# Technical report: Event-related fMRI adaptation paradigm on real and synesthetic colors

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## The Neural Bases of Grapheme-Color Synesthesia Are Not Localized in Real Color-Sensitive Areas

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## Introduction

Do the subjective experiences of real and synesthetic colors share the same neural representations of colors? A positive answer predicts that the 'normal' color system should be activated during the experience of synesthetic colors. The spatial resolution of fMRI is limited to the hemodynamic response of 'voxels', summarizing the energy demands of many thousands of neurons. In principle, fMRI results cannot decide whether the same neurons are involved in different processes. However, fMRI 'adaptation' protocols can suggest whether it is the case or not: the idea is that a decrease of the BOLD signal to the repetition of two different stimuli indicates an adaptation of the neuronal response. Such adaptation happens only if the neurons respond equally to the two stimuli – in our case that would be an equal response to e.g. real red and synesthetic red. Adaptation effects measured with fMRI are small and require performing analyses within regions of interest (ROI). The preliminary step was therefore to identify with fMRI the 'normal' color system.

Adaptation protocol is a method to test functional specificity with fMRI. Such a protocol is based on two properties: neuronal adaptation and linear summation of BOLD signals. Consider two populations of neurons sampled by two distinct voxels, the first population containing neurons coding colors irrespective of the stimulus shape while the second responding to shapes irrespective of their color. If a given colored shape (say a red shape) is presented twice in a row, neurons of both populations would fire less at the second presentation (due to adaptation). Now if the second stimulus has the same shape, but is green, neurons in the 'color voxel' that are selective to red will not respond anymore, but neurons selective to green will, with no adaptation. The induced summed hemodynamic response will therefore be larger than when the red shape was presented twice. In the 'shape' voxel however, the same neurons will respond to both the red and the green shapes, but less to the second stimulus because it has the same shape (adaptation), so the total BOLD signal will be smaller. For example, Kourtzi and Kanwisher (Kourtzi and Kanwisher 2001) applied this idea successfully to show specific adaptation to shape in the lateral occipital cortex (LOC), irrespective of contour information. To our knowledge, adaptation protocols have not been yet strictly tested on color selectivity. Cant and colleagues (Cant et al. 2009) and Cavina-Pratesi and colleagues (Cavina-Pratesi et al. 2010) used a modified version of the adaptation protocol on color, namely 'release of adaptation', where they compared the response to the exact repetition of the same stimulus to the response when changing only one attribute (like color). While the logic sounds similar, the protocol is

much less controlled, since for the observer there is either the repetition of the same stimulus or a stimulus change. It seems difficult to be able to control the attentional level over such different conditions. Moreover, when changing only the color of the stimulus, all neurons that are sensitive to color (for example neurons in V1 and V2) are going to show some release of adaptation, but that does not mean that these very neurons are going to respond equally to stimuli of the same color with different shapes – which is a much more stringent test of color coding. Mur and colleagues (Mur et al. 2010) have compared the results obtained with adaptation and release of adaptation protocols in a study on face identity. Not surprisingly, they observed much more widespread 'activations', some bearing little functional meaning, for the release of adaptation protocol.

Here we used the original adaptation protocol within an event related design, as described by Kourtzi and Kanwisher (Kourtzi and Kanwisher 2000). We designed a first protocol with pairs of colored pseudo-graphemes. Pseudo-graphemes in a pair were always different, but the color was the same or different. We also used this protocol to test adaptation to synesthetic colors. For each subject, we chose pairs of graphemes that had approximately the same synesthetic color. Fortunately, synesthetes had often such exact pairs (typically, a number would have the exact same synesthetic color as a vowel). Pairs were therefore always composed of different achromatic graphemes, leading to either the same or different synesthetic color. Finally, we designed a protocol mixing real and synesthetic colors, to be able to test whether there are neurons that code a color irrespective of whether it is real or synesthetic. Pairs then combined an achromatic grapheme with a colored pseudo-grapheme. Exact color matches of the synesthetic colors were performed in the scanner by the synesthetes before the recording sessions.

## Materials and Methods

*Subjects*

We tested the adaptation protocol on our ten synesthetes, but we included only nine of them in the analysis, excluding our color blind subject because we could not be sure of the matching between real and synesthetic colors; in his case, we could not be sure that the event-related protocol was well balanced across conditions.

*Stimuli*

The color, photism, and mixed protocols were combined. We had seven conditions: 'color' (real, synesthetic or mixed) was the same or different, and we had a 'null' condition (the fixation point briefly disappearing and reappearing). We used four possible colors (typically red, green, blue or yellow), whose exact hues and contrasts were chosen by each synesthete, depending on her/his synesthetic associations. Two pseudo-graphemes were associated to each color (to parallel the association of two graphemes to a specific synesthetic color). The design was perfectly balanced, in the sense that each condition was presented exactly 48 times, and all real and synesthetic colors were presented the same number of times, either in the first or the second position of the pair. This was very important for the design, since colors were not isoluminant and differed across subjects. A stimulus sequence with 24 repetitions of each condition was generated randomly but selected before the experiments so that trials from each of the seven conditions followed the other conditions equally often (Kourtzi and Kanwisher 2000). Balance across conditions was only approximately achieved for two trials back (Kourtzi and Kanwisher 2001), and the further preceding history of conditions was random. In order to correct for these minor imbalances, the sequence was also played backward. Each of both sequences was split in two runs, for a total of 4 scans each lasting 230s. Each scan started and ended by 10s showing only the fixation point. Each condition lasted 2.5s (duration was 2s in Kourtzi and Kanwisher, 2000 and 3s in Kourtzi and Kanwisher, 2001). Each image of a pair was presented for 300ms, with a blank interval of 400ms, similarly to Kourtzi and Kanwisher (Kourtzi and Kanwisher 2000, 2001). We verified for each synesthete that such a rapid presentation was still slow enough to generate systematically synesthetic colors.

*Attentional task*

We asked subjects to press a button each time a particular color (red, green, blue or yellow) was presented once and only once, whether it was the real or the synesthetic color. The subject had therefore to pay attention equally to the first and the second stimulus of each pair, and to both real and synesthetic colors. Since there were four runs, each color was once the target color. The synesthete read the instruction with each color to track before each run. They had been given a few practice trials outside of the scanner before the experiment. All synesthetes succeeded the task easily in the scanner (as verified by the recording of button presses) and enjoyed it. A non-synesthete can perform such a task only with much training (after learning by

heart the 8 synesthetic associations), the design leading to confusions when synesthetic colors are not perceived.

*Data analysis*

We computed the beta weights (corrected for serial correlations) in Brain Voyager for each condition in each individual color ROI (defined after the Mondrian protocol) as well as in retinotopic V4. To compute PSTHs, we extracted the BOLD signal of each z-score normalized run around each stimulus presentation, but we did not remove any baseline before the stimulus. All traces were reasonably close to zero around time zero (see Figures 1 and 2), meaning that our stimulus sequence was well enough balanced.

## Results

We first computed the PSTH for the seven conditions in retinotopic V4. Figure 1 shows that V4 response is the strongest for colored stimuli, not surprisingly. But we did not observe any color adaptation effect: the BOLD signal should have been weaker when the same color was repeated (red curve, to compare with the green curve with dark blue error bars), which was not really the case.

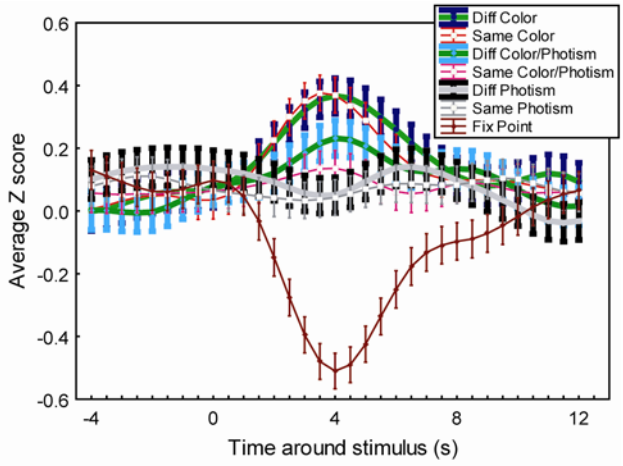


**Figure 1: average BOLD signal in retinotopic V4 for the Adaptation Protocol. Data averaged on both sides (we did not observe any difference) and across 9 synesthetes. Error bars represent 95% confidence intervals. Time zero corresponds to the beginning of the presentation of a pair of stimuli. The stimuli comprised either 2 colored pseudo-graphemes presented successively, 2 achromatic graphemes (generating synesthetic colors), or one grapheme and one colored pseudo-grapheme (mixed condition). The color (whether real or synesthetic) was either identical or different.**

We computed statistics on V4 beta weights of individual subjects. Table 1 displays the results for each critical comparison (same vs. different color, positive F values mean a stronger response for different colors). Non-Parametric Wilcoxon tests gave very similar values.

**Table 1: statistics on beta weights for color adaptation effects in retinotopic V4**

| Test | d.o.f. | F value | P value | $_p\varepsilon^2$ |
|---|---|---|---|---|
| Color | (1,8) | 0.41 | 0.54 | 0.05 |
| Mixed color & photism | (1,8) | 1.59 | 0.24 | 0.17 |
| Photism | (1,8) | 0.003 | 0.96 | 0 |

The lack of color adaptation in V4 means that we are not sure that colors are coded within V4 as an attribute independent of form. It is therefore not surprising that there is little or no response to synesthetic colors in retinotopic V4, as well as no adaptation to synesthetic colors.

We also did not observe any significant color adaptation in the average of 'color hot spots', whether within V4 or anterior to it (not shown). We did however observe a weaker response to repeated colors in some of these ROIs, for each subject. Since color ROIs were different across subjects, we could not select any given ROI (like the right VO1, for example) for adaptation across subjects. But we selected for each subject the ROI showing the greater suppression to color repetition, in order to test it for synesthetic response (Table 2). We reasoned that if we could select that way the region most specific to color perception in each subject, we may be able to observe some response and adaptation to synesthetic colors.

**Table 2: individual selected ROIs showing the strongest color adaptation.**

| Subject | VOI | t-value for color | Same Color | Diff Color | Same Photism | Diff Photism | Same Mixed | Diff Mixed | Fix Point |
|---|---|---|---|---|---|---|---|---|---|
| syn03 | V4r | 1.281 | 0.533 | 0.744 | 0.359 | 0.402 | 0.324 | 0.458 | -0.122 |
| syn04 | mondrian_VO2r | 0.286 | 0.622 | 0.669 | 0.325 | 0.323 | 0.312 | 0.311 | 0.345 |
| syn05 | V4l | 0.042 | 0.707 | 0.713 | 0.824 | 0.590 | 0.907 | 0.757 | 0.321 |
| syn07 | mondrian_VO2r | 0.433 | 0.560 | 0.624 | -0.155 | 0.155 | 0.315 | 0.409 | -0.123 |
| syn08 | mondrian_VO1l | 1.173 | 0.721 | 0.914 | 0.429 | 0.633 | 0.581 | 0.818 | 0.202 |
| syn09 | mondrian_VO2r | 1.234 | 1.179 | 1.341 | 0.504 | 0.765 | 0.883 | 1.044 | -0.049 |
| syn10 | mondrian_VO1r | 0.313 | 0.815 | 0.863 | 0.419 | 0.516 | 0.630 | 0.563 | 0.237 |
| syn11 | mondrian_V4r | 0.720 | 0.615 | 0.722 | 0.448 | 0.255 | 0.478 | 0.227 | -0.175 |
| syn12 | V4r | 0.493 | 0.471 | 0.548 | 0.164 | 0.370 | 0.068 | 0.220 | 0.032 |

**The second column in the table reports which Volume of Interest (VOI) had the stronger adaptation for colored stimuli, as indicated by the largest positive t-value (third column) when contrasting both color conditions (same vs. different colors). Area 'VO' was not defined retinotopically. Such a name was chosen by analogy with Brewer and colleagues (Brewer et al. 2005) and to give an indication of how anterior to V4topo the given ROI was (VO2, more anterior to V4 than VO1). The prefix 'Mondrian' means that the ROI was defined after the Mondrian protocol. The next columns report the beta weights, corrected for serial correlations, measured in those ROIs for each subject and condition. For further analyses, we excluded syn05 who showed no color adaptation at all, and syn04 who showed very little adaptation, together with as strong a response to the fixation point and to graphemes.**

Figure 2 shows the PSTHs obtained when averaging the response in the selected color ROIs of seven subjects (we did not include the ROIs of two subjects, as explained in the legend of Table 2; results were however similar when including these 2 subjects). We computed the PSTH for our three protocols.

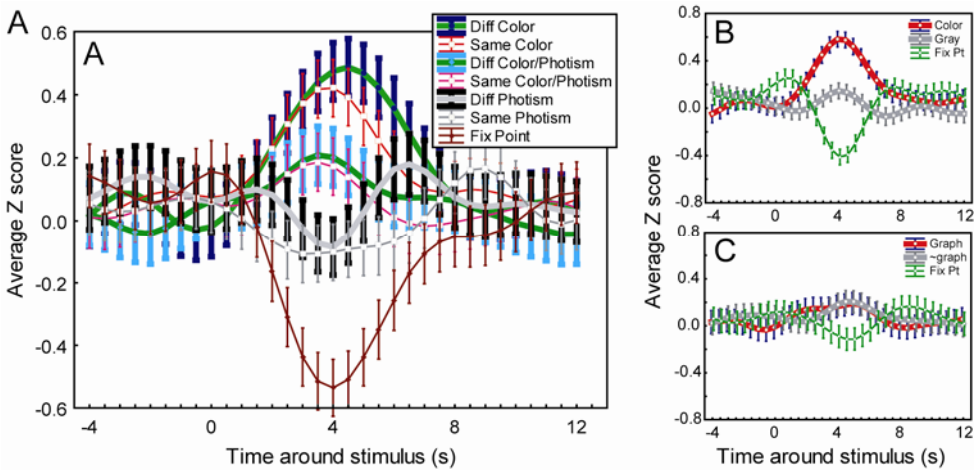


**Figure 2: average BOLD signal in the color ROIs indicated in Table 2 (7 subjects) for the adaptation, the Mondrian and the synesthesia protocols. A. The BOLD response is larger for different colors than repeated colors (first 2 curves), as expected. Remarkably, the time course of the difference is compatible with physiological adaptation, since only the late part of the red curve is below the green curve with dark blue error bars, meaning that the weaker BOLD response may be indeed due to a weaker BOLD response to the second stimulus of the pair when the color was the same (the peak response is delayed by about 500ms to 1s, while ISI was 700ms). B. The BOLD response is of course much larger for colored than gray Mondrian stimuli. C. The BOLD response is weak and similar for graphemes (inducing synesthetic colors) and pseudo-graphemes.**

For synesthetic colors (gray curves), the response looks weaker for repeated colors ('same Photism'), but the average time course difference does not look really compatible with adaptation mechanisms and failed to reach statistical significance (not by much, though: see Table 3). When mixing real and synesthetic colors, both response time courses are similar, but the late part of the BOLD signal is slightly larger for different colors. Beta weights are however not significantly different (Table 3)

**Table 3: statistics on beta weights for color adaptation in selected color ROIs**

| Test | d.o.f. | F value | P value | $_p\varepsilon^2$ |
|---|---|---|---|---|
| Color | (1,6) | 24.8 | 0.002 | 0.81 |
| Mixed color & photism | (1,6) | 1.07 | 0.34 | 0.15 |
| Photism | (1,6) | 4.25 | 0.085 | 0.41 |

The lack of significant adaptation for synesthetic colors may be due to a lack of power, since adaptation effects are weak and require both a strong BOLD response and averaging across

many subjects. We did not observe any specific response to synesthetic colors in those selected ROIs: the response to achromatic graphemes and pseudo-graphemes (measured during the 'synesthesia' protocols) were weak and similar (Figure 2, C). In order to test for adaptation effects of synesthetic colors, we would first have to localize regions that show a robust activation to synesthetic colors. Only then could we use the adaptation protocol in order to test whether such regions code synesthetic colors or other attributes related to the synesthetic experience. As a consequence, we did not run more subjects with this protocol.

## Discussion

This adaptation study did not reveal any clear adaptation for synesthetic colors in color ROIs. However, we also did not observe any systematic color adaptation in V4 or in color ROIs, so we could not test rigorously our hypothesis of adaptation across real and synesthetic colors. We tried an exploratory analysis by selecting in each subject the ROI that showed the stronger decrease for repeated colors compared to different real colors. Since the difference was never significant in individual subjects, and since we observed both increases and decreases depending on ROIs, such a post-hoc selection may just be random. Moreover, we decided to exclude two subjects where no ROI displayed any potential adaptation effect. Then we tested this tentative selection of color ROIs on the adaptation protocol with real and synesthetic colors. First of all, we were pleased to observe that the time course for the colored pairs was compatible with real adaptation effects – so our selection of ROIs might be meaningful. In addition, we did observe a weaker response for repeated synesthetic or mixed colors. The differences were not significant, but because of the lack of power (only 7 subjects) it is difficult to conclude either way. The weaker difference for mixed compared to synesthetic colors (Table 3) would argue against the hypothesis of the very same neurons being involved in both real and synesthetic colors. But all these conclusions are of course highly speculative.

To complete this analysis, we also performed statistical analyses on the critical part of the BOLD response rather than the full response as captured by beta weights. We used the color BOLD response to select the time points with the higher difference between same and repeated colors, that is around 6s after the presentation of the first grapheme. Figure 3 shows the average BOLD response for the different conditions.

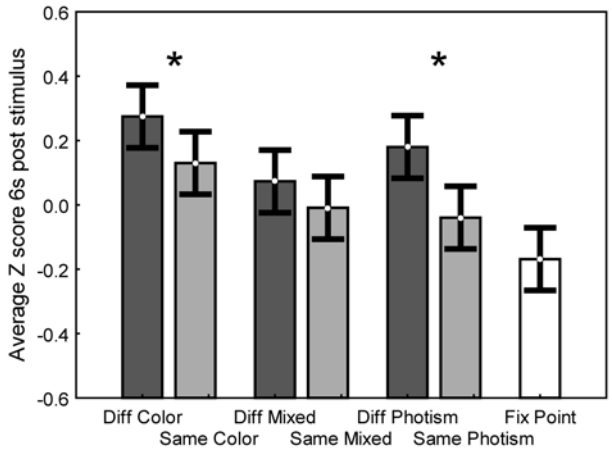


**Figure 3: average BOLD signal (+/- SEM) in the color ROIs indicated in Table 2 (7 subjects) 6s after the onset of the first grapheme. We computed an ANOVA on the responses of the seven conditions, with the variable subject considered as a random factor. There was a significant effect of the stimulus condition ($F(1,6)=8.7$, $p<0.0001$, ${}_p\varepsilon^2=0.59$). Post-Hoc comparisons revealed a significant difference between the first 2 conditions, as expected (LSD, $p = 0.04$), but also between the 2 photism conditions ($p = 0.002$), but not between the 2 mixed conditions ($p = 0.24$).**

Even though it would be tempting to conclude from that figure that regions that display specific color adaptation also display adaptation for synesthetic colors, such an interpretation is difficult to reconcile with the absence of specific response to graphemes in these regions (Figure 2 C). How can there be adaptation if there is no response? One may argue that ROIs are large enough so they can sample neurons that have different behaviors. The average signal may mask the signal while revealing adaptation. Such an interpretation is possible, but highly speculative, and should require further work using more refined techniques in order to be tested.

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