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Topography of Cortical Activation Differs for Fundamental and Harmonic Frequencies of the Steady-State Visual-Evoked Responses. An EEG and PET $H_2^{15}O$ Study

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In humans, visual flicker stimuli of graded frequency (2–90 Hz) elicit an electroencephalographic (EEG) steady-state visual-evoked response (SSVER) with the same fundamental frequency as the stimulus and, in addition, a series of harmonic responses. The fundamental component of the SSVER is generated by increased synaptic activity in primary visual cortex (V1). We set out to determine the cortical origin of the harmonic responses in humans. For this purpose, we recorded the SSVERs at 5 different frequencies (5, 10, 15, 25, and 40 Hz) and measured regional cerebral blood flow (rCBF) with positron emission tomography-$H_2^{15}O$ at rest and during visual stimulation at the same frequencies. The rCBF contrast weighted by the amplitude of the SSVERs first harmonics showed activation of a swath of cortex perpendicular to V1, including mostly the inferior half of the parieto-occipital sulcus. This area overlapped minimally with the primary visual cortex activated by the fundamental frequency. A different method, estimating EEG cortical source current density with low-resolution brain electromagnetic tomography, gave the same results. Our finding suggests that the inferior portion of the banks of the parieto-occipital sulci contains association visual cortex involved in the processing of stimuli that can be as simple as a flickering light source.

Keywords: LORETA, parieto-occipital sulcus, positron emission tomography, regional cerebral blood flow, steady-state visual-evoked potentials, visual cortex

Introduction

Oscillatory activity plays a critical role in neuronal communication and thereby in the codification of information by the brain (Vogels et al. 2005). In the visual system, the position of an object in space, its color, and its movement are coded by oscillatory activity, which can be recorded by intracerebral electrodes in experimental animals (Gray et al. 1989; Engel et al. 1991; Victor et al. 1994; Gegenfurtner et al. 1997). In humans, the oscillatory activity of neuronal assemblies in response to visual stimuli can be recorded by electroencephalography (EEG) (Tallon-Baudry 2003). One of the oldest paradigms of visual stimulation, both in the clinical and research settings, is the application of long trains of repetitive stimuli of variable frequency using a stroboscope (Regan 1966). The resulting brain response recorded by EEG is sinusoidal, with the greatest amplitude at the stimulation frequency. As this activity becomes regular about 200 ms after the onset of stimulation and remains steady for the duration of stimulation, it has been termed the steady-state visual-evoked potential or response (SSVER) (Regan 1977). Fast Fourier analysis of this brain-generated signal discloses that it is composed of a larger fundamental frequency, with the same frequency as the stimulus, and a series of harmonic frequencies of the fundamental frequency (Fig. 1*A1*). When, instead of using a stroboscope, the stimulation is performed with a square-wave reversing checkerboard, the largest oscillatory response beats at double the stimulation frequency, corresponding to the "on" and "off" discharges of the ganglion cells in the retina (Fawcett et al. 2004). In this case, the first harmonic response beats at 4 times the rate of the stimulation frequency (Fawcett et al. 2004). Harmonic responses after repetitive stimuli of visual areas have been documented in animal studies (Rager and Singer 1998), as well as EEG (Herrmann 2001; Pei et al. 2002) and magnetoencephalographic (MEG) (Fawcett et al. 2004) studies of the human SSVER. The characteristics of these harmonic responses suggest that they represent true brain activity and not just a repetitive stimulation artifact (Fawcett et al. 2004). The first harmonic response of the fundamental frequency in the visual system is particularly robust, both in experimental animals (Rager and Singer 1998) and in humans (Herrmann 2001; Pei et al. 2002; Fawcett et al. 2004).

The cortical origin of the fundamental frequency of the SSVER has been addressed in studies with MEG (Fylan et al. 1997; Fawcett et al. 2004), functional magnetic resonance imaging (fMRI) (Kwong et al. 1992; Mentis et al. 1997; Thomas and Menon 1998; Zhu et al. 1998; Hoge et al. 1999; Kaufmann et al. 2001; Ozus et al. 2001; Hagenbeek et al. 2002; Singh et al. 2003; Parkes et al. 2004), and positron emission tomography (PET) (Fox and Raichle 1985; Fox et al. 1987; Pastor et al. 2003; Feng et al. 2004). With a square-wave reversing checkerboard, Fawcett et al. (2004) stimulated the lower visual quadrant and using synthetic aperture magnetometry observed an activation of the upper portion of the calcarine sulcus, suggesting a V1 origin for the fundamental frequency. As visual stimulation with fMRI and PET elicits activation of both primary and association visual cortices, these methods by themselves do not allow a precise estimation of the cortical source of the fundamental frequency. Localization with fMRI could be improved by combining the use of interleaved EEG recording (Bonmassar et al. 2001) or simultaneous fMRI-EEG acquisition (Garreffa et al. 2004), although technical problems remain to be solved. Comparing the behavior of the conventional EEG response with the pattern of brain activation determined by $H_2^{15}O$ PET, we found that the amplitude of the fundamental frequency of the steady-state oscillatory response is related to increased synaptic activity in primary cortex, for both the auditory (A1) and visual (V1, Brodmann's area [BA] 17) cortices (Pastor et al. 2002, 2003).

In contrast to the localization of the cortical origin of the fundamental frequency, it is not known what is the cortical region most involved in the generation of the first harmonic response of the SSVER. With MEG, harmonic responses have been found in the same voxels giving rise to the fundamental response (Fawcett et al. 2004), but this finding does not exclude the possibility that other brain areas participate even more

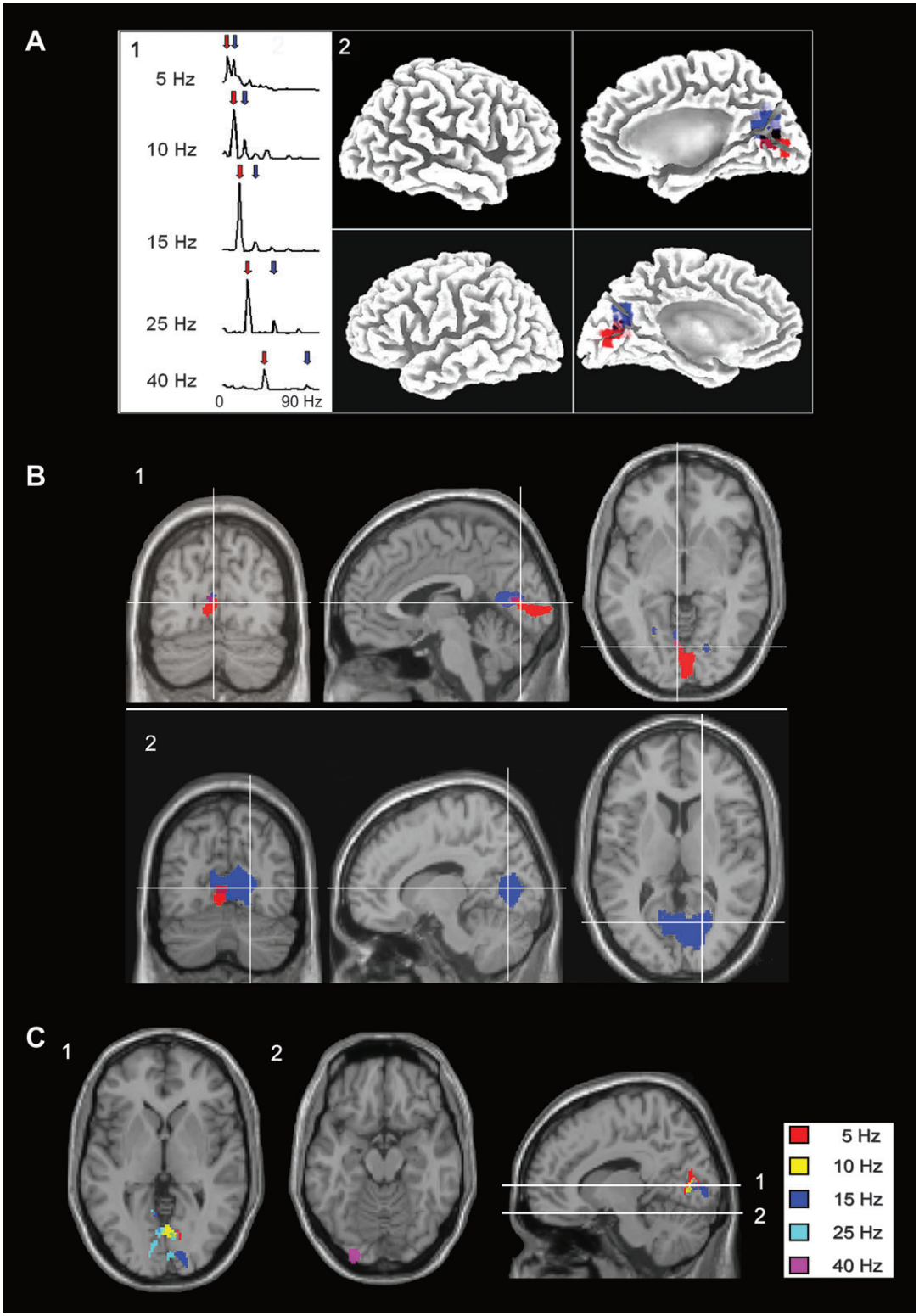

Figure 1. (*A1*) Grand average of the individual FFTs of the mean SSVERs recorded at electrodes O2, Oz, and O1. The amplitudes at each frequency (in microvolts) are listed in Table 1. Red arrow: fundamental frequency. Blue arrow: first harmonics. (*A2*) Render image of the cerebral cortex showing the grand mean of the fundamental component and harmonic generators calculated with the LORETA method (Pascual-Marqui et al. 1999) in one subject (red: fundamental component; blue: first harmonic). (*B*) Areas with significant changes in rCBF during visual stimulation, projected upon T_1-weighted coronal, sagittal, and transversal canonical images of the human brain. In red, activation in the contrast weighted by the amplitude of the fundamental frequency EEG occipital SSVER—contrast 2 in the text. In blue, activation in the contrast weighted by the amplitude of the first harmonic EEG occipital SSVER—contrast 3 in the text. The crosshairs indicate the plane of the sections, though the maxima voxel of each of the 2 activations are (*B1*) fundamental frequency and (*B2*) first harmonics. As canonical images depict activation exclusively in the plane of section, some of the activated areas are not shown. (*C*) Areas with significant changes in rCBF during visual stimulation at individual frequencies compared with rest (5 Hz: red; 10 Hz: yellow; 15 Hz: cyan; 25 Hz: blue; and 40 Hz: magenta), projected upon T_1-weighted sagittal (left) and transverse canonical images of the human brain. The level of the transverse sections (1 and 2) is indicated on the sagittal section. For each frequency, *Z* scores, significance level, and the coordinates of the maxima voxel in Talairach's standard stereotaxic space are listed in Table 2.

vigorously in generating the harmonic response. Thus, we decided to try to determine the cortical generator for this oscillatory brain activity. Our working hypothesis, which was proven incorrect by our experiments, was that the source of the first harmonic response would coincide with the source of the fundamental frequency. We used a similar technique as the one we applied to study the cortical generator of the fundamental response (Pastor et al. 2003). Normal subjects received stroboscopic stimulation at 5 different frequencies while recording EEG and, at a different time but with an identical stimulation paradigm, regional cerebral blood flow (rCBF) with $H_2^{15}O$ PET. With EEG, we measured the amplitude (square root of power) of the first harmonics for each of the electrodes and frequencies in each subject. We then determined the cortical region in which the rCBF paralleled the behavior of the amplitude of the SSVER at the different stimulation frequencies. In this region, a higher amplitude of the SSVER would correspond to a higher rCBF and a lower amplitude to a lower rCBF. We used this method because our previous studies indicated that, for each of the stimulation frequencies, there was a positive correlation between the amplitude of the evoked response on EEG and the rCBF (Pastor et al. 2002, 2003). Thus, if the rCBF in a specific region of the brain in response to stimulation by a series of different frequencies parallels the amplitude recorded on EEG for those same frequencies, it is logical to conclude that this specific region, rather than the rest of the cortex that behaves differently, is linked to the generation of the EEG response.

In addition to the rCBF determination weighted by the EEG amplitude, we used another method to localize the cortical origin of the first harmonic response of the SSVER: we estimated the cortical generators of the surface EEG signal using 48-electrode digital EEG and the "low-resolution brain electromagnetic tomography (LORETA)" methods (Pascual-Marqui et al. 1999). Both methods identified the same region as the cortical source of the first harmonic response of the SSVER.

Materials and Methods

Subjects

For the first experiment, with EEG and PET, we studied 9 normal volunteers (4 females, mean age = 30.4 years; standard deviation [SD] = 4.8). For the second experiment, we studied a different set of 7 normal volunteers (2 females, mean age = 28.7 years; SD = 4.7). All subjects were right handed by the Edinburgh Handedness Inventory (Oldfield 1971) with no history of ophthalmological or neurological disease and normal general and neurological examinations. The protocol was approved by the Ethics Committee of the Hospital of the University of Navarra, and all subjects gave their written informed consent for the study, according to the declaration of Helsinki, after its nature was fully explained to them.

First Experiment: rCBF Weighted by the Amplitude of the Fundamental and Harmonic Components of the SSVER

EEG Study: Amplitude of the Fundamental and Harmonic Components of the SSVER

SSVERs during wakefulness were elicited by white flashes delivered by a strobe lamp placed 40 cm away and facing the subject, with an intensity of 10 lumen/s/ft^2 and a duration of 0.5 ms for each flash. Responses were digitized at 500-Hz bandpass and filtered between 1 and 200 Hz. The sampling frequency was 500 Hz (512 points in 500-ms epochs), for a frequency resolution of 0.976 Hz per point. A total of 500 responses, in epochs of 500 ms, were averaged for each stimulus frequency. Responses were recorded at 21 scalp locations (10–20 EEG international system), all referred to a balanced noncephalic reference. Electrode impedance was kept below 5000 Ω. The frequency was defined as the number of flashes presented binocularly per second and was controlled by varying the interval between flash pulses, resulting in stimulation frequencies of 5, 10, 15, 25, and 40 Hz. Individual 500-ms trials that contained blink or electromyographic (EMG) artifacts were rejected from additional analysis. We chose the stimulation frequencies among the relevant EEG visual steady-state responses: 5 Hz as a low frequency, 10 Hz as the occipital activity at rest, 15 Hz as the highest amplitude SSVER and sensitization frequency for photoparoxysmal responses, 25 Hz as inducing the highest response in frontal areas (F3 and F4), and 40 Hz as the frequency most frequently recorded in human occipital cortex during tasks involving visual integration and characteristic of the fast rhythmic bursting cells, widely distributed in visual cortex (Gray et al. 1989; Cardin et al. 2005). Because the luminous intensity of each flash remained constant, increasing flash frequency by decreasing the flash interval resulted in a steadily increasing luminance at higher frequencies.

Having obtained the average SSVER per stimulation frequency in each subject, we calculated the dominant frequencies of power spectra and the amplitude (square root of power) per stimulation frequency at each of the 21 electrodes. For this purpose, a fast Fourier transform (FFT) was applied to the averaged SSVER using the Brain Atlas-III (Biologic, Mundelein, IL) software. All the dominant response peaks corresponding to each stimulation frequency (fundamental frequency) and their harmonic responses were measured. Isopotential maps of the visual-evoked potentials were obtained using Brain Atlas-III, which made a 4-point linear interpolation at 0.5-ms intervals. An FFT brain map was obtained for each stimulus rate and frequency peak.

Because the highest amplitude for the steady-state–evoked potentials was recorded at O1, O2, and OZ, we took an average of the amplitude (square root of the power of the FFTs) of the SSVER fundamental component and first harmonics at the 3 occipital electrodes to compare it with the rCBF data. The procedure (contrast weighted by the amplitude of the SSVER) is described with the PET methods.

Determination of rCBF with $H_2^{15}O$ PET

In a different session, but at a similar time in the afternoon as the EEG study, we measured rCBF with PET–$H_2^{15}O$ at rest and during visual stimulation at the same 5 different frequencies used in that study: 5, 10, 15, 25, and 40 Hz.

The PET scans were performed with an ECAT EXACT HR+ (Siemens/CTI, Knoxville, TN) that collected 63 simultaneous parallel planes over a 15.2-cm axial field of view. The tomographic resolution was 4.5 mm. Transmission scanning was done prior to radiopharmaceutical injection using 3 rotating rods (^{68}Ge source). The subject was positioned so that the entire intracranial volume was included in the field of view.

The subjects lay comfortably in a supine position in a room dimly and uniformly lighted. A small catheter was placed in the left cubital vein for the injection of the radioisotope. Series of flashes were delivered from a stroboscope as described in the methodology for the EEG recording experiments. Each subject underwent 6 consecutive scans at 20-min intervals, one at rest and one for each frequency of visual stimulation (5, 10, 15, 25, and 40 Hz). The order of the different frequency stimulation and baseline scans was randomized across subjects to avoid an order effect. For the baseline scan, subjects lay quietly. After 60 s at rest or, for the stimulation conditions, after onset of visual stimulation, subjects received 370 MBq of $H_2^{15}O$ as an intravenous bolus. Scans were initiated automatically when the radioactive count rate in the brain reached a threshold value of 100 K counts per second, approximately 20 s after intravenous injection, and continued for 60 s. Visual stimulation was maintained during scanning. Emission data were corrected for attenuation by means of a transmission scan. There were no explicit task requirements.

PET scans were analyzed using statistical parametric mapping (SPM2b, an update of SPM99, Wellcome Department of Cognitive Neurology, London, UK) (Friston, Holmes, et al. 1995; Friston et al.1996) on a Matlab 5.3 platform (Mathworks Inc. Natick, MA). Head movement was corrected by rigid alignment (Friston, Ashburner, et al. 1995). The scans were then spatially normalized using the template from the Montreal Neurological Institute series and the reference system of Talairach and Tournoux atlas (Talairach and Tournoux 1988). All the scans were smoothed using a Gaussian filter set at 10 mm full-width at half-maximum in plane, to increase the signal-to-noise ratio.

Data were analyzed after construction of a design matrix for the analysis of group data for conditions and covariates. All scans were subjected to an analysis of covariance (ANCOVA). This procedure removes the confounding effect of differences in global activity across scans and normalizes global activity (measured as radioactive counts) to a notional mean rCBF of 50 mL/dL/min. For each voxel, the ANCOVA generated 6 condition-specific mean rCBF values and associated error variances.

With SPM, we compared the mean blood flow elicited by the different stimulation frequencies on a voxel-to-voxel basis. The condition means were weighted by the appropriate contrast, generating a statistical parametric map of the *t*-values. A *P* value was computed for each *t*-score. To make inferences about stimulus-frequency–dependent rCBF responses, and thereby test our hypotheses, we specified a series of contrasts pertaining to the condition-specific effects, as follows:

1. First, we compared rCBF visual-evoked responses across all frequencies relative to rest. We used the ensuing (T) statistical parametric map to identify the visual cortex region showing the greatest rCBF response (Pastor et al. 2003).
2. To assess the correlation between the EEG response and rCBF in the visual cortex activated in the previous contrast, we specified a second contrast. Its weights were obtained by linear interpolation for the 5 frequencies used in the PET experiment from the amplitude of the occipital EEG steady-state responses fundamental component, using the data presented in Table 1. The resulting 5 amplitudes were then mean corrected to a mean of 0 and the rest condition discounted using a contrast weight of 0. Thus, the contrast tested the hypothesis that the EEG steady-state response could predict rCBF activation. The contrasts weighted were 0, –1.16, 0.14, 2.14, 0.54, and –1.66. It is important to note that this contrast is orthogonal with the previous contrast applied. In other words, the identification of the voxels most responsive to visual stimuli is independent of frequency-specific effects.
3. To assess the correlation between the SSVER first harmonics and rCBF in the visual cortex activated in the first contrast, we specified a third contrast. Its weights were obtained by linear interpolation for the 5 frequencies used in the PET experiment from the amplitude of the first harmonic steady-state responses, using the data presented in Table 1. The resulting 5 amplitudes were then mean corrected to a mean of 0 and the rest condition discounted using a contrast weight of 0. Thus, the contrast tested the hypothesis that the amplitude of the first harmonic steady-state EEG response could predict rCBF activation in the same or a different area as the fundamental component. The contrasts weighted were 0, 0.83, 0.46, –0.36, –0.14, and –0.79.
4. To explore whether the area activated in the previous contrast simply represented the greater weight of some individual frequencies among the harmonic frequencies tested, we performed 5 contrasts comparing the topography of rCBF changes during stimulation at each of the 5 individual frequencies (5, 10, 15, 25, and 40 Hz) versus baseline.

For contrast 1, we used a familywise error-corrected (FWE) *P* value of $P < 0.01$. For contrasts 2 and 3, we corrected for the volume of interest (a sphere of 40 mm of radius, centered at $x = 0$, $y = -84$, $z = 0$), and used a threshold of $P < 0.001$, because our inferences were restricted to the cerebral visual areas activated by contrast 1, and contrasts 2 and 3 are orthogonal to contrast 1. For the 5 contrasts indicated in (4), we used a FWE $P < 0.001$, raising the threshold in order to assess the cortical area predominantly activated by each frequency. To simplify the presentation of the data, only clusters with a size (k) >20 are shown in the figures.

Table 1
Amplitude—microvolt (standard error)—of the SSVERs components

Stimulation frequency	5 Hz	10 Hz	15 Hz	25 Hz	40 Hz
Fundamental peak	173 (1.3)	301 (0.3)	502 (32.1)	340 (24.2)	123 (5.3)
First harmonic peak	168 (13.9)	131 (12.4)	49 (1.5)	71 (1.8)	6 (0.76)

Second Experiment: Estimation of Cortical Source Current Density for the Fundamental and Harmonic Components

To determine the cortical sources of the fundamental and harmonic components of the SSVERs, we used the LORETA software and method (Pascual-Marqui et al. 1999). SSVERs during wakefulness were recorded at 58 scalp locations (10–120 EEG international system) and referred to a linked earlobes reference. Each session began with a 3-dimensional (3D) digitization of the positions of the electrodes, using the Zebris equipment and software (Zebris Medical GmbH, Max-Eyth-Weg 42, Isny, Germany). Reference marks on nasion, inion, and both ears helped to guarantee a correct registration to the Talairach human brain atlas (Talairach and Tournoux 1988).

Luminous stimuli and electrode impedance were kept as in the previous experiment. To facilitate comparing the source of fundamental and harmonic responses, we stimulated not only with the frequencies used in the previous experiment (except 25 Hz, to limit the stimulation time per subject) but also with their first harmonics (e.g., 10 Hz as the first harmonic of 5 Hz). Thus, we stimulated at 5, 10, 15, 20, 30, and 40 Hz. This procedure facilitated comparing the cortical origin of the same frequency as fundamental frequency and as first harmonic at stimulus presentation. Responses were digitized at 500 Hz with filter settings of 1 and 200 Hz. A total of 400 responses, in epochs of 500 ms, were averaged for each stimulus frequency. The sampling frequency was 500 Hz (512 points in 500-ms epochs) for a frequency resolution of 0.976 Hz per point. Individual trials that contained blink or EMG artifacts were rejected from additional analysis.

Using LORETA frequency domain analyses, we estimated the cortical generators of the surface EEG signal related to the fundamental component and first harmonic frequencies for each individual and condition (Pascual-Marqui et al. 1994; Frei et al. 2001). This LORETA version (Pascual-Marqui et al. 1999) employed a 3-shell spherical head model registered to the Talairach human brain atlas (Brain Imaging Center, Montreal Neurological Institute). First, each 3D individual electrode position was fitted to the standardized Talairach skull surface. As described in Pascual-Marqui et al. (1999), a total of 2394 voxels at 7 mm spatial resolution were produced under this neuroanatomical constraint.

For each LORETA image, we calculated the coordinates of the "centroid" for the M voxels with highest activity according to

$$(c_x, c_y, c_z) = \frac{\sum_{i=1}^{M} (x_i, y_i, z_i) \cdot A_{(x_i,y_i,z_i)}}{\sum_{i=1}^{M} A_{(x_i,y_i,z_i)}},$$

where $A_{(x_i,y_i,z_i)}$ is the activity at the (x_i, y_i, z_i) coordinates for the ith voxel, and (c_x, c_y, c_z) are the coordinates for the centroid.

We calculated the centroids for $M = 30$ and $M = 50$ voxels over the entire volume of the brain and over a region of interest constrained to the occipital lobe. The region of interest was selected because it was maximally activated with the SSVER in our previous study (Pastor et al. 2003). An N-way analysis of variance (ANOVA) (with $N = 3$) was used to assess the significant dependence of the cortical activation topography according to subject, frequency rate, or fundamental component versus harmonic condition.

Results

First Experiment: rCBF Weighted by the Amplitude of the Fundamental and Harmonic Components of the SSVER

EEG Study: Amplitude of the Fundamental and Harmonic Components of the SSVER

An oscillatory EEG response, phase locked with the presented flash frequency, was recorded at 5, 10, 15, 25, and 40 Hz. As a result of a rapidly repeated visual stimulus application, an initial transient response evolved into a steady-state waveform with the same frequency as the stimulus. The oscillatory response reached the greatest amplitude at approximately 15 Hz in the occipital area and decreased at higher click rates. The FFT of the

recordings at these 5 frequencies in occipital electrodes showed a fundamental component frequency locked with the stimulus rate and a series of harmonics (Fig. 1*A1*). Their amplitudes are listed in Table 1.

Determination of rCBF with $H_2^{15}O$ PET

Contrast 1. Compared with rest, repetitive visual stimulation with all 5 frequencies, considered as a group, increased the rCBF in primary and association visual cortices, mostly of the occipital lobe, but reaching into the temporal and parietal lobes. Specific values have been reported previously (Pastor et al. 2003).

Contrast 2. The t-SPM from the contrast weighted by the amplitude of the SSVER fundamental component, which illustrates the positive correlation between the EEG response and changes in rCBF, defined an activation cluster in pericalcarine occipital visual cortex (maximum voxel—x: –6, y: –74, z: 2; centroid—x: 0, y: –84, z: –8) (BA 17, V1) (Fig. 1B), as previously reported (Pastor et al. 2003).

Contrast 3. The t-SPM from the contrast weighted by the amplitude of the SSVER first harmonics highlighted parieto-occipital cortex partially overlapping the cortex activated by contrast 2, but mostly rostrodorsal to it. It corresponded to the most rostral portion of the calcarine cortex, extending rostrally into both banks of the parieto-occipital sulcus and then superolaterally into the inferior half of the dorsal bank of the parieto-occipital sulcus (maximum voxel—x: 18, y: –66, z: 10; centroid—x: 5, y: –70, z: 10) (Fig. 1B). This area corresponded to the area generating the EEG signal defined by the second EEG experiment with LORETA localization, and differed from the primary visual area, maximally activated at the main component of the SSVER. This contrast showed also activation of the frontal eye fields. The coordinates (x, y, z) of the voxel of greatest activation in the left frontal eye field were –48, 0, 50 (Z = 2.7; P = 0.047) and in the right 52, 2, 52 (Z = 2.5; P = 0.042).

Contrast 4. Figure 1C depicts the significant rCBF changes during visual stimulation at 5 (red), 10 (yellow), 15 (cyan), 25 (blue), and 40 Hz (magenta) versus baseline. All main activations were within V1 (Fig. 1C and Table 2). Specifically, the cortex of the parieto-occipital sulci (the area activated by contrast 3) was not activated by lower frequencies (5 and 10 Hz), which had the highest first harmonic power. This finding confirmed the relationship between the areas activated in contrast 3 and the response to the first harmonic, discarding any influence of the higher relative weight of lower frequencies on the results.

Second Experiment: Estimation of Cortical Source Current Density for the Fundamental and Harmonic Components

Fundamental and harmonic components of the SSVER emerged from different regions of the parieto-occipital cortex. In each of the subjects, the generator for the harmonic frequency was significantly more anterior and dorsal than for the fundamental frequency. For the group, the Talairach coordinates of the LORETA centroid for the harmonic condition were placed rostrodorsally to those corresponding to the fundamental component (Table 3). Applying an ANOVA, a marked difference was found for the z ($P < 0.0064$) and y coordinates ($P < 0.0029$) of these centroids, which did not differ in their x coordinates ($P < 0.5154$). As expected, intersubject differences were also significant for the location of the centroids ($P < 0.05$, not shown here); however, there was no significant interaction between subject and centroid location for the fundamental versus harmonic frequency conditions (Fig. 1*A2*).

Table 2
Cerebral region activated with the contrasts comparing visual repetitive stimulation at the given frequency versus the rest condition

Stimulation frequency (Hz)	*Z*	*P*	*x*,	*y*,	*z*
5	6.46	0.01	14,	−78,	6
10	6.05	0.01	−6,	−94,	−10
15	5.72	0.01	14,	−78,	6
25	5.83	0.01	−2,	−70,	2
40	5.73	0.01	30,	−96,	−18

Note: Data include *Z* scores, significance level (*P* corrected), and the coordinates (*x*, *y*, *z*) of the maxima voxel in standard stereotaxic space (Talairach and Tournoux 1988).

Table 3
Mean centroid position for the fundamental and harmonic responses (LORETA)

	Fundamental response	Harmonic response	*F*	*P* value
Coordinate *x*	0.8830 ± 0.3195	0.8355 ± 0.3465	0.42816	0.5154
Coordinate *y*	−70.7702 ± 4.0837	−68.2522 ± 6.3295	9.6583	0.0029
Coordinate *z*	16.9295 ± 7.7862	20.8738 ± 10.6150	7.969	0.0064

Discussion

Two independent methods, the localization of cortical sources of the EEG signal with the LORETA method and the rCBF data showing cortical activation on $H_2^{15}O$ PET, defined the same parieto-occipital area as linked to the first harmonic frequencies of the SSVER (Fig. 1*A2,B*). It must be noted that the location of the maxima voxels differs for the 2 techniques. This is not surprising, as PET measures rCBF and the LORETA method calculates the EEG cortical source current density, which need not peak in the same voxel. This also precludes a direct comparison of the centroids obtained with either technique. Additionally, the measurements of PET activation have higher spatial resolution than those of LORETA. Despite the different nature and resolution of both techniques, the region related to the harmonic frequency was highly concordant, as can be seen comparing Figure 1*A,B*. This area minimally overlaps with, but is clearly different from, V1, the area generating the fundamental component of the SSVER (Fig. 1*A2,B*) (Pastor et al. 2003). It could be argued that this phenomenon merely reflects the overrepresentation among the first harmonics of frequencies that activate an area different from V1. However, the individual frequencies having the highest amplitudes among the first harmonics, when tested as fundamental frequencies, activate maximally V1 not the area activated by the first harmonics as such. This point was proven independently by our second experiment and by contrast 4 of the PET study.

In our first experiment, the EEG and the PET studies are necessarily linked and were performed in the same subjects because, as explained in the Introduction, the region with rCBF that paralleled the amplitude of the EEG harmonic responses at the different stimulation frequencies could be considered the

cortical region giving rise to those responses. The second experiment is completely independent and separate from the first EEG–PET experiment. For this second experiment, we selected a different set of subjects. Of course, the stimulation paradigm and the frequencies chosen were the same as for the first experiment. That the same cortical region was identified by 2 independent methods and in 2 different subject samples reinforces the notion that this region represents the cortical origin of the harmonic response of the SSVER.

Our finding is quite striking, but there are other examples of selective processing by the brain of frequencies encoded in repetitive stimuli. For instance, the binaural application of 2 simultaneous tones with a mean frequency of 400 Hz, but differing in frequency by 40 Hz, produces an auditory steady-state–evoked response (ASSER) on EEG at 40 Hz (Schwarz and Taylor 2005). The evoked response at 40 Hz grows in amplitude over the first 200 ms and is sustained during the rest of the stimulation, a behavior remarkably similar to that of the ASSER produced by stimulation at 40 Hz and to the behavior of the visual-evoked responses studied here. Also in the auditory realm, a MEG study (Fujioka et al. 2003) showed that the magnetic source in auditory cortex was different for stimulation with a pure tone (at fundamental frequencies ranging from 250 to 1000 Hz) or with a complex sound having the same pitch as the pure tone (virtual pitch) but made up of 4 harmonics of the pure tone and missing the fundamental frequency. In this study, as in most activation experiments, a single task is explored with each activation paradigm. The diverse behavior of harmonic brain responses has also been documented in the visual system. Using an ingenious stimulation paradigm that combines 2 different frequencies in the same stimulus, Pei et al. (2002) found that attention enhanced the amplitude of a brain response, but not its higher order harmonic. The design of our experiment allowed us to relate precisely the areas activated with 2 different brain events resulting from the same activation paradigm: a flickering light induced both a synchronous EEG response and a harmonic response.

Although we have defined the cortical origin of the harmonic response on EEG, the pathways leading to it remain unknown. It is possible that there is a differential projection from the lateral geniculate nucleus or, more likely, that the activation of the parastriatal cortex to produce the harmonic response occurs through corticocortical pathways. In this regard, it is intriguing that the lateral geniculate nucleus in the cat has 3 neuronal populations, each with a predominant oscillatory activity (Podvigin et al. 2004). The numerical relationship between these predominant frequencies is 1:2:3, that is, a harmonic relationship.

Another possibility is that the harmonic responses are related to the top–down visual control system. Stimulation of the frontal eye fields by transcranial magnetic stimulation, with a current low enough to prevent causing eye movements, elicits nonetheless activation of an area that overlaps with the area we found activated in relation to the harmonic responses (Ruff et al. 2005). In support of this possibility is our finding that the frontal eye fields are activated in the PET contrast locked to the amplitude of the harmonic brain responses.

The area activated by the first harmonic includes the most rostral tip of the calcarine cortex, a small portion of V1, and the most rostral portions of V2d, V3, and V3a (Tootell et al. 1997) but mostly lies in the inferior half of the parieto-occipital sulcus, on both banks inferiorly while tapering to occupy the dorsal bank superiorly. In macaque monkeys, it corresponds to the anterior extent of V2 or V3 (Galletti et al. 2005). The relatively few studies available on this region suggest that it probably mediates motion detection and facilitates the coordination of visual with somatosensory information (Galletti et al. 2003). In humans, it is activated more readily by luminance stimulation than by checkerboard stimulation, in contrast to primary visual areas (Dechent and Frahm 2003). However, in our experiment, the amplitude of the harmonic response decreased at higher frequencies with higher luminance, and therefore, the effect of luminance is not likely to have influenced the localization of the cortical generator of the first harmonic response. Together with primary visual areas, this region is activated by viewing a pattern that conveys to the subject the feeling of vection, particularly rollvection (Deutschlander et al. 2004). A transient activation of this region, detected by MEG 100–400 ms after flicker onset stimulation, has been interpreted as inhibitory over V1, supposedly protecting from cortical hyperactivity due to chromatic flicker (Watanabe et al. 2002).

In summary, steady-state stimulation of the visual cortex elicits responses with at least 2 different cortical sources: 1) the fundamental components originating from primary visual cortex and 2) the first harmonic components originating from the rostral portion of the calcarine sulcus but mostly from the inferior half of the parieto-occipital sulcus. The localization of the harmonic source is not related to the weight of the individual harmonic frequencies but to the characteristics of these frequencies as being harmonics of the stimulation frequencies. Our finding calls attention to a seldom-studied region of the brain, the inferior portion of the banks of the parieto-occipital sulcus, and identifies it as associated with visual information processing.

Notes

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