Is colour modulation an independent factor in human visual photosensitivity?

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Considering that the role of colour in photosensitive epilepsy (PSE) remains unclear, we designed a study to determine the potential of different colours, colour combinations and white light to trigger photoparoxysmal responses (PPRs) under stringent controlled conditions. After assessing their photosensitivity to stroboscopic white light and black and white patterns, we studied 43 consecutive PSE patients (mean age 19 years, 34 women), using a specially designed colour stimulator. Stimuli included: pulse trains between 10 and 30 Hz of white light and of all primary colours, and also isoluminant alternating time-sequences of colours. Illuminance was kept constant at 100 lux. A progressive stepwise increase of the modulation-depth (MD) of the stimuli was used to determine PPRs threshold. Whereas all the 43 patients were found to be sensitive during the stroboscopic and pattern protocol, only 25 showed PPRs (Waltz’s score ≥2) at least in one session when studied with the colour stimulator. Coloured stimuli elicited PPRs in all these patients, whereas white light did so only in 17 patients. Of the primary colours, red elicited more PPRs (54 in 22 patients) and at a lower MD (max Z-score 0.93 at 10 Hz). Of the alternating sequences, the red–blue was the most provocative stimulus, especially below 30 Hz (100% of patients, max Z-score: 1.65 at 15 Hz). Blue–green was the least provocative stimulus, since it elicited only seven PPRs in seven (28%) patients (max Z-score 0.44 at 10 Hz). Sensitivity to alternating colours was not correlated to sensitivity to individual colours. We conclude that colour sensitivity follows two different mechanisms: one, dependent on colour modulation, plays a role at lower frequencies (<30 Hz). Another, dependent on single-colour light intensity modulation correlates to white light sensitivity and is activated at higher frequencies. Our results suggest that the prescription of spectacles with coloured lenses, tailored to the patient, can be an effective preventative measure against visually induced seizures.

Keywords: photosensitive epilepsy; seizure prevention; photoparoxysmal response; chromatosensitive epilepsy; tinted lenses

Abbreviations: AEDs = antiepileptic drugs; ILAE = International League Against Epilepsy; IPS = Intermittent Photic Stimulation; LEDs = light emitting diodes; MD = modulation-depth; PPRs = photoparoxysmal responses; PSE = photosensitive epilepsy; PSS = photosensitivity score

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Introduction

Photosensitive epilepsy (PSE) is the most common form of reflex epilepsy, affecting up to 10% of children with epilepsy (Harding and Jeavons, 1994; Ferlazzo et al., 2005; Fisher et al., 2005). Moreover, between 4 and 9% of the population carries this risk factor, and may be unaware of this risk (Erba, 2001). Despite the high prevalence of this disorder, little is known about the mechanisms of human PSE.

The role of colour in pathogenesis of this disorder has received renewed attention in recent years after the Pokémon incident, which caused an unprecedented number of seizures among Japanese children (Harding, 1998), even though previous studies showed the importance of highly saturated red in triggering photoparoxysmal responses (PPRs) (Takahashi et al., 1976; Rubboli et al., 2004). This incident also prompted the review of the protective guidelines to include colour as a provocative stimulus and highlighted the need to develop preventative measures that can be implemented to protect not only the patients but also those subjects who carry the trait but are virtually unaware of it (Erba, 2001). The term...
chromatosensitive epilepsy was coined by Japanese authors to designate the condition of those patients in which changes in colour modulation constitutes the most important triggering factor (Tobimatsu et al., 1999).

Takahashi et al. (1999) proposed the participation of different mechanisms in the genesis of PSE, one dependent on luminance changes (quantity of light) and the other on wavelength of the stimuli that could be correlated with the clinical characteristics and severity of the epilepsy in these patients. Furthermore, the patient’s colour sensitivity might be an important feature to further define the phenotypical features of these patients in the quest for the genetic substrate of this condition, and it may be useful to plan adequate therapeutic strategies.

Taking good note of the increasing importance of colour in PSE, the recent ILAE proposal for a classification scheme for people with epileptic seizures and with epilepsy (Engel, 2001) mentions explicitly that ‘sensitivity to colour will be specified when possible’ in patients with reflex seizures precipitated by light.

However, despite of the increasing evidence suggesting the important role of colour, its contribution to human photosensitivity remains obscure (Fisher et al., 2005). We designed this study to investigate how different colours, colour combinations and white light contribute to trigger PPRs under strict controlled conditions.

**Material and Methods**

**Patient population**

Forty-three consecutive patients (35 women, mean age 19 years, range 9–56 years) with known history of visually induced seizures and diagnosis of idiopathic PSE (Guerrini and Genton, 2004) were prospectively studied at our centre (Table 1). These patients had history of absences, myoclonic, generalized tonic-clonic or occipital seizures triggered by visual stimuli. Twenty-six of those had spontaneous (not visually induced) seizures as well. The diagnosis of occipital seizures was made on the basis of their typical ictal semiology (Guerrini et al., 1995, 1998; Panayiotopoulos, 2002; Guerrini and Genton, 2004). All patients had normal colour vision as defined by the Ishihara test. They were specifically referred by their neurologist for an extensive investigation of visual sensitivity in order to assess the degree of photosensitivity.

**Assessment of the patients’ photosensitivity: stroboscopic white light intermittent photic stimulation (IPS) and pattern sensitivity**

All these 43 patients underwent a standardized extended visual sensitivity examination, which is designed to assess the potential provocative factors that the patients may encounter in their daily life (Rubboli et al., 2004). The main basis of the stimulation protocol used here was published earlier in an educative video (Zifkin and Kastelein-Nolst Trenite, 2000). This first phase of the investigation was carried on by means of a commercially available stroboscopic photic stimulator (Grass PS33). Briefly, the patients are seated on a chair in front of the stroboscopic stimulator at 30 cm from their eyes. During this procedure, the patients were stimulated during 5 s, or shorter if a PPR was elicited, in the following conditions: during eye closure, with eyes closed, with eyes open and finally, with eyes open covered by a diffuser (Leijten et al., 1998). We used the following frequencies: 2, 5, 10, 15, 20, 25, 30, 40, 50 and 60 Hz. The flashes had a duration of 10 ms. The PPRs elicited were scored according to the Waltz classification (Waltz et al., 1992). The photosensitivity range was calculated considering the lower and higher frequency of a standard flickering light, at which the PPR response was higher than Waltz’s type 2. In addition, pattern sensitivity was assessed by presenting black and white striped patterns with vertical and horizontal orientations and a square luminance profile, with a spatial frequency ranging from one to five cycles per degree (Wilkins, 1995). These patterns were generated on a computer running a home-made program based on MATLAB environment (The Mathworks, Inc., MA, USA) specifically designed for this purpose. These patterns were presented to the patient on a plasma television screen. The spatial frequency of the patterns varied in a stepwise fashion at steps of 0.5 degree difference. Each pattern was maintained for 10 s or shorter if a PPR was elicited. The patterns bore a central fixation point at which the patient was instructed to gaze. We also tested the effects of different programs on 50 and 100 Hz television sets, to show the patients and families the potentially different effects of these two devices. Our ethical commission approved this clinical protocol. Informed consent from all adult patients and the parents of subjects younger than 18 years was obtained.

**Colour sensitivity investigation**

**Technical specifications of the colour stimulator**

Following the recommendations of the ILAE (Engel, 2001), a fully dedicated colour stimulator was built by the Medical Physics Department at our Institute (Figure 1). This device was made of 1008 equally distributed electronic lamps or Light Emitting Diodes (LEDs) of the three primary colours (red, green and blue). The LEDs were presented over a curved surface in order to maximize the angle of stimulation to 180°. Its dimensions are 55 x 123 x 66 cm. In order to produce a diffuse light source, an internal reflector and a diffuse foil were placed behind the layer of LEDs. The device was calibrated by means of a spectral radiometer, RPS 380 (380–780 nm) from International Light, Inc. (Newburyport, MA, USA). The spectrum of the different LEDs is shown in Figure 1C. The system was driven by a home-made program based on MATLAB environment specifically designed for this purpose. A computer with an interface card with four analogue outputs was used to control the stimulator. Three outputs were connected to the power supply-unit with ‘Pulse Width Modulators’ to control the light-intensity of the LEDs, the fourth output was connected to the EEG ‘mark input’ which generated pulses synchronized with the stimulus signal. A small digital camera was placed in front of the patient’s face to monitor any clinical response.

**Protocol of chromatic stimulation**

The patients were seated on a chair with the eyes opened and the head positioned at 30 cm distance of the LEDs surface (Figure 1). The ambient light of the room was set to standardized scotopic conditions. Patients were stimulated at different frequencies...
(10, 15, 20 and 30 Hz). Stimuli included: square pulse trains of white light, all primary colours (red, green and blue) and isoluminant alternating time-sequences of these. In addition, we also tested yellow light as it is considered a primary colour in psychophysicist experiments and it seems to be conveyed together with blue light through a specific visual pathway in the human visual system (koniocellular pathway) (Hendry and Reid, 2000).

A progressive stepwise increase of the modulation-depth (MD) of the stimuli was used to determine PPRs threshold at a certain frequency. MD is defined as the ratio between the amplitude (difference between maximal and minimal intensity) of the signal and the maximal intensity of the signal (Figure 2).

\[
\text{MD} = \frac{L_{\text{max}} - L_{\text{min}}}{L_{\text{max}}}
\]

Where \(L_{\text{min}}\) and \(L_{\text{max}}\) are correspondingly the minimal and maximal luminescence of the stimulus or of any of the colour components in case of isoluminant colour stimulations. As we showed in a previous paper (Parra et al., 2005), MD constitutes by itself an important factor in the ability of the stimuli to trigger PPRs.

We used squared-pulses stimuli with a 50% duty-cycle in four increasing steps of the MD (25, 50, 75 and 100%) for every single-colour, white light and alternating colour combination tested. Maximal luminance (\(L_{\text{max}}\)) of the stimuli was kept constant at 100 lux and \(L_{\text{min}}\) was adjusted to produce the required MD. To avoid the development of habituation to the frequency of stimulation (Topalkara et al., 1998), the five-seconds trains of flashing stimulus at increasing modulation depths were separated by 5 s periods, in which the stimulus presented was a static, non-flashing version of the stimulus. PPRs were scored according to the Waltz’s classification (Waltz et al., 1992). Only type 3 and 4 of the Waltz’s classification were tabulated and used in the evaluation of the results. Following the same standard as in the IPS protocol with stroboscopic light described earlier (Rubboli et al., 2004), the threshold for every condition of stimulation was set at the level of the MD at which a reproducible generalized PPR (type 4 response from Waltz PPR) was triggered. At this point the stimulation was discontinued for that condition. The latency to the occurrence of the PPR was also measured, as it may constitute a relevant parameter with respect to the sensitivity to the stimuli.

Our Institutional Review Board and ethical commission approved this clinical protocol. Informed consent from all adult patients and the parents of subjects younger than 18 years was obtained.

**Statistical analysis**

Non-parametric tests for paired samples were used for comparisons between groups (Mann–Whitney U test, Kolmogorov–Smirnoff, Kruskal–Willis). Spearman rho’s correlation was used

| Table 1 Clinical data of the patients and results to stroboscopic IPS and patterns protocol |
|---------------------------------|---------------------------------|------------------|------------------|
| Variable                        | Patients non-sensitive to colour\( (n = 18)\) | Colour-sensitive patients\( (n = 25)\) |
| Gender \( (\text{women/men})\)   | 14/4                           | 20/5             |
| Age in years \( (\text{mean } \pm \text{SD})\) | 17.8 ± 5.2                    | 22 ± 11.5        |
| Age of seizure onset in years \( (\text{mean } \pm \text{SD})\) | 12.1 ± 6.0                    | 13 ± 9.7         |
| Seizure types                   |                                |                  |
| Febrile seizures                | 2                              | 1                |
| Occipital seizures              | 8                              | 5                |
| Absences                        | 7                              | 10               |
| Tonic–clonic seizures           | 16                             | 15               |
| Myoclonias                      | 7                              | 11               |
| Number of patients with paroxysmal eyelid movements | 4                             | 5                |
| Seizures non-induced by visual stimuli | 12                        | 14               |
| Antiepileptic drugs             |                                |                  |
| None                            | 6                              | 9                |
| One                             | 8                              | 14               |
| Two or more                     | 4                              | 2                |
| EEG                             |                                |                  |
| Number of patients sensitive during eye closure \( (\text{mode of the frequency range})\) | 16 \( (10–30 \text{ Hz})\) | 24 \( (10–60 \text{ Hz})\) |
| Number of patients sensitive during eye open stimulation \( (\text{mode of the frequency range})\) | 6 \( (\text{non-sensitive})\) | 8 \( (\text{non-sensitive})\) |
| Number of patients sensitive during eyes-closed stimulation \( (\text{mode of the frequency range})\) | 13 \( (30 \text{ Hz})\) | 22 \( (10–30 \text{ Hz})\) |
| Number of patients sensitive during eyes-open with diffuser \( (\text{mode of the frequency range})\) | 13 \( (60 \text{ Hz})\) | 20 \( (10–60 \text{ Hz})\) |
| Number of patients sensitive to horizontal black and white patterns\* | 1                             | 8                |
| Number of patients sensitive to vertical black and white patterns\*                  | 2                             | 12               |
| Number of patients sensitive to 50 Hz TV | 2                             | 8                |
| Number of patients sensitive to Pokemon scene\*                               | 10                            | 22               |

* \(P < 0.05\) Fisher exact test, one-tailed.
to assess correlations between groups. t-Test for independent samples was used to compare latencies. The statistically significant level was set at $P<0.05$. We calculated $Z$-scores per frequency and per colour condition, on the basis of the maximum sensitivity defined by the minimum MD able to trigger a PPR. Cross-correlations were run to assess possible correlations between sensitivity to different colours. In addition, a non-linear association index ($h^2$) (Pijn et al., 1990) was calculated to assess asymmetrical non-linear relationships between primary colours and colour combinations, providing in this way an extra dimension for analysing the paired statistics of photosensitivity (Kalitzin et al., 2007). Statistical significance of paired statistics was calculated using Monte Carlo iteration methods. For each pair 10000 Monte Carlo iterations were performed.

Further we extrapolate between the integer sensitivity weights using a sensitivity correction factor according to the time delays from the beginning of the stimulation until the PPR was recorded. As we stimulated for no more than 5 s, the relative fraction delay/5 s was subtracted from the discrete sensitivity values. Thus, we defined the photosensitivity score (PSS) as follows:

$$PSS = \left( \frac{125 - MD_{\text{min}}}{25 - T/5} \right)$$

where $MD_{\text{min}}$ is the minimal modulation depth (in%) at which PPRs are triggered; $T$ is the elapsed time between the start of the stimulation and the PPR in seconds. The correction factor $T/5$ was introduced to account for the fact that a PPR at a short latency should correspond to a larger PSS index than a PPR at the same $MD_{\text{min}}$ but at a longer latency. As we have used four discrete values for $MD = (25, 50, 75, 100)$, and if we ignore the delay $T$, the above formula produces values for the sensitivity of 1, 2, 3 and 4, correspondingly. The delay factor produces a decrement correction (the longer the delay the less sensitive is the patient) between 0 (immediate response) and 1 (response after 5 s stimulation, the maximal stimulation duration). Therefore the quantity PSS takes values between 0 and 4. For example if stimulation at

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**Fig. 1** View of the colour LEDs stimulator showing its internal design and concept (A), patient position and control during stimulation procedure (B) and the calibration spectra of the different LEDs (C).


**Fig. 2** Illustration of the concept of modulation depth (MD).
Here two sinusoidal luminous stimuli of a similar maximal intensity (100 lux) but of different MD are represented. MD is defined as the ratio between the amplitude (difference between maximal and minimal intensity) of the signal and the maximal intensity of the signal; this ratio can vary from 0 (no modulation) to 100%. In the figure the purple line represents a sinusoidal signal of 100% MD while the green line represents signal of 50% MD. The phase difference is introduced just for visualization purposes.

50% MD elicits a PPR after exposure (delay) of 3 s, this would result in 
\[ PSS = \frac{(125 - 50)}{25} - \frac{3}{5} = 2.4. \]

**Results**

**Results of stroboscopic IPS and pattern stimulation in colour and non-colour sensitive patients**

Whereas all the 43 PSE patients examined showed PPRs during the assessment of their photosensitivity with stroboscopic IPS and black and white pattern stimulation, only 25 (58%) of those displayed PPRs when the colour stimulator was used (Table 1). The two groups, however, showed a different photosensitivity profile during the standard IPS stimulation protocol with stroboscopic light. Patients who were found sensitive with the colour stimulator exhibited more PPRs during the IPS procedure, being sensitive to more frequencies and to more test conditions (Table 1). Specifically, they were significantly more sensitive during the stimulation at eye closure and eyes open with diffuser \((P < 0.05)\). They were also more sensitive to stimulation with black and white striped patterns, which was statistically significant for the vertical ones \((P < 0.05)\). The question whether these two groups were different with respect to gender, age, age of onset and clinical characteristics of the seizures could be answered negatively.

**Results of the chromatic stimulation protocol**

Twenty-five of the 43 PSE patients screened with stroboscopic IPS and pattern stimulation showed PPRs (Waltz’s score >2) during the chromatic stimulation protocol. Coloured stimuli elicited PPRs in all these 25 patients with a median of 14 PPRs per patient (range 1–36 PPR per patient), whereas stimulation with white light with this stimulator elicited PPRs in just 17 of these patients with a median of 3 PPRs per patient (range 1–4). The data is summarized in Table 2, broken into white light, single colour and alternating colour stimulations.

**White flicker versus single-colour stimulations (Table 2, Figure 3)**

Single-coloured flashes provoked 178 PPRs in 23 patients (median per patient 8 PPRs, range 1–16). All of these single-coloured stimuli elicited PPRs, with red being the most provocative, precipitating 54 PPRs in 22 patients (median per patient 2.5 PPRs, range 1–4), whereas yellow was the least provocative with 38 PPRs in 15 patients (median per patient 2 PPRs, range 1–6). White stimulation with this stimulator provoked 50 PPRs in 17 patients (median per patient 3 PPRs, range 1–4). Single-colour stimulation triggered 44 PPRs at 10 Hz and 36 PPRs at 15 Hz, whereas stimulating with white light only accounted for 9 and 10 PPRs in each frequency. Twenty-six (48%) of the PPRs triggered by red flashes were in this frequency range. Nonetheless, 20 and 30 Hz were the frequencies that triggered more PPRs for both, single colour and white stimulations. The average latency of the PPRs was significantly longer for single-colour flashes (mean 0.79 s, 95% CI 0.66–0.93) than the latencies of the PPRs triggered by the white flashes generated by this stimulator (mean 0.55 s, 95% CI 0.34–0.75) \((t\text{-test}, P = 0.048)\). When each colour was compared independently versus white light, only PPRs elicited by red flashes showed a statistically significant longer averaged latency \((t\text{-test}, P = 0.013)\). Even though, in absolute numbers, 100% MD was the most provocative MD amongst single-colour stimulation, 24.7% of the PPRs were elicited at 25 and 50% MD. This figure was highest with red colour which triggered 35.2% of their PPRs at these low MDs. In contrast, only 12% of the PPRs elicited with white light were elicited at these low MDs. Considering the minimum MD to elicit a PPR, red flashes elicited more PPRs and at a lower MD (max \(Z\)-score 0.93 at 10 Hz) (Figure 3).

**Alternating colour stimulation versus white flicker and single-colour stimulations**

Isoluminant stimulation with alternating colours elicited 136 PPRs in all 25 patients (median per patient 5 PPRs, range 1–13) with a mean latency of 1.44 s (95% CI 1.26–1.62). Fifty-two PPRs were elicited at 15 Hz stimulation, whereas just nine PPRs were elicited at 30 Hz stimulation. The distribution of the latencies of the PPRs obtained during stimulation with alternating colours was significantly different to those of single colour and white stimulations (Kruskal-Wallis \(P < 0.004\) and \(P < 0.001\), respectively).
respectively). Considering the minimum MD to elicit a PPR, alternating red–blue was the most provocative stimulus of all the stimuli used during the chromatic investigation, eliciting PPRs in 100% patients (Table 2), with a maximum Z-score: 1.65 at 15 Hz (Figure 3). With alternating red and blue, only 11 PPRs were elicited at 20 Hz (six of them at MD ≥75%) and just two at 30 Hz, all of them at 100% MD. On the other hand, blue–green stimulation was the least provocative stimulus, eliciting only seven PPRs in seven patients, always at 100% MD (max Z-score 0.44 at 10 Hz). There was a statistically significant negative correlation between latencies to PPRs and MD, with higher MDs eliciting PPRs with shorter latencies (Spearman Rho, one-tailed, −0.127, P<0.01).

**Cross-correlations**

There were significant cross-correlations between the responses to all the single-colour stimuli and white light that reached, in general, larger values than those between single colour and stimulations with alternating colours and among alternating colour stimulations, with the exception of the correlation between red and alternating red–green flashes (Figure 4). The stimulation with alternating red–blue did not show any significant correlation with

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**Table 2** Results to colour stimulation

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Patients (n)</th>
<th>PPRs (n)</th>
<th>Frequencies which provoked most PPRs (% of all provoked PPRs)</th>
<th>Latency in s: mean (95% CI)</th>
<th>Most provocative modulation depth (MD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>17</td>
<td>50</td>
<td>62% at 20 and 30 Hz</td>
<td>0.55 (0.34–0.75)</td>
<td>80% PPRs at 75% MD</td>
</tr>
<tr>
<td>Blue</td>
<td>18</td>
<td>47</td>
<td>57.4% at 20 and 30 Hz</td>
<td>0.75 (0.48–1.02)</td>
<td>51.1% PPRs at 100% MD</td>
</tr>
<tr>
<td>Red</td>
<td>22</td>
<td>54</td>
<td>51.8% at 20 and 30 Hz</td>
<td>0.99 (0.7–1.3)</td>
<td>33.3% PPRs at 100% MD</td>
</tr>
<tr>
<td>Yellow</td>
<td>15</td>
<td>38</td>
<td>52.7% at 20 and 30 Hz</td>
<td>0.88 (0.56–1.2)</td>
<td>55.3% PPRs at 100% MD</td>
</tr>
<tr>
<td>Green</td>
<td>16</td>
<td>39</td>
<td>59% at 20 and 30 Hz</td>
<td>0.50 (0.31–0.68)</td>
<td>61.5% PPRs at 100% MD</td>
</tr>
<tr>
<td>Alternating colour stimulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue + red</td>
<td>25</td>
<td>50</td>
<td>72% at 10 and 15 Hz</td>
<td>1.59 (1.23–1.29)</td>
<td>38% PPRs at 75% MD</td>
</tr>
<tr>
<td>Red + green</td>
<td>20</td>
<td>42</td>
<td>73.8% at 10 and 15 Hz</td>
<td>1.14 (0.9–1.38)</td>
<td>40.5% PPRs at 100% MD</td>
</tr>
<tr>
<td>Blue + green</td>
<td>7</td>
<td>7</td>
<td>85.7% at 10 and 15 Hz</td>
<td>2.34 (0.42–4.43)</td>
<td>100% PPRs at 100% MD</td>
</tr>
<tr>
<td>Blue + yellow</td>
<td>17</td>
<td>37</td>
<td>67.6% at 10 and 15 Hz</td>
<td>1.46 (1.1–1.84)</td>
<td>56.8% PPRs at 50% MD</td>
</tr>
<tr>
<td>Total</td>
<td>25</td>
<td>364</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 3** Summary of the colour sensitivity per colour and frequency as expressed in its Z-score values averaged through all patients. The highest Z-score corresponded to red-blue stimulation at 15 Hz. Note the remarkable low Z-score values during green-blue stimulation at all frequencies.
any single-colour stimuli, neither with red nor with blue, and just a moderate correlation with alternating red–green. In other words, the effects of the red and blue stimulation can not be anticipated by the sensitivity to any of these colours taken separately. This finding emphasizes that this colour combination is a rather unique and powerful stimulus, based on a synergistic effect of these colour combinations that goes beyond the summation of the effects of the individual colours. We used in this study a unidirectional association measure ($h^2$) that yields an extra dimension to the analysis of the paired statistics of photosensitivity. Indeed we can deduce from Fig. 5 that the associations between the sensitivities to various stimulation parameters are not symmetric. Whereas sensitivity to any single-colour stimulus can be anticipated from the sensitivity to white flicker, the sensitivity to any single-colour flicker does not determine uniquely the sensitivity to white-light stimulation. The sensitivity to yellow and green stimuli can be deduced from the red–green sensitivity but not vice-versa. On the other hand, the sensitivity to alternating red–blue flashes could not be accurately anticipated by the sensitivity to blue or red alone.

**Virtual correction of photosensitivity by emulated coloured lenses**

We could simulate the virtual effect of coloured lenses on the overall degree of photosensitivity per individual by appropriately substituting the colour(s) of the stimulus.

Based on the theory of colour opponency (Gegenfurtner, 2003), sensitivity to red–blue flicker filtered through cyan lenses, for example, is assumed to be equivalent to that of blue monochromatic stimulation. In this way we could elaborate a hypothetical prediction (Figure 6) regarding the tinted lens that could have more chances to decrease the photosensitivity to white-light stimulation under strict isoluminance conditions. Based on these calculations we hypothesized that blocking the red component, for instance by using turquoise lenses, could have the most effective value in our patient population as they could be able to totally block all PPRs in five patients and decrease in more than 50% the degree of photosensitivity in another six patients. Blocking the blue component, for instance with yellow lenses, could be helpful to decrease the photosensitivity degree in four patients. Finally, blocking the green component, by use of purple lenses for instance, could provide significant benefit for one additional patient.

Further studies are necessary to corroborate these assumptions as well as to establish the potential benefits of the long-term use of tinted lenses in patients with PSE.

**Discussion**

The main findings of the present study concern the role of chromatic stimulation in photosensitive epilepsy. These observations enhance our understanding of the concept chromatosensitive epilepsy that was introduced earlier...
Our results argue for the contribution of two types of pathophysiological mechanisms in human photosensitivity that may contribute either synergistically, or independently, to elicit a PPR: one dependent on pure luminance changes or quantity-of-light (black and white) and another one dependent on the wavelength of the stimuli (Takahashi et al., 1999). Previous studies showed how low-luminance, highly saturated red, with a wavelength spectrum around 700 nm was more provocative than black and white light flicker (Takahashi and Tsukahara, 1976; Rubboli et al., 2004). Binnie and collaborators found that stimulation of either red or green cones by the silent substitution method may produce epileptiform discharges, there being a slight (and not significant) excess of patients showing a greater sensitivity for green than for red cone stimulation (Binnie et al., 1984). This effect of colour was later further emphasized after the occurrence of the Pokemon incident (Harding, 1998), which effect was attributed to a dominant stimulation of the red cones which are the most numerous type of cones in the retina, disabling the compensatory mechanism of stimulating complementary cones.

Both types of pathophysiological mechanisms can be highly intermingled in many patients and they might be very difficult to segregate. In fact, we were able to characterize the role of specific wavelengths (i.e. colour) in 58% of our PSE patients. For obvious reasons, the colour stimulation was carried out while the patient was looking at the stimuli, i.e. with eyes opened. This condition is known to be the least provocative during the routine testing of visual sensitivity with stroboscopic light (Rubboli et al., 2004). Whereas eye closure at the time of the stimulation is by far the most provocative condition to elicit PPRs, it would have introduced a bias during the stimulation because the blood vessels of the eyelids introduce a red filter to the light impinging on the retina. Taking these factors into consideration, it is remarkable that this form of stimulation was able to trigger PPRs in 10 patients who did not show any PPR under the eyes-open condition during the routine stroboscopic stimulation, a much more powerful source of light. Nevertheless, the low-intensity stimulation used in this study is a likely limiting factor that might have influenced the relatively low frequency of PPRs elicited by stimulation with colour. Had we used higher intensity stimulation would likely have increased the number of patients showing sensitivity to colour modulations. This fact might have underestimated the real prevalence of colour sensitivity. An alternative hypothesis would be the existence of a specific subset of patients sensitive to pure chromatic stimulation.

The patients that finally showed PPRs with the colour stimulator also showed more PPRs during black and white pattern stimulation than those who did not, which can be interpreted as manifestation of a more severe

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**Fig. 5** Significant ($p < 0.001$) non-linear cross-correlations ($h^2$) of the sensitivity between white light, single-colour and alternating colour stimulations. Diagonals were set to zero to improve visualization of adjacent significant values. From the upper left corner is clear that the sensitivity to any monochromatic stimulus can be predicted (high $h^2$ in the first column) from the sensitivity to white flicker. The associations in the inverse direction (the first raw) are much weaker which shows that sensitivity to any monochromatic flicker does not determine uniquely the sensitivity to white light stimulation. This observation is rather intuitive as the white light is composed of all the chromatic components. Similarly, the sensitivity to yellow and green stimuli can be predicted from the alternating red-green sensitivity but not vice-versa.
photosensitivity. Vertical patterns were especially provocative in the colour sensitive patients than in non-colour sensitive patients. Differences in activation based on orientation of the pattern have been found to be important in some patients but not in others, and it can even vary with time in the same patient (Klass, 2000). Although the selectivity of the sensitivity to one direction of the pattern can be the consequence of poor focusing to one orientation (astigmatism), this orientation selectivity might have a neurological basis as well (Wilkins, 1995). Neurons in the visual cortex respond selectively to line contours with a limited range of orientations. As a consequence, some patients can be sensitive to a limited range of pattern orientations. This is consistent with the interpretation that the mechanism responsible for eliciting PPRs involves neurons of the visual cortex (Wilkins, 1995).

Physiopathological considerations

Two findings are particularly worth putting in evidence: (i) that the effect of alternating colour modulations, virtually vanishes above 20 Hz, in contrast with both white and red stimulations (Figure 3) and (ii) that the associations among PSS of single-colour stimuli are much larger than between alternating colour modulations and single-colour stimulations, with the exception of red versus red–green. These findings indicate that the mechanisms underlying alternating colour stimulations differ from those responsible for single-colour and white-light stimulations with respect to the elicitation of PPRs. In addition, the former are dominant at relatively low stimulation frequencies (< 20 Hz), whereas the latter are most evident at higher frequencies (> 20 Hz). It is noteworthy to examine these findings in the framework of the basic organization of the visual system (Gegenfurtner, 2003). The magnocellular pathway is mostly sensitive to luminance and responds to high temporal frequencies, whereas the parvocellular pathway is sensitive to red–green (Long-middle wavelength, L&M) and the koniocellular pathway to blue-yellow (Short wavelength S) signals and respond mainly at low temporal frequencies. Interestingly, stimuli initiated by photon absorptions in the S-cones appear to move more slowly than stimuli with equal contrast but initiated in the L- or M-cones (Dougherty et al., 1999). At the cortical level, cells that add L and M cone inputs are called luminance cells, and cells that subtract L- or M- or S-cone inputs are called colour cells. Functional MRI studies in human have shown that, under a wide range of conditions human primary visual cortex responds more powerfully (per unit cone contrast) to chromatic signals (the difference of L and M cones or red–green stimuli) than to luminance signals (the sum of L and M cones) (Kleinschmidt et al., 1996; Engel et al., 1997). Furthermore clear responses are also seen with blue–yellow stimuli but these responses decline rapidly with temporal frequency (Engel et al., 1997). There is only a weak response in MT+ to the signals initiated in the S-cones, whereas the other two colour dimensions (light/dark) and (red/green) evoke powerful responses in this region (Liu and Wandell, 2005). Conway (2001) found in monkey’s visual cortex that most red–green cells, the activity of which is modulated by L- and M-cone stimuli, are also modulated by the S-cone stimulus, and thus may be called red–cyan (cyan being green plus blue) cells. This is in line with our finding that alternating red–blue stimulations were mainly effective at relatively low frequencies. The finding, that the most effective stimulus to elicit PPRs in our patients was red–blue alternating pattern at 15 Hz, suggests that this sort of stimulation would recruit a more extensive population of neurons spread of a wider cortical space than white or single-colour stimuli. Thus, activation of the ventral occipital cortex including the foveal representations of visual field maps hV4, VO-1 and VO-2 (Brewer et al., 2005) and probably MT+ would play a critical role in the genesis of these discharges in these patients. These considerations are also in agreement with the hypothesis of a selective participation of specific visual pathways in the genesis of the PPRs, being the one mediated by the parvocellular pathway by far the strongest (Harding and Fylan, 1999).

The finding of a greater latency with coloured stimuli deserves some comments, as it may seem surprising. Note that only stimuli that triggered a PPR had latency to be computed. If the stimuli did not elicit a PPR, no latency
was computed. The data of Figure 3 and Table 2, show that colour stimulation, triggered more PPRs between 10 and 15 Hz than white light. Above this frequency, between 20 and 30 Hz, all the single-colour stimulations were as provocative as white light, whereas alternating colours were significantly less provocative. The latency of the PPRs at 10 and 15 Hz frequencies was longer than the latency at 30 Hz. This suggests that chromatic stimuli represent an independent mechanism in the genesis of the PPR that is active at these low frequencies, which would fit with the properties of the parvocellular pathway. At higher frequencies, luminance would play a more active role in the genesis of the PPR.

Our findings show evidence for a synergistic effect in triggering PPRs for certain colour modulations, especially those combining red light with other colour. This effect was most evident with red-and-blue modulations at 15 Hz. Our findings suggest a truly synergistic effect of alternating flickering light of both colours, in the same line that was pointed out by others (Shirakawa et al., 2001). Red and green isoluminance patterns, on the other hand, have been previously reported not to evoke any epileptiform activity (Wilkins et al., 1979) and gratings of these colours did not affect the latency and amplitude of the visual evoked potential (Porciatti et al., 2000). These last authors demonstrated a contrast gain defect in a selected group of patients with idiopathic occipital epilepsy compared to normal controls, measuring the effects of sinusoidal gratings on the evoked potential, with a sub-threshold paradigm, i.e. without eliciting PPRs. They tested black and white, and red and green gratings. No other colours were tested. Obviously, the different methodologies used in this study and in ours do not allow a direct comparison of the results. Thus, it remains speculative in which way our patients do share the same mechanisms as those described by these authors. However, major neurophysiological differences must be taken into account when considering the role of colour in the contrast gain mechanisms, namely with respect to the different responses shown by the magnocellular and parvocellular pathway, being high for magnocellular cells at low contrasts, and low for parvocellular cells at all contrasts (Kaplan, 2004). We may relate these findings with the observations reported by Gardner et al. (2005). On event-related fMRI human studies, these authors showed that while cortical areas V1–V3 presented decreases or increases in BOLD responses to contrast increments or decrements, respectively, area V4 (hV4), the cortical area that seems to play a major role in colour processing, responded always with positive responses to both contrast increments and contrast decrements.

The role of the modulation depth of the stimulus

Our results also emphasize the role of MD, an important parameter for the occurrence of PPRs as we already showed in a previous study (Parra et al., 2005). The use of this intrinsic parameter of the stimulus should be added to improve the current guidelines over visual material to avoid the occurrence of seizures (Harding and Takahashi, 2004; Rubboli et al., 2004; Harding et al., 2005). The implementation of such an invariant parameter may help to adapt the current guidelines to the increasing diversity of audiovisual platforms each of them with different properties regarding size, luminance and chromatic spectra.

Clinical value: tinted lenses

Finally, the definition of the colour sensitivity in subjects with photosensitive epilepsy goes beyond the academic purpose of defining the pathophysiology of this reflex epileptic disorder as it represents an important additional tool in the treatment of patients with PSE (Takahashi and Tsukahara, 1992; Wilkins et al., 1999; Kepecs et al., 2004; Capovilla et al., 2006). Our results support the overall prevalent view that lenses that filter out red light (such as those in blue tones) would be the more efficient ones, although the results should be individualized per patient. Studies over the long-term effects of the prolonged use of such lenses are still necessary.

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