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Invisible visual stimuli elicit increases in alpha-band power

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Bareither I, Chaumon M, Bernasconi F, Villringer A, Busch NA. Invisible visual stimuli elicit increases in alpha-band power. *J Neurophysiol* 112: 1082–1090, 2014. First published May 28, 2014; doi:10.1152/jn.00550.2013.—The cerebral cortex responds to stimuli of a wide range of intensities. Previous studies have demonstrated that undetectably weak somatosensory stimuli cause a functional deactivation or inhibition in somatosensory cortex. In the present study, we tested whether invisible visual stimuli lead to similar responses, indicated by an increase in EEG alpha-band power—an index of cortical excitability. We presented subliminal and supraliminal visual stimuli after estimating each participant's detection threshold. Stimuli consisted of peripherally presented small circular patches that differed in their contrast to a background consisting of a random white noise pattern. We demonstrate that subliminal and supraliminal stimuli each elicit specific neuronal response patterns. Supraliminal stimuli evoked an early, strongly phase-locked lower-frequency response representing the evoked potential and induced a decrease in alpha-band power from 400 ms on. By contrast, subliminal visual stimuli induced an increase of non-phase-locked power around 300 ms that was maximal within the alpha-band. This response might be due to an inhibitory mechanism, which reduces spurious visual activation that is unlikely to result from external stimuli.

alpha-band; EEG; event-related synchronization; oscillations; subthreshold stimulation

VISUAL EVENTS come at an enormous range of intensities—from a faint glow in the dark to the blinding light of the midday sun. How does the visual system respond to stimuli of such different intensities? A number of experiments have reported weaker, but similar, brain responses for visible compared with invisible stimuli when stimuli were made invisible by masking (Harris et al. 2011; Haynes and Rees 2005). Notably, these stimuli were of strong intensity, but their processing was interrupted and thus made invisible by an intervening stimulus (the mask). It is currently unknown whether the visual system responds in a similar fashion to stimuli that are invisible because of their low contrast.

For somatosensory stimuli, Blankenburg et al. (2003) demonstrated a specific response pattern for low-intensity stimuli: subthreshold stimulation caused blood oxygenation level-dependent (BOLD) signal decreases in somatosensory cortical areas relative to a baseline condition, while suprathreshold stimulation usually results in BOLD signal increases. This deactivation in response to subthreshold stimuli was interpreted as a focal inhibition mechanism that protects the cortex against spurious activation by noise. Here we investigated whether specific responses to subliminal stimuli of subthreshold intensity are also observable within the visual system.

By using EEG, an equivalent inhibitory response can be found within the alpha-band. Elevated ongoing alpha-band power at stimulus onset inhibits processing of visual stimuli (Busch et al. 2009; Ergenoglu et al. 2004), and selective attention increases alpha-band power in order to inhibit task-irrelevant stimuli (Busch and VanRullen 2010; Foxe et al. 1998; Klimesch et al. 2007). In contrast, processing of suprathreshold or task-relevant stimuli is characterized by an event-related alpha-band power decrease (also called desynchronization) (Adrian and Matthews 1934). Furthermore, simultaneous recordings of fMRI and EEG have demonstrated an inverse correlation of the BOLD signal with alpha-band power (8–12 Hz); while fMRI activation is associated with reduced alpha-band power in cortical areas, fMRI deactivation is associated with elevated alpha-band power (Goldman et al. 2002; Moosmann et al. 2003).

In the present study, we investigated electrophysiological responses to subliminal and supraliminal visual stimuli, as well as ongoing activity in blank trials as a control. We demonstrate that low- and high-intensity stimuli elicit specific effects within the alpha-band: Supraliminal stimuli resulted in a strongly phase-locked lower-frequency response representing the evoked potential and induced a prominent decrease of alpha-band power from 400 ms on. In contrast, subliminal visual stimuli caused a non-phase-locked increase maximal within alpha-band power around 300 ms, possibly reflecting an inhibitory mechanism for reducing spurious visual activation. As suggested previously by Blankenburg et al. (2003), the functional role of such an inhibitory mechanism in response to weak stimuli could be a protection against activation that is unlikely to be caused by real events in the world.

MATERIALS AND METHODS

The purpose of this study was to investigate how the visual system responds to subliminal stimuli and characterize how the response differs from that to supraliminal stimuli. In *phase I* of the experiment, we estimated each participant's visual sensitivity threshold. In *phase II*, we recorded EEG responses to subliminal and supraliminal stimuli. After the EEG recording, in *phase III* of the experiment, we repeated the threshold estimation in order to verify that visual thresholds had not changed during EEG acquisition.

Participants. EEG signals were recorded from 21 participants. Three had to be discarded from further analysis because of substantial noise in the data and/or eye movements; 18 participants remained (age 18–32 yr, mean age 25 yr; 8 women, 10 men; 17 right-handed). None of the participants reported a history of neurological or psychiatric

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disorders, and all had normal or corrected-to-normal vision. Informed consent was obtained from each subject after explanation of the study. The experiment was approved by the Ethics Committee of the Charité-Universitätsmedizin Berlin.

Stimuli and apparatus. The experiment was written in MATLAB (MathWorks) with the Psychophysics Toolbox (Brainard 1997). Participants were seated in a dark, electrically shielded, sound-attenuated chamber. Stimuli were presented on a calibrated 19-in. CRT monitor with 1,280 × 1,024 resolution and refresh rate of 100 Hz, located 56 cm from the participants' eyes. Head position was stabilized with a chin rest. For all phases of the experiment, the background consisted of a random white noise pattern with a mean RGB value of [38,38,38], standard deviation 11. Mean luminance of the background pattern was 10.76 cd/m^2. A central fixation cross and peripheral markers above and below potential stimulus locations were continuously present on the screen. Stimuli were circular patches, 0.38° in diameter, presented for 30 ms on the horizontal meridian at an eccentricity of 18.3° to the right or the left of the fixation cross. Participants were instructed to always maintain central fixation.

Procedure phase I—threshold estimation. In *phase I*, we estimated each participant's sensitivity threshold. Trials started with the presentation of a fixation cross and peripheral markers, as described above. After a variable delay (range: 1–1.5 s), stimuli were presented in 87.5% of the trials. The rest were stimulus-absent "catch" trials to estimate the false alarm rate. Stimuli were presented at seven different intensities (12.28, 12.66, 12.89, 13.3, 13.76, 14.17, 14.88 cd/m^2), 20 repetitions each. After a delay of 1 s, the fixation cross turned into a question mark. Participants were asked to indicate whether they saw a stimulus via button press.

To determine sensitivity thresholds, we calculated A-prime (A′) at each of the different stimulus intensities using the participant's hit rate for each intensity and the global false alarm rate. In contrast to d′, A′ is a nonparametric measure of sensitivity. In an experiment with infinite number of trials, an A′ of 0.5 for a given intensity indicates that an observer cannot distinguish a stimulus of that intensity from noise whereas an A′ of 1 indicates perfect performance (Stanislaw and Todorov 1999). However, with a finite number of trials, it is possible that an observer with zero sensitivity achieves an A′ above 0.5 because of lucky guessing. To account for guessing, we simulated the performance of an observer with zero sensitivity in a detection task with the same number of signal trials and catch trials as in the present experiment. This simulation was repeated 100,000 times, yielding a distribution of expected A′ values for an observer with zero sensitivity in this experiment. This allowed us to compute for each of the different stimulus intensities the probability that a participant's empirical A′ was achieved despite zero sensitivity. A participant's threshold was defined as the minimal stimulus intensity required for an A′ for which this probability was <0.01.

Phase II—target detection task. In *phase II*, we recorded electrophysiological responses to subliminal and supraliminal stimuli. The contrast of subliminal stimuli was set at 25% of the participant's sensitivity threshold; the contrast of supraliminal stimuli was set at five times the threshold.

Subliminal and supraliminal stimuli were each presented for 30 ms on one-third of all trials. The remaining one-third were "blank" trials on which no stimulus was presented. As described above, a central fixation cross and peripheral markers were continuously present on the screen. The intertrial interval ranged from 2,000 to 2,500 ms (see Fig. 1).

In contrast to *phase I*, participants were not required to detect or report the peripheral stimuli. Instead, we introduced an additional target detection task in order to maintain participants' vigilance. To that end, we presented 100 circles (12.5 cd/m^2; diameter 1.6°) at fixation, randomly interspersed between presentations of the peripheral stimuli. Participants were asked to count these targets and report the number during the breaks. We deliberately chose not to have

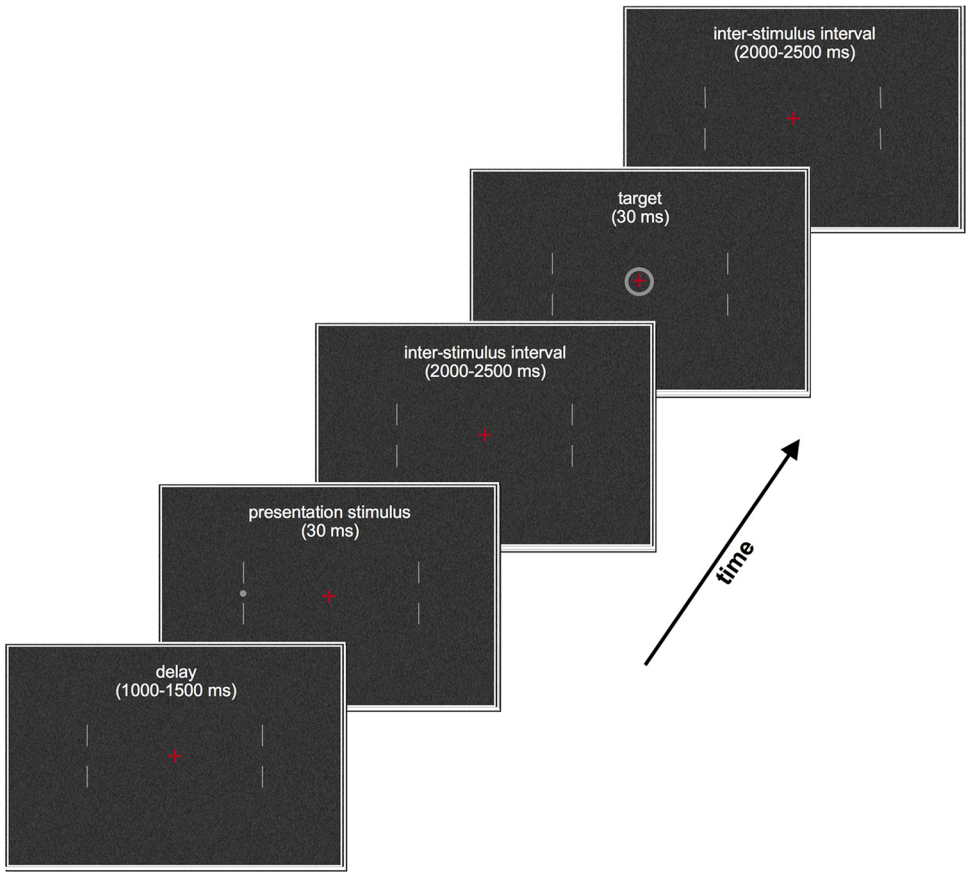

Fig. 1. Illustration of the paradigm during *phase II* (EEG recording). Trials started with the presentation of a fixation cross and peripheral markers. After a variable delay, stimuli were presented in 1/3 of the trials. The remaining trials were stimulus-absent blank trials. Participants were asked to count targets that were randomly interspersed at fixation between peripheral presentations.

participants detect the presence of the peripheral stimuli for two reasons. First, stimuli were presented in a continuous stream of 1,300 stimuli, interrupted only by eight breaks. Thus it would have been impossible for participants to know when they were supposed to report the absence of a stimulus (be it a subliminal stimulus or a blank trial). Second, the comparison of neural responses to subliminal and supraliminal stimuli would have been complicated by additional signals related to response selection, execution, and confidence, such as the P300 event-related potential (ERP) component. Participants accomplished the task of counting 100 circles in total with high accuracy (mean 99.17, SD 5.9; min = 90, max = 112).

Phase III—postthreshold test. After EEG acquisition, we tested whether participants' visual sensitivity had changed during the experiment. In particular, we had to ascertain that stimuli labeled as "subliminal" were still below the participant's threshold. The procedure was identical to the threshold estimation of *phase I*.

EEG acquisition. During *phase II* of the experiment, EEG was acquired with a 64-channel ActiveTwo system (Biosemi). Electrodes were placed according to the International 10-20 electrode placement system. The vertical and horizontal electrooculogram (EOG) was recorded by additional electrodes below the right eye and at the outer canthi of both eyes. Signals were digitized at 1,024 Hz with 24-bit conversion resolution and filtered between 0.16 Hz and 100 Hz.

EEG analysis. Signals were filtered off-line between 0.5 and 40 Hz, downsampled to 512 Hz, referenced to the average signal, and epoched from −1,000 ms before to 1,500 ms after stimulus onset. An automatic artifact rejection excluded epochs in which the signal exceeded ±120 μV, and the remaining data were screened manually for residual artifacts.

ERPs and global field power (GFP) were used as a measure for the broadband EEG response. GFP is a global measure that does not depend on an arbitrary selection of electrodes and reflects the spatial standard deviation of the ERP across all electrodes (Skrandies 2005). We tested whether the GFP for subliminal or supraliminal stimuli was different from the GFP on blank trials. First, paired-sample *t*-tests were calculated for each sampling point, comparing the difference between the blank and supraliminal conditions as well as the blank and subliminal conditions. The distribution of *P* values was corrected for multiple comparisons according to the false discovery rate (FDR) procedure (Benjamini and Hochberg 1995). We computed a q threshold that set the expected rate of false discoveries to 7%.

The focus of our analysis was on effects of supraliminal and subliminal stimulation (as compared to blank trials) on oscillatory neural responses (power and phase locking). We used a continuous wavelet transform of single-trial data for the frequency range 2–40 Hz. The length of the wavelets increased linearly from two cycles at 2 Hz to four cycles at 40 Hz. At each time *t* and frequency *f*, the result of the wavelet transform for trial *k* is a complex number in which *A* represents the amplitude of the signal and ϕ its phase:

$$A_{k(t,f)}e^{i\varphi k(t,f)}$$

To test effects of supraliminal and subliminal stimuli on oscillatory power, we used the nonparametric randomization method introduced by Maris and Oostenveld (2007), which controls for multiple comparisons by identifying clusters of significant condition differences over time and frequency. To increase the sensitivity of this test, we performed this analysis for power averaged across those electrodes that showed strong power (8–12 Hz; 0–700 ms) in both conditions tested (i.e., supraliminal vs. blank trials and subliminal vs. blank trials). We first identified contiguous clusters of time points and frequencies at which the *P* value of a paired-sample *t*-test comparing subliminal vs. blank and supraliminal vs. blank was <0.05. The cluster-level statistic was defined as the sum of the *t*-statistics of all time points and frequencies within a cluster. These cluster-level statistics were then compared against the permutation distribution, which was approximated by drawing 1,000 random permutations of the observed data (i.e., randomization between conditions). Thus the *P* values obtained in the permutation procedure represent the probability under the null hypothesis of observing a cluster-level statistic that is as strong as or stronger than the observed cluster-level statistics. Significant clusters are highlighted in Fig. 4*A*.

Phase locking was quantified by calculating the phase-locking factor PLF (Tallon-Baudry et al. 1996) as

$$\mathrm{PLF} = \left| \frac{1}{n} \sum_{k=1}^{n} e^{i\varphi k} \right|,$$

where ϕ is the phase angle and *n* is the number of trials. The phase-locking factors in each condition were tested with a nonparametric method, similar to the test for effects on power described above. However, since phase locking describes the distribution of phase angles across single trials, the procedure differed slightly from the cluster analysis of oscillatory power described by Maris and Oostenveld (2007). Phase-locked responses are characterized by an alignment of the phases of the complex wavelet transform. We thus tested at each channel whether there was a consistent influence of stimulus presentation on the distribution of phases across single trials in each of the three conditions. Our null hypothesis was that the phase of the oscillatory activity was random across trials. To construct a random partition of this null hypothesis, we used the following procedure in each condition separately. For each participant, we computed a series of 1,000 surrogate PLF values (Tallon-Baudry et al. 1996) by adding random phase shifts (uniformly distributed between 0 and 2 π radians) to the phases of each single trial before computing the PLF. This phase shift was identical for all time points and frequencies within one trial, but random across trials, so as to preserve the phase autocorrelation across time and frequencies of the actual data. We then identified contiguous clusters of time points and frequencies at which phase locking (averaged across participants) exceeded a threshold corresponding to the 95th percentile of the surrogate data. The cluster-level test statistics were defined as the sum of PLF values within a cluster. These cluster-level statistics were then compared to the distribution of the sums of PLF values found in clusters obtained under the null hypothesis. Only the clusters whose summed PLF values exceeded that of 95% of the clusters found under the null hypothesis were considered significant and are reported here (see Fig. 4*B*).

RESULTS

Behavioral results. In *phase I* of the experiment, we estimated individual perception thresholds by calculating A′ as a sensitivity index. By comparing empirical sensitivity to that of a simulated observer with zero sensitivity, we found that the minimal stimulus intensity required for above-chance performance (threshold) was 13.75 cd/m^2 (min = 12.7, max = 14.2 cd/m^2), corresponding to a Weber contrast of 0.28. Thus the contrast of subliminal stimuli was set to 1.4 and that for supraliminal stimuli was set to 0.07.

After the EEG experiment, we presented participants again with the subliminal and supraliminal stimuli as well as blank trials to test whether perception thresholds changed during the experiment. Participants were still unable to discriminate subliminal stimuli from blank trials (mean A′ = 0.5, SD = 0.0032), while performing almost perfectly for supraliminal stimuli (mean = 0.98, SD = 0.0104). Compared with a simulated observer with zero sensitivity, participants' performance was significantly better for supraliminal stimuli ($P < 0.001$) but not for subliminal stimuli ($P = 0.97$).

EEG results. In *phase II* of the experiment, we recorded EEG responses to subliminal and supraliminal visual stimuli. The earliest detectable component of the ERP was a negative-

going deflection with a peak latency of ~200 ms with a posterior topographical distribution (see Fig. 2). The latency and topography of this deflection resembled the N2 component. We tested for the presence of the N2 by comparing the average ERP amplitude for supraliminal and subliminal stimuli at posterior channels (see Fig. 2) in the time window from 190 to 230 ms to the signal on blank trials. A significant N2 was found only for supraliminal stimuli [$t(17) = -2.80$; $P = 0.012$; 2-tailed t-test] but not for subliminal stimuli [$t(17) = -0.337$; $P = 0.710$].

Furthermore, we observed a positive deflection at central channels starting at ~300 ms, resembling the P3 component. We tested for the presence of the P3 by comparing the ERP for supraliminal and subliminal stimuli to the signal on blank trials at central channels (see Fig. 2) in the time range from 300 to 500 ms. Again, a significant P3 was found only for supraliminal stimuli [$t(17) = 4.76$; $P < 0.001$] but not for subliminal stimuli [$t(17) = -0.96$; $P = 0.347$].

We also compared subliminal and blank trials in the same time range, in which a significant increase in alpha-band power occurred for subliminal stimuli (200–400 ms, see below). No significant differences were found at posterior [$t(17) = -1.685$; $P = 0.15$] and central [$t(17) = -1.5$; $P = 0.12$] channels. In addition, we analyzed the GFP. Supraliminal stimuli evoked a GFP component peaking at 550 ms. No response was observed for subliminal stimuli (Fig. 3*A*). To confirm this impression statistically, we computed samplewise t-tests comparing the subliminal condition to blank trials, with statistical significance set at $P < 0.05$ (Fig. 3*B*). We found 23 (of a total of 616) significant sampling points that were distributed across the whole epoch (−400 to 800 ms). After correction for multiple comparisons, we found no significant differences between the subliminal and blank conditions at any time. For comparison, the same analysis was performed for the difference between the supraliminal and blank conditions. Here, of 616 sampling points, 325 were significant after correction for multiple comparisons with the same method. This result seems to indicate that stimuli too weak to elicit a conscious percept also do not evoke any neuronal response. However, our samplewise analysis of GFP might not have been powerful enough for detecting a weak response. Therefore, we also tested mean GFP amplitudes averaged in the time range from 200 to 800 ms—the time range with the strongest response to supraliminal stimuli. The difference between supraliminal stimuli and blank trials in this time window was again highly significant [$t(17) = 7.67$; $P < 0.001$]. However, still no

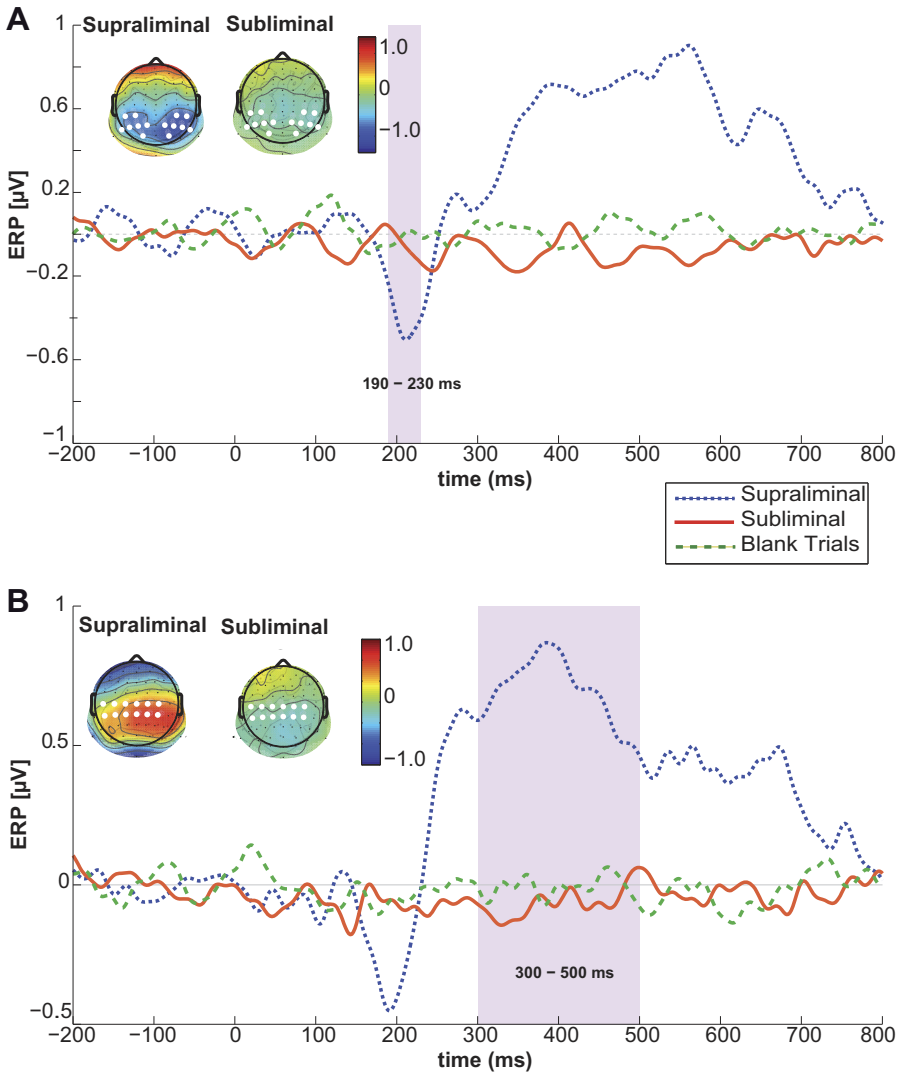

Fig. 2. Event-related potential (ERP) analysis. *A*: comparison of the average ERP amplitude for supraliminal and subliminal stimuli at posterior channels (white dots) in the marked time window (190–230 ms) to the signal on blank trials revealed a significant N2 only for supraliminal stimuli but not for subliminal stimuli. *B*: comparison of ERPs for supraliminal and subliminal stimuli at central channels (white dots) revealed a significant P3 component only for supraliminal but not for subliminal stimuli (300–500 ms).

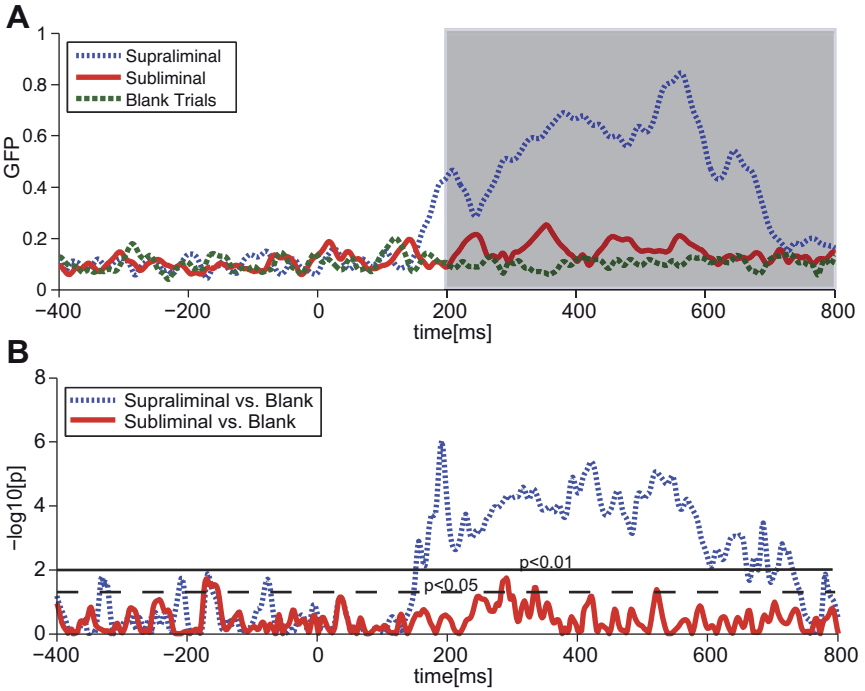

Fig. 3. *A*: grand-average global field power (GFP). Within the time range of interest (gray segment), a strong response was observed for supraliminal but not for subliminal stimuli. *B*: paired-sample *t*-tests at each sampling point for the supraliminal as well as subliminal conditions, both compared with the blank condition. Black lines indicate *P* values of 0.01 (solid line) and 0.05 (dashed line). After correction for multiple comparisons, no significant differences between the subliminal and blank conditions were found at any time, whereas differences between the supraliminal and blank conditions are evident.

significant effect was found between subliminal stimuli and blank trials [$t(17) = 1.31$; $P = 0.2$].

Results of the nonparametric analysis of oscillatory power revealed distinct activity patterns for each paired comparison (supraliminal stimulation vs. blank trials over electrodes P07, PO3, O1, PO4, PO8; subliminal stimulation vs. blank trials over electrodes CP1 and CPz). Compared with blank trials, supraliminal stimulation elicited a significant increase of lower-frequency power and a decrease that is maximal at the alpha-band power after 400 ms (Fig. 4*A*, *left*), including beta and higher theta bands. By contrast, subliminal stimuli elicited a significant increase with a maximum in the alpha-band between 200 and 400 ms (Fig. 4*A*, *right*), including the beta band.

The topography for supraliminal stimulation (500–700 ms) vs. blank trials (0–700 ms) revealed an occipital desynchroni-

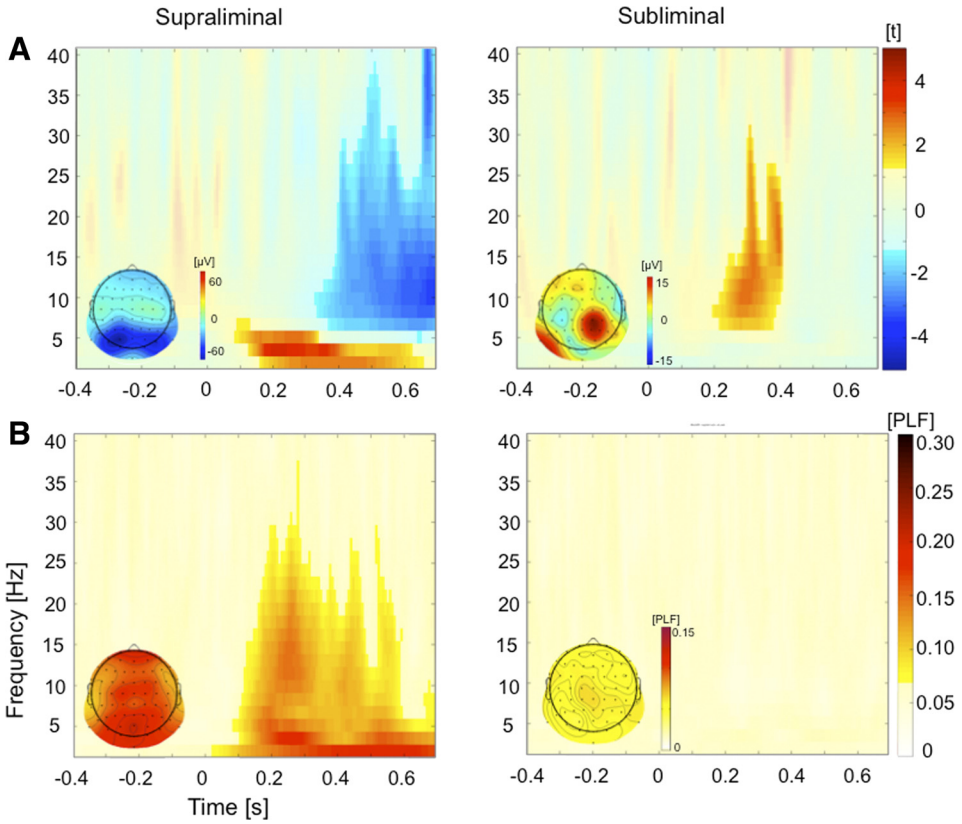

Fig. 4. Analysis of oscillatory power. *A*: time-frequency representation of event-related power changes averaged across channels with strongest alpha power, showing oscillatory responses to supraliminal stimuli (*left*) as well as subliminal stimuli (*right*) compared with blank trials. Nonsignificant regions are dimmed to improve visibility of the significant time-frequency clusters. Topographical distributions reveal an occipital desynchronization for supraliminal stimulation. Subliminal stimulation results in 2 maxima, synchronizing over parietal-occipital areas. *B*: time-frequency representation of the phase-locking factor (PLF) for supraliminal (*left*) and subliminal (*right*) stimuli. Nonsignificant regions are dimmed to improve visibility of the significant time-frequency clusters. Note that the same analysis conducted on blank trials also resulted in no significant phase-locked response, either (not shown). Topographies show the distribution of PLF values within the alpha frequency range (8–12 Hz; 200–400 ms).

zation. The topography for subliminal stimulation (200–400 ms) vs. blank trials revealed two maxima, synchronizing over occipital-parietal and left posterior channels (see Fig. 4).

To evaluate whether any phase-locked activity occurred in response to subliminal stimuli, we quantified phase locking with the PLF (see MATERIALS AND METHODS) for each condition and found that only supraliminal stimuli (Fig. 4*B*, *left*) elicited a significant phase locking after stimulus onset. There was no increase in phase locking in response to subliminal stimuli (Fig. 4*B*, *right*) or during blank trials (not shown). Topographies showing the distribution of PLF values (8–12 Hz; 200–400 ms) support the claim that the subliminal stimulation does not lead to any phase-locked response.

We also studied the consistency of these response patterns across participants. In particular, we looked at the consistency of responses in two time windows: 200–400 ms and 400–700 ms at frequencies from 8 to 12 Hz, averaged across electrodes of interest as indicated in Fig. 5. These time-frequency windows correspond to the early alpha-band synchronization in response to subliminal stimuli and the late alpha-band desynchronization in response to supraliminal stimuli, respectively. Effects in single participants are expected to be more variable and noisier than the grand average. However, the late alpha-band desynchronization was found in a clear majority of subjects in response to supraliminal stimuli (i.e., the direction

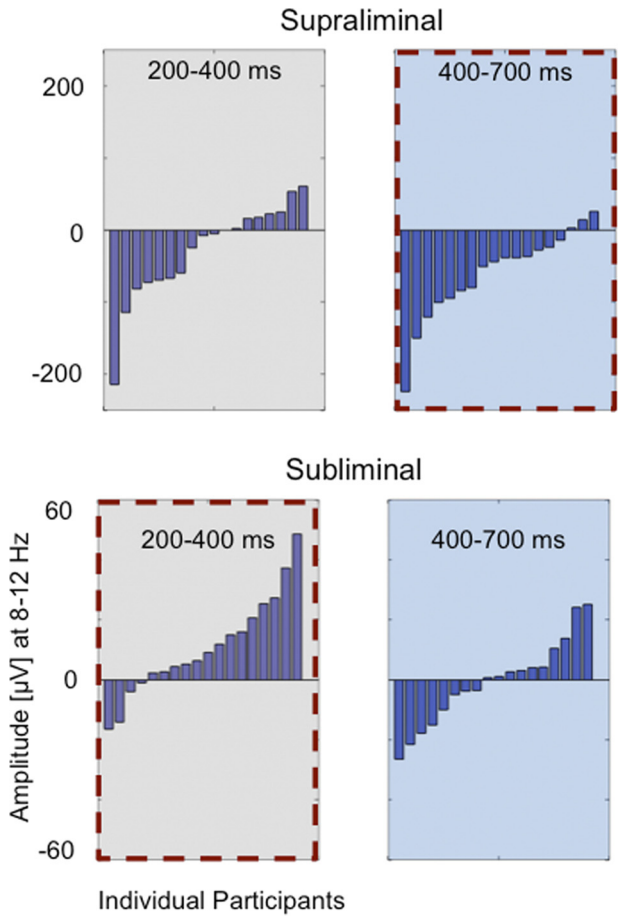

Fig. 5. Single-subject analysis. Alpha-band (8–12 Hz) power changes for each participant in ascending order, averaged across time ranges of interest (red dashed boxes). As a comparison, the respective other time ranges of interest are shown.

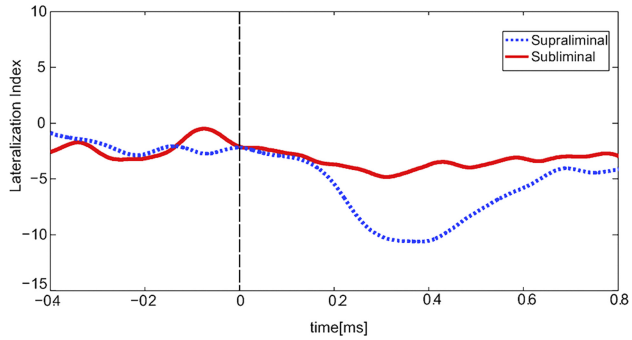

Fig. 6. Grand-averaged lateralization index averaged across all homologous electrode pairs for supraliminal and subliminal stimuli in the alpha frequency range (8–12 Hz). Positive values reflect stronger power contralateral to the stimulated hemifield. Accordingly, the negative lateralization values for supraliminal stimuli indicate stronger alpha desynchronization (power decrease) contralateral to the stimulated hemifield.

of the effect was the same in 15 of 18 participants, Fig. 5, *top*; analyzing each subject separately, this increase was significantly different from baseline in 13 of these 18 subjects). A χ^2-test of the goodness of fit—testing whether or not the observed frequency distribution differs from chance—indicates that this result is very unlikely given the null hypothesis of no effect [$\chi^2(1,N = 18) = 8$, $P < 0.01$]. For comparison we analyzed the same time range for subliminal stimulation and found no consistency across participants [$\chi^2(1,N = 18) = 0.889$, $P < 0.346$]. Similarly, in the early time-frequency window, almost all of the participants [14 of 18, $\chi^2(1,N = 18) = 5.556$, $P < 0.05$; Fig. 5, *bottom*] responded to subliminal stimuli with an increase in alpha-band power (although, analyzing each subject separately, a significant increase compared with the baseline could be found in only 3 of the 18 participants). No consistency regarding the direction of the effect (i.e., alpha synchronization or desynchronization) was observed for supraliminal trials within this time range [$\chi^2(1,N = 18) = 0.222$, $P < 0.627$].

We tested whether individual perception thresholds as estimated in *phase I* of the experiment influence power changes due to subliminal (8–12 Hz; 200–400 ms) or supraliminal (8–12 Hz, 400–700 ms) stimulation, but no significant correlation was found for subliminal ($r = -0.2686$, $P = 0.3$) or supraliminal ($r = 0.2158$, $P = 0.4$) stimulation (Pearson product-moment correlation).

Furthermore, we calculated a lateralization index for responses to supraliminal and subliminal stimuli by subtracting power at ipsilateral electrodes (relative to the hemifield in which the stimulus was presented) from the power at homologous contralateral electrodes (Fig. 6). Supraliminal stimuli elicited strong lateralization. The lateralization index took negative values because the lateralization is related to the stimulus-induced alpha desynchronization. Thus negative values indicate stronger desynchronization within the hemisphere contralateral to the stimulus. By contrast, no lateralization was found for the increase in alpha power from 200 to 400 ms induced by subliminal stimuli.

DISCUSSION

We investigated how the visual system responds to stimuli that are invisible because of their low contrast and character-

ized how this response differs from the response to visible, supraliminal stimuli.

Supraliminal stimuli triggered a well-known response: a significant increase in lower frequencies (representing the evoked potential) as well as a reduction in power at frequencies within the alpha-band and higher frequencies after 400 ms—a typical effect after visual stimulation (Adrian and Matthews 1934), also known as event-related desynchronization. By contrast, subliminal stimuli elicited a distinct power increase that showed a maximum in the alpha-band around 300 ms.

These results indicate that brain responses to stimuli that are not consciously perceived seem to depend on the nature of the stimulus and the reason for its invisibility: A stimulus that is invisible because of masking has been shown to elicit weaker, but similar, responses compared with unmasked stimuli (Harris et al. 2011; Haynes and Rees 2005). Likewise, responses to undetected periliminal stimuli are weaker, but qualitatively similar, compared with responses to detected stimuli (Pins and Ffytche 2003). Importantly, however, stimuli in both paradigms are usually of such intensity that they would be always visible when unmasked or at least sometimes visible if periliminal. Here we demonstrate that stimuli that are never visible because of their low contrast, and thus imperceptible under any circumstance, elicit a specific response that is different from that to visible stimuli.

Interestingly, this response was not lateralized for subliminal stimulation, as indicated by the lateralization index. We believe that our failure to find a significant lateralization for subliminal stimuli is due to the low signal-to-noise ratio. The response to a subliminal stimulus is very weak to start with, so that any potential lateralization will be even weaker. Moreover, analyzing stimuli presented to the left and right hemifields separately inevitably reduces the number of trials by half and so decreases the signal-to-noise ratio even further.

Did participants really not perceive any of the stimuli that were labeled as "subliminal" in our study? We used several methods to ascertain that stimuli labeled as "subliminal" were below the participant's detection threshold while stimuli labeled as "supraliminal" were above: First, to measure sensitivity for a certain stimulus independently from a participant's response criterion, we analyzed A′ as a sensitivity index. This measure is based on signal detection theory and controls for a potential bias of a participant toward a certain response category (Stanislaw and Todorov 1999). Second, we calculated an absolute detection threshold by comparing participants' empirical sensitivity to that of a simulated observer with zero sensitivity. Third, the contrast of subliminal stimuli was set at only 25%, while the contrast of supraliminal stimuli was 500% of the threshold intensity, hence ensuring that stimulus intensities were far below and above the threshold, respectively. Fourth, we retested participants' thresholds after the EEG recording to ensure that thresholds had not changed in the course of the experiment.

In addition to these methodological considerations, several of our results indicate that participants did not, in fact, perceive any of the subliminal stimuli: First, responses in the GFP and ERPs were found only for supraliminal but not for subliminal stimuli. In particular, only supraliminal stimuli evoked a significant N2 and P3 response. Earlier ERP components were most likely too small to be detected because of the small size and low contrast of the stimuli (see Busch et al. 2004; Schadow et al. 2007). The absence of the N2 and P3 for subliminal stimuli is in line with previous reports associating the N2 (also termed "visual awareness negativity") with conscious stimulus detection and attentional selection and the P3 with conscious access, decision making, and response confidence (Busch et al. 2010; Railo et al. 2011; Sergent et al. 2005). Second, our main result demonstrates qualitative differences in the responses to supraliminal and subliminal stimuli: While a phase-locked lower-frequency response and desynchronization within the alpha-band and higher frequencies followed supraliminal stimulation, a non-phase-locked increase within the alpha-band, including higher theta and lower beta power, followed subliminal stimulation. If participants had consciously perceived stimuli labeled as "subliminal," the pattern of responses to subliminal stimuli should closely resemble the responses to supraliminal stimuli. Together, these findings confirm that subliminal stimuli were in fact invisible, rather than just less visible than supraliminal stimuli.

Our results show that the consequence of a weak, subliminal stimulus is not simply weak sensory activation (or just no activation at all). Rather, a weak subliminal stimulus induces a response that could indicate a brief sensory inhibition. We suggest that the alpha-band power increase in response to subliminal visual stimuli corresponds to fMRI deactivation found for subliminal somatosensory stimuli reported by Blankenburg et al. (2003) and Taskin et al. (2008) for the following reasons. First, alpha oscillations have long been known to inhibit visual perception and stimulus processing (Foxe and Snyder 2011; Klimesch et al. 2007). Second, cortical activation in fMRI is negatively correlated with spontaneous fluctuations of alpha-band power in EEG (Goldman et al. 2002; Moosmann et al. 2003). Finally, elevated power of spontaneous alpha oscillations just before stimulus onset inhibits perception in visual detection tasks (Busch et al. 2009; Ergenoglu et al. 2004). This inhibitory effect not only co-occurs with spontaneous fluctuations of alpha oscillations but is also related to voluntary shifts of attention (Busch and VanRullen 2010; Foxe and Snyder 2011; Klimesch et al. 2007; Worden et al. 2000), possibly related to anticipatory gating of visual processing by parieto-occipital structures (Foxe and Snyder 2011)—brain areas that are well in line with our topographical results in response to subliminal stimulation. The topography of the alpha power increase induced by subliminal stimulation differed from the topography of alpha desynchronization after supraliminal stimulation. While the former had two maxima over parietal-occipital areas, the latter was clearly located over occipital areas. While the topography of the response to subliminal stimuli indicates a distribution over visual areas, we believe that methods exhibiting higher spatial resolution are necessary to arrive at more reliable conclusions about the origins of the very weak signal in response to low-contrast subliminal stimulation. In any case, the topographical difference between responses to subliminal and supraliminal stimuli implies different underlying neural generators.

Generally, an alpha rhythm increase indicates suppression of cortical brain areas. By suppression of cortical brain areas, alpha-band activity can subserve a "gating function" (Lopes da Silva 1991)—studies on anesthetized animals have shown that when alpha-like 10-Hz sleep spindles occur, thalamocortical interneurons are mainly hyperpolarized and thus exhibit a decreased probability of responding to external inputs (Foxe

and Snyder 2011; Jahnsen and Llinás 1984). Furthermore, gamma activity that has been related to engagement and stimulus processing (Fries et al. 2007; Jensen et al. 2007) is modulated by alpha activity (Osipova et al. 2008; Voytek et al. 2010). According to the "pulsed inhibition" theory of Jensen and Mazaheri (2010), alpha activity produces periods of inhibition that rhythmically reduce gamma activity, possibly due to GABAergic feedback from inhibitory interneurons (Lorincz et al. 2009). Inhibition due to subliminal stimulation is in line with previous findings that inhibitory cortical interneurons in primary somatosensory cortex (SI) as well as primary visual cortex (V1) have lower stimulation thresholds compared with excitatory neurons (Swadlow 2003; Zhuang et al. 2013). Thus subliminal sensory stimulation might lead to a favored activation of feedforward inhibitory interneurons within V1, which in turn triggers an increase of alpha rhythm.

A precise localization of the alpha power increase in response to subliminal stimuli cannot be easily obtained because of the small magnitude of this effect and the inherent spatial imprecision of electroencephalography. The more anterior location in response to subliminal stimulation compared with supraliminal stimulation might indicate a suppression of areas that were irrelevant for, and thus potentially interfering with, the processing of this specific stimulus (thus potentially serving to improve stimulus detection). This would be analogous to studies showing that perceptual and motor tasks induce alpha-band synchronization reflecting the inhibition of stimulus-irrelevant sensory modalities while they induce alpha-band desynchronization in relevant brain areas (Klimesch et al. 2007).

An alternative explanation is that subliminal stimulation induces a downregulation of areas involved in stimulus processing. This effect would serve a suppression of input noise that has been described previously in the somatosensory system (Blankenburg et al. 2003; Taskin et al. 2008). We propose that the function of this inhibitory response could be a protection of the visual system against spurious channel noise. Such a protection mechanism would be akin to automatic and unconscious inference processes that are abundant in low-level visual processing (Rock 1983). Prior to inferences about the features of a visual event, the visual system needs to decide based on visual input whether any visual event has occurred at all, or whether the "activation" is just noise. In some situations, the visual system may infer that a sensory signal is not plausible or strong enough to indicate that a visual event has occurred and therefore inhibit further processing of this event. One example of such a decision is saccadic suppression of object displacement. Generally, observers are very poor at detecting the displacement of an object during a saccade. However, detection of object displacement is unimpaired when the object is briefly blanked right after the saccade (Deubel et al. 1996). This finding implies that insensitivity to object displacement is not simply due to a loss of information during the saccade. Rather, the visual system seems to operate under the null hypothesis that objects in the world normally maintain their locations and that small position shifts are artifacts caused by the saccade. Thus if a minor displacement is detected, the visual system seems to infer that the object has not moved and the displacement is "deliberately" discarded as noise. A similar inference mechanism has been suggested by New and Scholl (2008) as an explanation for motion-induced blindness, in which salient target objects in full view fluctuate into and out of awareness when superimposed onto a global moving pattern (Bonneh et al. 2001; Wu et al. 2009). According to his proposal, the visual system filters out input signals that are more likely due to imperfections of the eyeball or retina than to real events in the world. Thus when visual input indicates a small object, which is invariant with respect to salient, global stimulus changes, the visual system makes the inference that this input is unlikely to result from a real object, to the effect that processing of the target is inhibited and the target object is not consciously perceived. Likewise, in the case of very weak, subliminal stimuli, the visual system might also infer that the input signal is not of sufficient strength to indicate a real visual stimulus and inhibit further processing.

This hypothesis stands in contrast to other theories of visual awareness or consciousness. It has been proposed that visual perception is based on the interplay between feedforward processing from lower to higher visual areas and feedback or reentrant processing (Bullier 2001; Lamme and Roelfsema 2000). In particular, visual awareness is thought to be dependent on reentrant processing and ongoing communication between higher- and lower-level visual areas. Several authors have proposed that visual masking reduces awareness by disrupting reentrant processing or by providing a mismatch between feedforward and reentrant signals (Crouzet et al. 2014; Di Lollo et al. 2000; Lamme and Roelfsema 2000). By contrast, subliminal stimuli in the present study were imperceptible because of their low contrast, not because of an additional mask stimulus. We suggest that the finding of specific neural signatures for weak subliminal stimuli indicates that—while there is neuronal processing of the stimulus in the visual system—the feedforward sweep is interrupted at a cortical level such that no reentrant process occurs. Furthermore, the transiently induced enhancement of alpha rhythm may correspond to a mechanism of the visual system that usually inhibits noise.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

Author contributions: I.B., A.V., and N.A.B. conception and design of research; I.B. performed experiments; I.B., M.C., F.B., and N.A.B. analyzed data; I.B., A.V., and N.A.B. interpreted results of experiments; I.B. and M.C. prepared figures; I.B. and N.A.B. drafted manuscript; I.B., M.C., F.B., A.V., and N.A.B. edited and revised manuscript; I.B., M.C., F.B., A.V., and N.A.B. approved final version of manuscript.

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