**INTRODUCTION**

Synaesthesia is a heterogeneous phenomenon (Baron-Cohen et al., 1987; Cytowic, 1989; Grossenbacher and Lovelace, 2001; Emrich et al., 2002; Rich and Mattingley, 2002) in which, specific events in one sensory modality involuntarily and automatically induce experiences in another. There is robust experimental evidence for the specificity and stability of synaesthetic experiences (Baron-Cohen and Harrison, 1997; Cytowic, 1989). However, recent psychophysical findings suggest that mechanisms of attention also influence synaesthetic experiences (Rich and Mattingley, 2003) and that synaesthesia depends on the conceptual level of representation (Ward and Simner, 2003).

In this study, we focus on colour-graphemic synaesthesia, a condition in which synaesthetes report vivid colour experiences induced by written letters. Our working hypothesis is that these colour experiences are associated with the activation of these parts of the visual cortex that are held responsible for the processing of colour information (Nunn et al., 2002). In the literature these areas have been identified as V4 (Zeki and Marini, 1998; Bartels and Zeki, 2000) or V8 (Hadjikhani et al., 1998). Based on the evidence that these areas (V4/V8) are relevant for colour perception (Schoenfeld et al., 2002; Morland et al., 1999), we focused on a region of interest (ROI) analysis of this specific area.

Recent functional imaging studies have investigated the activation of the human colour centre in the context of coloured-hearing, another form of synaesthesia. In this case spoken words induce colour experiences (Baron-Cohen et al., 1987). Using Positron Emission Tomography (PET) Paulesu et al. (1995) observed activation of visual association cortex in coloured-hearing synaesthetes. However, in this study no significant activation of lower visual areas, including areas V1, V2 or V4/V8 has been detected. Taking advantage of the greater spatial resolution and sensitivity of fMRI, Nunn et al. (2002) repeated the experiments by comparing brain activation patterns elicited by spoken words versus tones in synaesthetes and controls. Their findings revealed activation of V4/V8 in the left hemispheres of synaesthetes but not controls.

Using fMRI, Elias et al. (2003) demonstrated differences in brain activation during psychophysical tasks performed by a colour-graphemic synaesthete and a control. The synaesthete showed significant activation along the left dorsal visual stream (including Brodmann’s Area 19, 7, 39 and 40), while there was no such activation in the control. Whether colour-graphemic synaesthesia elicited by written letters is also associated with an activation of V4/V8 is unknown. The purpose of the present study was to resolve this question.

**AIMS AND DESIGN**

Using fMRI we investigated whether colour experiences of synaesthetes elicited by written
letters are accompanied by an activation of the human colour centre V4/V8.

**Retinotopic Mapping**

In order to determine the borders of V4/V8 for each of our subjects (Sereno, 1994; Hadjikhani et al., 1998) we applied the technique of polar-angle mapping of early visual areas as reported by Goebel et al. (1998).

**Colour Mapping**

Additionally we have used colour mapping in order to confirm that colour stimuli specifically activate the areas identified as V4/V8 in each of our subjects. Blocks of coloured and achromatic Mondrians were presented in alternation in an AB boxcar design (a modified version of the colour mapping experiment of Nunn et al., 2002), five of each type over 260 scans (520 sec). Luminance of coloured and achromatic Mondrians was adapted using a LiteMate/SpoteMate photometer system (Photo Research Inc., CA, USA). Ten Mondrians were presented in each block, each for 3 sec. These were followed by a grey isoluminant screen for 1 sec to avoid motion cues at transitions to the new patterns. Between each block a fixation cross was presented for 10 sec.

**Colour-Graphemic Synaesthesia**

In order to determine whether the colour centre identified by retinotopic and colour mapping is activated during the experience of colour-graphemic synaesthesia, we used an AB boxcar design with blocks of letters that elicited a synaesthetic colour experience (condition A) and blocks of letters that did not (condition B). Each subject gave us a list of three letters inducing colour-graphemic synaesthesia. Attempting to control for shape complexity, we additionally selected three letters that did not induce colour-graphemic synaesthesia but were similar in shape. These letters possessed achromatic synaesthetic qualities (the experience of transparent grey or anthracite). Blocks of the same type (A or B) were shown five times over 204 scans (408 sec). Ten letters were presented in each block in pseudorandom order, each for 2.5 sec, followed by a grey isoluminant screen for .5 sec. Between each block a fixation cross was presented for 10 sec.

**METHOD**

All fMRI measurements were performed with a 1.5 T MAGNETOM Vision MRI scanner (Siemens Medical Systems, Erlangen, Germany) equipped with a standard head coil.

For functional imaging, we used a gradient echo planar imaging (EPI) sequence [(TR/TE) = 2000 msec / 60 msec, (FA) = 90°, (FoV) = 200 × 200 mm², voxel size 3.13 × 3.13 × 3 mm³]. Each scan comprised the acquisition of 260 volumes (colour-mapping experiment) and 204 volumes (colour-graphemic synaesthesia experiment), respectively (one volume = 16 axial slices covering the occipital and inferior temporal cortex). A T1-weighted anatomical FLASH (fast low-angle shot) scan was recorded in the same session for each subject (matrix = 256 × 256; slice thickness 1 mm, 180 slices; voxel dimensions 1 × 1 × 1 mm). Data analysis, registration and visualisation were performed with the fMRI-software package BrainVoyager QX (Brain Innovation, Maastricht, the Netherlands) (Goebel et al., 1998).

**Anatomical Data**

**Talairach Transformation**

For each subject the 3D-FLASH recordings were transformed into Talairach space. This Talairach transformation was performed in two steps. At first the 3D data set of each subject was aligned with the stereotaxic axes by specifying the location of the anterior (AC) and posterior commissure (PC) manually. In the second step the
extreme points of the cerebrum were specified and used together with AC and PC coordinates to scale the 3D data sets into the dimensions of the standard brain of the Talairach and Tournaux atlas (1988).

**Surface Reconstruction**

The 3D-FLASH recordings were used for surface reconstruction of both hemispheres (Muckli et al., 2002; Kriegeskorte and Goebel, 2001; Linden et al., 1999). The white/grey matter border was segmented with a region-growing method. It was tessellated using two triangles for each side of a voxel located at the margin of white matter. The reconstructed surface was then subject to iterative corrective smoothing. This iterative morphing algorithm (Goebel et al., 1998) let the surface grow smoothly into the grey matter. The resulting surface was used as a reference mesh for the visualization of functional data. The iterative morphing algorithm was further used to inflate each hemisphere. Each inflated hemisphere possesses a link to the folded reference mesh so that functional data can be shown at the correct position of the inflated representation. Displaying functional maps on an inflated hemisphere permits the topographic representation of the three-dimensional pattern of cortical activation without loosing the lobular structure and the gyral patterns of the telencephalon.

**Functional Data**

Prior to statistical analysis, the functional data were preprocessed as follows: the temporal slice scan time shift was corrected by using the first scan time within a volume for alignment by linear interpolation in the following slices of that volume. A Talairach transformation (Prvulovic et al., 2002) was performed for the complete set of functional data of the subject (three functional runs), yielding a 4-D data representation (volume time course: 3 × space, 1 × time). The time-series of functional images was aligned in order to minimize the effects of head movements. The central volume of the time-series was used as a reference volume to which all other volumes were aligned, using a 3-D motion correction that estimates the three translation and three rotation parameters of rigid body transformation. Temporal smoothing was applied to EPI images with linear trend removal and temporal frequency-based (fast Fourier transform) high-pass filter of 3 cycles per time course.

The statistical analysis was based on the application of multiple regression analysis to time series of task-related functional activation (Friston et al., 1995). The general linear model (GLM) of the experiment was computed for each of the 3 volume time courses (colour mapping, colour-graphemic synaesthesia and retinotopic mapping).

**Polar Angle Mapping**

Retinotopy of polar angle was revealed with cross correlation analysis selecting the lag value resulting in the highest correlation value for a particular voxel. The obtained lag value finally determined the pseudocolour for that voxel as well as for corresponding polygons on reconstructed surfaces (Goebel et al., 1998; Muckli et al., 2002).

**Colour Mapping and Colour-Graphemic Synaesthesia**

In the colour mapping experiment, the signal values during the coloured and the isoluminant achromatic Mondrian stimuli were considered the effects of interest. In the colour-graphemic synaesthesia experiment the effect of interest were the signal values during the presentation of letters that did and did not induce synaesthetic experiences, respectively. The corresponding predictors, obtained by convolution of an ideal box-car response (assuming a value 1 for the volume of task presentation and a volume of 0 for the remaining time points) with a linear model of the hemodynamic response (Boynton et al., 1996), were used to build the design matrix of the experiment. To analyze the effect of conditions compared to baseline, 3-D statistical maps were generated by associating each voxel with the F value corresponding to the specific set of predictors and calculated on the basis of the least mean squares solution of the GLM. The obtained p values were then corrected using the false discovery rate (FDR) (Genovese et al., 2002). The statistical maps are based on a fixed effects analysis. Statistical results were visualized as 3-D statistical maps on a surface reconstruction of the subject’s brain. Effects were only shown if p (corrected for FDR) was < .05 for one of the conditions compared to baseline.

As ROI we defined the significantly activated clusters in left and right V4/V8 (as revealed by retinotopic mapping) which showed overlapping colour-specific activity in the colour mapping as well as in the colour-graphemic synaesthesia experiment.

We analyzed the signal of every ROI by first averaging the data (time courses) of all voxels constituting the ROI and then computing statistical parameters for the time course on the basis of the GLM. The ROI-GLMs were corrected for serial correlations within the ROI time course. The ROI-GLM results included the F value for the explained variance of the model (after the variance explained by the different signal levels had been removed) and the t value for the comparisons of the beta weights of each current predictor (effect of conditions compared to baseline). We also performed contrast analysis, based on the t test of differences (a priori alpha error of p < .05)
between the beta weights of both predictors to identify clusters that showed a higher activity for letters inducing synaesthesia when compared to letters not inducing such experiences (colour-graphemic synaesthesia experiment). The same contrast analysis was repeated to identify clusters that showed higher activity in the colour Mondrian condition than the achromatic Mondrian condition (colour mapping experiment).

In order to rule out whether a global BOLD change during the colour-graphemic synaesthesia condition led to a local change in V4/V8 we did a region of interest analysis on other visual areas as well (V1, VP). The published coordinates (V1, VP) of Hasnain et al. (1998) were used as a reference.

**RESULTS**

In all subjects the polar-angle retinotopic map permitted us to delineate V4/V8 in both hemispheres (see Figure 1 as example) and to confirm that the ROIs used for contrast analysis overlapped with area V4/V8 as identified with retinotopic mapping.

As exemplified in Figure 2 the significant BOLD signal changes for coloured Mondrians versus baseline (A) also overlapped with the significant BOLD changes, for letters inducing synaesthetic colour experiences versus baseline (B) (p < .05, corrected for FDR in all conditions).

Thus, regions identified as V4/V8 were selectively activated by coloured stimuli and the presentation of letters giving rise to the experience of colour.

The comparison of β-values between the condition that gave rise to the experience of colour and the control condition in other selected regions of the visual system (V1, VP) did not show consistent increase during the colour-graphemic synaesthesia condition comparable to the identified increase in V4/V8. Even a decrease during colour-graphemic synaesthesia can be reported in VP.

Tables I and II summarize the Talairach coordinates of the activated ROIs with the statistical values of the ROI-GLM and the results of the contrast analyses between the corresponding conditions.

In the colour mapping experiment the ROI-GLM based contrast analysis between coloured and achromatic Mondrians revealed a significantly higher BOLD-signal activation for the coloured Mondrian condition in both, left and right V4/V8. In the colour-graphemic synaesthesia experiment there was a higher BOLD-signal change in V4/V8 for the synaesthesia-inducing letters than for the letters not inducing the experience of colour. This direct comparison was significant (p < .05, after correction for serial correlations) in two subjects.

Additionally we found in both subjects (1 and 2) the frontal cortex, the insula, the superior and inferior temporal cortex to be activated in a contrast analysis between the letters evoking...
colour-graphemic synaesthesia and letters that did not (see Appendix for details).

Subject 3 only showed significant activation of the inferior frontal and inferior temporal lobe. There were no clusters with significant contrast in the data of subject 4. V4/V8 activation for subject 3 and 4 failed to reach the chosen level of significance in the contrast analysis after correction of serial correlations (data not shown). Direct group analysis was hampered because of insufficient spatial overlap of the ROIs.

**DISCUSSION**

**Cortical Activation during Colour-Graphemic Synaesthesia**

**Activation of V4/V8 during Colour-Graphemic Synaesthesia**

According to reports of synaesthetes their colour experiences resemble real colour percepts rather than colour imagery (Emrich et al., 2002). This
TABLE I
Statistical analysis of V4/V8 activation in colour-mapping and colour-graphemic synesthesia.

SUBJECT 1

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Area</th>
<th>Coordinates x</th>
<th>y</th>
<th>z</th>
<th>Model</th>
<th>F</th>
<th>Predictor 1 t</th>
<th>beta</th>
<th>Predictor 2 t</th>
<th>beta</th>
<th>Contrast t</th>
<th>p</th>
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<tbody>
<tr>
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<td>−68</td>
<td>−17</td>
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<td>3.51</td>
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<td>.29</td>
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<td></td>
<td>rV8</td>
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<td>−17</td>
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<td>4.33</td>
<td>1.09</td>
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<td>−2.528</td>
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<th>z</th>
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<th>Contrast t</th>
<th>p</th>
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<td>5.62</td>
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<td>rV8</td>
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<td>−15</td>
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<td>3.14</td>
<td>3.58</td>
<td>4.021</td>
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Talairach coordinates for centres of mass of ROIs and ROI-GLM results of the different conditions versus baseline: Predictor 1 (colour mapping) = coloured Mondrians versus baseline; Predictor 2 (colour-mapping) = achromatic Mondrians versus baseline. Predictor 1 (colour-graphemic synesthesia) = colour-graphemic synesthesia-inducing letters versus baseline; Predictor 2 (colour-graphemic synesthesia) = non colour-graphemic synesthesia-inducing letters versus baseline. ROI-GLM results included statistical values for the explained variance of the model (F values; p < .05, corrected for serial correlation), the beta weights for each predictor (t values; p < .001 corrected for serial correlation) and contrast analysis between the two predictors in each experiment (t values; significant at p < .05). Each selected cluster comprised an area of max 5 × 5 × 5 mm in Talairach space.

raises the question how true colour vision, colour imagery and synaesthetic experiences differ in their neuronal manifestation (Rich and Mattingley, 2002).

If synaesthetic colour-graphemeric experience is in fact similar to true colour percepts, one should expect activation of the human colour centre during the experience of colour by synaesthetes.

In the PET experiment of Paulesu et al. (1995) synaesthetic colour-graphemeric experiences have been found to be associated with activation of regions of the visual cortex addressed as visual association cortex. However, the low resolution and sensitivity of PET (Aine, 1995) precluded identification of the exact areas of the visual cortex. Data had to be subject to group-analysis, and because the position of the colour centre in the human brain differs between subjects (Bartels and Zeki, 2000), precise location of the expectedly small activation foci was not possible. In contrast to these PET data the fMRI-findings of Nunn et al. (2002) suggest that synaesthetic colour experiences are associated with activation of earlier levels of visual cortex. In the condition of coloured hearing these authors found activation of the human colour centre in the left hemisphere. Elias et al. (2003) demonstrated that activation patterns in a colour-graphemeric synaesthete and a control differed during psychophysical experiments including a dice arithmetic task and an eyes-closed addition task. However, the design of their study did not enable them to detect activation in early visual areas.

In the present study we first applied retinotopic colour mapping in order to confirm that colour stimuli specifically activate area V4/V8. We then compared activation patterns induced by letters that did or did not evoke synaesthetic experiences and found that the former led to a significantly higher activation of V4/V8 than the latter. These findings support the hypothesis that the grapheme-induced colour experiences in synaesthesia arise from a coactivation of the colour areas V4/V8 of extrastriate visual cortex. Interestingly, V4/V8 is not differentially activated in colour imagery tasks (Howard et al., 1998). Thus, the neural substrate of synaesthetic colour-phonemic as well as colour-graphemeric experience seems to be different from that supporting colour imagery. The fact that synaesthetic experiences go along with activation of an area that is at a low level of the processing hierarchy suggests that synaesthetic experiences are closer to hallucinations (ffytche et al., 1998) than to colour imagery. As shown in a recent fMRI study in schizophrenic patients (Diersk et al., 1999) auditory hallucinations are associated with activation of early sensory areas, in this case the primary auditory cortex.

**Lateralization**

Analysing colour-phonemic synaesthesia, activation of visual cortex was restricted to the left hemisphere (Nunn et al., 2002). The authors hypothesized that normal colour perception competes with synaesthetic perception in left V4/V8. In our study activation was equally significant in V4/V8 of both hemispheres in Subject 1 and 2. Further experiments with lateralized presentation of the visual stimuli might resolve this issue.

**Differences between Subjects**

Two of our subjects (subjects 1 and 2) showed significant activation of V4/V8 after correction for serial correlations. Interestingly these subjects reported to perceive a screen in their mind’s eye which gets completely coloured whenever seeing a letter inducing synaesthesia. The two other subjects
Concluding Remarks

This study is the first demonstration of the coactivation of the human colour centre in colour-graphemic synaesthesia. It confirms and extends previous evidence obtained with colour-phonemic synaesthesia (Nunn et al., 2002). A challenge for future studies of synaesthesia will be the identification of the pathways that mediate this activation of early visual areas and to determine whether this coactivation occurs before or after semantic decoding of the colour inducing stimuli. Applying advanced imaging techniques such as event-related fMRI with high temporal resolution might be one of the options to resolve these questions.
REFERENCES


Addendum

After our manuscript was accepted for publication we learned of the paper by Hubbard et al. (Neuron, 45: 975-985, 2005) reporting similar findings. For your information, the full reference is: Hubbard EM, Arman AC, Ramachandran VS and Boynton GM. Individual differences among grapheme-color synesthetes: Brain-behavior correlations. Neuron, 45: 975-985, 2005.
### APPENDIX

**TABLE III**
Additionally activated areas (Contrast map); Subject 1

<table>
<thead>
<tr>
<th>Area</th>
<th>Cluster size</th>
<th>Coordinates</th>
<th>Model</th>
<th>Contrast</th>
</tr>
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<tr>
<td>IFG</td>
<td>745</td>
<td>–32 48 19</td>
<td>20.187</td>
<td>6.358 .00</td>
</tr>
<tr>
<td></td>
<td>679</td>
<td>30 49 19</td>
<td>5.173</td>
<td>2.971 .003329</td>
</tr>
<tr>
<td>Insula</td>
<td>703</td>
<td>–41 8 10</td>
<td>9.179</td>
<td>4.214 .000038</td>
</tr>
<tr>
<td></td>
<td>711</td>
<td>44 7 0</td>
<td>3.904</td>
<td>2.782 .005930</td>
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<tr>
<td>STG</td>
<td>790</td>
<td>–48 10 4</td>
<td>2.618</td>
<td>2.286 .023293</td>
</tr>
<tr>
<td></td>
<td>541</td>
<td>52 14 –2</td>
<td>5.435</td>
<td>3.111 .002137</td>
</tr>
<tr>
<td>ITG</td>
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<td>–56 –15 –16</td>
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**TABLE IV**
Additionally activated areas (Contrast map); Subject 2

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<th>Model</th>
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<td>IFG</td>
<td>104</td>
<td>–44 19 7</td>
<td>5.694</td>
<td>3.086 .002317</td>
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<td></td>
<td>378</td>
<td>42 40 18</td>
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<td>Insula</td>
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<td>39 3 4</td>
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<tr>
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<td>ITG</td>
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**TABLE V**
Additionally activated areas (Contrast map); Subject 3

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Talairach coordinates for centres of mass of clusters with significant contrast between different conditions: Predictor 1 (colour-graphemic synesthesia) = colour-graphemic synesthesia-inducing letters versus baseline; Predictor 2 (colour-graphemic synesthesia) = non colour graphemic synesthesia-inducing letters versus baseline. ROI-GLM results included statistical values for the explained variance of the model (F values; p < .05, corrected for serial correlation), the beta weights for each predictor (t values; p < .001 corrected for serial correlation) and contrast analysis between the two predictors in each experiment (t values; significant at p < .05). Each selected cluster comprised an area of max 5 × 5 × 5 mm in Talairach space. IFG (inferior frontal gyrus), STG (superior temporal gyrus), ITG (inferior temporal gyrus).