$, magnitude: $r = .80$, $t(10) = 4.18$, $p < 0.01$, small stimuli: frequency could only be ascertained in four participants, magnitude: $r = -.04$, $t(10) = 0.12$), but not total γ activity (big stimuli: frequency could only be ascertained in five participants, magnitude: $r = .45$, $t(10) = 1.59$, small stimuli: frequency could only be ascertained in five participants, magnitude: $r = -.73$, $t(10) = -3.43$).

3.7. Reliabilities of broad band γ activity

We performed the same analysis as above with the amplitudes averaged across the whole time–frequency range of interest (50–130 ms, 28–90 Hz). Correlations were significant under the same conditions as with peak frequency analysis. However, these correlations were much lower (on average .17 less than for peak frequencies).

4. Discussion

In the current study we investigated the reliability of early, phase-locked γ activity in response to different stimulus sizes. We observed that for large stimuli a reliable measurement of longer lasting properties of evoked, phase-locked γ activity is possible. Total γ activity was not significantly correlated between the two sessions.

It has been demonstrated previously, that evoked γ oscillations are highly dependent upon parameters of the

Table 1
Correlations between first and second session, evoked indicates evoked activity, PL indicates phase-locking and total indicates total activity

	Big stimuli			Small stimuli		
	Correlation r	t value	p value	Correlation r	t value	p value
Response frequencies						
Evoked	0.85	5.1	0.001	Not enough observations		
PL	0.95	8.95	0.0001	Not enough observations		
Total	Not enough observations			Not enough observations		
Response magnitudes						
Evoked	0.82	4.55	0.01	0.30	1.1	n.s.
PL	0.88	5.98	0.001	0.21	0.64	n.s.
Total	0.31	0.99	n.s.	0.49	1.67	n.s.

Note that frequency was assumed to be 40 Hz for the magnitude analysis if no clear peak was visible. However, frequency could not be analyzed in these cases.

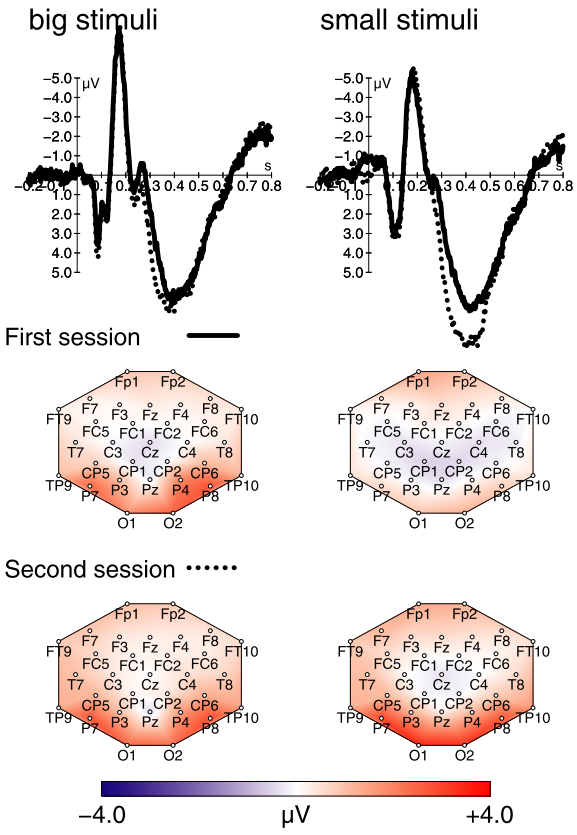

Fig. 5. Event-related potentials after stimulation with big stimuli (left column) and after stimulation with small stimuli (right column). The top row shows time courses of the event-related potential, the second row shows topographic maps of activity between 80 and 120 ms in the first session and the third row shows topographic maps of the same time window in the second session.

stimulation procedure like size or eccentricity (Busch et al., 2004). For small stimuli subtending only 1° visual arc, the eGBR hardly exceeds the noise level. We found the same in our current data and could even extend these findings by showing that for small stimuli stable detection of γ responses across two recording sessions is not possible either.

From the fact that phase-locking in our data was generally more reliable than total γ activity, we infer that the reliable part of the eGBR was due to phase-locking rather than modulation of amplitudes in single trials. This is in line with findings indicating that phase-locking and power modulations of early γ responses are independent parameters of cortical information processing (Yordanova et al., 1997; Fell et al., 2005). Total γ activity, which correspond to induced activity, was not significantly correlated between sessions. This might explain, why Keil et al. (2003) found only weak reliabilities for power increases of early γ activity.

Phase-locked γ activity has been regarded as being mainly related to sensory processing (Karakaş and Başar, 1998), which is modulated by top-down processes like memory (Herrmann et al., 2004b) or attention (Busch et al., 2006; Tiitinen et al., 1993; Debener et al., 2003; Fell et al., 2003). Phase dynamics of high frequency brain oscillations have gained interest in recent years (Makarenko and Llinás, 1998; Kazantsev et al., 2004; Freeman and Rogers, 2002). The phase of ongoing oscillations in the γ–β range is spontaneously reset at a frequency of approximately 5 Hz (Freeman and Rogers, 2002). These authors observed that phase-resets occur simultaneously at different spatial scales of cortical processing and can be very rapid. Such phase-resets have been studied in much detail in the inferior olive. The subthreshold oscillations of neurons in the inferior olive can be reset rapidly to a value that is determined by properties of the stimulus (Kazantsev et al., 2004). Makarenko and Llinás, 1998 suggested that such mechanisms might be found in different brain structures in which subthreshold oscillations can be detected. Thus, it might be possible, that resetting the phase of ongoing high frequency oscillations provides a mechanism for rapid classification of visual stimuli, (Körner et al., 1999). These authors further argue, that for the very same oscillation phase-locking to the stimulus might become weaker with time, while the cells are synchronized to each other by a common subthreshold oscillation. From this point of view it seems reasonable to expect phase-locking to be reliable in an early time window, whereas amplitudes (i.e. local synchronization between many neurons) become more reliable in later time windows as has been shown before (Hoogenboom et al., 2006; Keil et al., 2003).

In a line of experiments, we studied the conditions for the detection of eGBRs. We especially reported that measurement of eGBRs is only possible, if the stimulation is appropriate (Busch et al., 2004, 2006). The current results extend these findings by demonstrating that γ oscillations can also be reliably detected if the stimulation is appropriate. Thus, a clinical application of eGBRs as a diagnostic tool seems realistic. Especially schizophrenia has been associated with abnormalities of γ oscillations (Lee et al., 2003). For schizophrenic patients deficits in early visual processing have been demonstrated (Brand et al., 2005), that could also account for cognitive impairments in higher order processes like social cognition (Sergi and Green, 2002). It has been argued that these deficits in early visual processing could be explained by dysfunction of the magnocellular pathway in schizophrenic patients (Schechter et al., 2005). It has been suggested that GBRs can be associated with activity in the magnocellular pathway (Sewards and Sewards, 1999). Indeed it was reported that phase-locking of oscillatory γ activity is abnormal in schizophrenic patients and that these abnormalities are correlated with symptoms of schizophrenia (Spencer et al., 2003, 2004). The current findings might thus be speculated to open new paths for a diagnosis of magnocellular dysfunction in schizophrenia. However, to date no specific links between eGBRs and magnocellular function have been established.

We conclude that using appropriate stimulation it is possible to obtain reliable measurements of evoked, phase-locked γ oscillations in EEG measurements.

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