Supplementary Information for "Retinotopic activity in V1 reflects the perceived not the retinal size of an afterimage"

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1. Behavioral Experiments

Emmert's law

Subjects. Eight right-handed subjects (4 females; age range: 18-22 years old; mean = 19) with normal or corrected-to-normal visual acuity took part in this experiment. Informed consent was obtained from all participants according to procedures and protocols approved by the Research Ethics Board at The University of Western Ontario.

Experimental design. Emmert’s law was tested outside of the MRI scanner under natural viewing conditions. Using the same set-up as in the fMRI afterimage experiment (see Methods), subjects matched the perceived size of afterimages “projected” at different viewing distances to one of five circles presented on a computer monitor at a fixed viewing distance (57 cm) by pressing the corresponding keys on a PC keyboard (1, 2, 3, 4 or 5).

Results. The results reveal that the perceived size of the afterimage increased with viewing distance in a linear manner as one would predict with Emmert’s law ($r_{(3)} = 1.0$, $P < 0.0001$; see Suppl. Fig. 1). Such a finding is in agreement with previous work showing that under natural viewing conditions the perceived size of an afterimage is a perfect fit to Emmert’s law.

Suppl. Fig. 1. Emmert's law. Results from a behavioral experiment performed in the laboratory in which we had subjects report by means of a size matching task the perceived size of afterimages. As predicted by Emmert’s law, the data show a positive correlation between the mean perceived size and distance. A) Results for each subject at the different viewing distances. B) Group results for each of the different viewing distances.
Subjective description of afterimages

Subjects. Six right-handed subjects (3 females, age range: 23-32 years old, mean = 26.3) with normal or corrected-to-normal visual acuity took part in this experiment. Informed consent was obtained from all participants according to procedures and protocols approved by the Research Ethics Board at The University of Western Ontario.

Experimental design. We induced afterimages using the same set-up as in the fMRI afterimage experiment (see Methods) and asked the subjects what the afterimages looked like.

Results. All subjects consistently reported an afterimage of a greyish-purple filled circle no matter what viewing distance was used (see Suppl. Table 1).

<table>
<thead>
<tr>
<th>Subjective report</th>
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<tbody>
<tr>
<td>S1  Dark grey-black filled circle</td>
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<tr>
<td>S2  It's a blackish circle</td>
</tr>
<tr>
<td>S3  I see a purple solid circle</td>
</tr>
<tr>
<td>S4  I see a dark grey solid circle</td>
</tr>
<tr>
<td>S5  It's circular, it's kind of a dark-purplish colour</td>
</tr>
<tr>
<td>S6  It's a dark filled circle</td>
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Suppl. Table 1. Subject reports on the appearance of afterimages. We carried out a behavioral experiment in which we induced afterimages at various distances and asked subjects to describe what they saw. All subjects consistently reported an afterimage of a dark filled circle no matter what the viewing distance was.

2. Localizer Experiment to Define ROIs

Experimental design. This experiment defined regions-of-interest (ROIs) in V1, V2, and V3. E-Prime (Psychology Software Tools, Pittsburgh, PA, USA) was used to front-project videos of annuli that flickered at 8 Hz onto a screen placed at 53 cm from the subjects’ eyes. These videos were presented for blocks of 8 s every 22 s. The annuli were designed to locate ROIs in V1, V2, and V3 that corresponded to the five different eccentricities along the expected diameter of the induced afterimages. The diameter of each annulus was calculated using Emmert’s equation:

\[ s = d \cdot \tan \theta \]

where \( s \) is the apparent size of the afterimage, \( d \) is the apparent distance between the observer and the surface upon which the afterimage is “projected”, and \( \theta \) is the visual angle subtended by the afterimage. When referring to perceived (or apparent) distance rather than actual (or projected) distance, this relationship is formally equivalent to the size-distance invariance hypothesis (SDIH), a more recent account of size constancy. On the basis of this equation, we created videos of five flickering annuli that subtended 4.1°, 5.7°, 7.4°, 9° and 11.1° of visual angle. Each of the five different flickering annuli was presented five times in a counter-balanced design. This localizer experiment also

Nature Neuroscience: doi:10.1038/nn.3069
served as a training phase so that subjects could familiarize themselves with the five different sizes of the eccentricity annuli and subsequently use this knowledge as an index when making estimations of afterimage size during the afterimage experiment. The fMRI experiment consisted of one run with 290 volumes.

**Data analysis.** We carried out a parametric analysis in SPM8 to formulate the eccentricity ROIs. Namely, we examined the relationship between the BOLD response and the increasing preference for a particular eccentricity across different parametric conditions; in this case, the parameter estimates represented the covariation of the BOLD response with a linear measure. The *t* statistical maps obtained from these localizer contrasts were then superimposed over each subject’s anatomical MRI. We then created 8 mm³ cubic (i.e. one 2mm isotropic voxel) ROIs centered on the ‘hotspot’ for each contrast provided that the activity reached $P_{\text{uncorr}} < 0.001$ and fell within the boundaries of either V1, V2, and V3 as defined in the next experiment (see next section). Using these criteria, we were able to formulate all eccentricity ROIs in all of the subjects. Suppl. Fig. 2 illustrates the location of V1 ROIs that we created in one individual along the calcarine sulcus. As one would expect, the localized ROIs were found consistently in all subjects more posteriorly along the calcarine sulcus as the annuli decreased in radius.

**Suppl. Fig. 2. ROIs.** (a) Flickering annuli of five different eccentricities presented in blocks were used to define ROIs during a “localizer” run. We used Emmert’s Law to calculate the diameter of the annuli corresponding to the perceived size of the afterimage at each of the five different viewing distances. (b) The location of the different ROIs that were created in one subject (red, Ecc 1 = 4.1°; orange, Ecc 2 = 5.7°; green, Ecc 3 = 7.4°; blue, Ecc 4 = 9°; purple, Ecc 5 = 11.1°). Note that the ROIs were defined in a volume-based as opposed to a surface-based space and hence these circles are approximations of where we extracted the BOLD signal in this individual.
3. fMRI results in V2 and V3

We found that the retinotopic coding of an afterimage in V1 changed with perceived size even when the size of the retinal image remained constant: namely, V1 activation invoked during afterimages took place in regions of V1 representing the perceived but not the retinal image sizes. As shown in Suppl. Figs. 3 and 4, this was not the case for either V2 or V3.

Suppl. Fig. 3. Time course of the mean (±SEM) BOLD percent signal change relative to baseline for V2d and V2v. ANOVAs on the extracted time course revealed significant three-way Eccentricity ROI x Distance x Time Point interactions for V2d (F(224,1344) =1.623, p<.0001) and V2v (F(224,1344) =2.193, p<.0001). Asterisks (*) denote significant pair-wise comparisons after corrections were made for multiple comparisons using the Bonferroni method (P < 0.05). Note that the largest BOLD response to the inducing light occurred for the Ecc 1 ROI (the red curve) at all viewing distances. Thus, BOLD responses in V2d and V2v were mainly driven by the light. There were no clear indications that these areas responded in a way that reflected size constancy.
Suppl. Fig. 4. Time course of the mean (±SEM) BOLD percent signal change relative to baseline for V3d and V3v. ANOVAs on the extracted time course revealed significant (or close to significant) three-way Eccentricity ROI x Distance x Time Point interactions for V3d ($F_{(224,1344)} = 1.159$, $p=0.07$) and V3v ($F_{(224,1344)} = 2.133$, $p<0.0001$). Asterisks (*) denote significant pair-wise comparisons after corrections were made for multiple comparisons using the Bonferroni method ($P < 0.05$). There were no clear indications that these areas responded in a way that reflected size constancy.

4. Vergence Experiment

Experimental design. The procedures were similar to those performed during the afterimage experiment with the following exceptions: 1) we did not present a light; and, 2) we tested three as opposed to five viewing distances. Subjects closed their eyes for 4 s (during the same time period in which we turned on the light in the afterimage experiment) and then fixated for 26 seconds on a small fixation dot presented on the back screen. The back screen was placed at three different viewing distances (53, 97 and 144.5 cm). Each distance was repeated in a counter-balanced design five times in
each functional run. Subjects were cued through headphones about when to open and close their eyes. The experimenter was cued similarly through headphones about when and where to move the back screen. We used E-Prime to present auditory instructions to both the subjects and the experimenter. The fMRI experiment consisted of four runs with 288 volumes in each run.

**Results.** BOLD extracted from the ROIs in V1 for this experiment was compared with the afterimage experiment. ANOVA revealed a four-way interaction (Experiment x Eccentricity ROI x Distance x Time Point; $F_{(56,448)} = 1.542$, $p = 0.01$; Supp. Fig. 5). The results reveal that the BOLD response in V1 was not modulated in the same manner as it was in the afterimage experiment. Instead, the only effect that vergence seemed to have on V1 BOLD was a general suppression in neural activity as the eyes converged at closer distances (Suppl. Fig. 5).

![Suppl. Fig. 5. Effects of vergence compared to viewing afterimages at different distances.](image)

The panels show the time course of the mean (±SEM) BOLD percent signal change relative to baseline for the afterimage (top row) versus the vergence (bottom row) experiments. Note that, during the afterimage phase, particularly for Distance 5, there is a marked difference in the BOLD signal between the eccentricity ROIs (1 vs. 5) in the afterimage experiment but not in the vergence experiment. Asterisks (*) denote significant pair-wise comparisons after corrections were made for multiple comparisons using the Bonferroni method ($P < 0.05$).

5. **Simulated Afterimage Experiment**

**Experimental design.** The procedures were similar to those performed during the afterimage experiment with the following exceptions: 1) we did not present an inducing light; 2) we presented real stimuli that simulated the afterimages that subjects reported seeing in the afterimage experiment (i.e. the stimuli were scaled according to Emmert's Law) and, 3) we tested only three as opposed to five viewing distances. The real stimuli consisted of filled black circles that were glued to a removable screen. The sizes of the circles were 3.8 cm (small), 6.9 cm (medium), and 10.3 cm (big). Subjects closed their eyes for 14 s (to allow the experimenter time to change the viewing distance of the screen) and then fixated for 8 seconds on a small fixation dot presented in the centre of the circle. The screen
was placed at three viewing distances (53 cm for the small circle, 97 cm for the medium circle, and 144.5 cm for the big circle) so that retinal image size was maintained at 4.1 degrees of visual angle. Each distance was repeated in a counter-balanced design six times during each functional run. Subjects were cued through headphones about when to open and close their eyes and the experimenter was cued through headphones about when to change the stimulus and where to move the screen. We used E-Prime to present auditory instructions to both the subjects and the experimenter. The fMRI experiment consisted of four runs with 206 volumes in each run.

Results. BOLD signal was extracted from the same Eccentricity ROIs in V1 that were defined in the localizer experiment. ANOVA revealed a three-way interaction (Eccentricity ROI x Distance x Time Point; \(F_{(36,144)} = 4.496, p < 0.0001; \) Supp. Fig. 6). In contrast to the vergence experiment, the results reveal that the BOLD response in V1 was modulated in the same manner as it was in the afterimage experiment. As shown in Suppl. Fig. 6, pair-wise comparisons using the Tukey's HSD method to correct for multiple comparisons revealed that the BOLD response was greater for the small circle presented at the closest viewing distance in the smallest Eccentricity ROI as compared to the other Eccentricity ROIs. In contrast, Tukey's HSD pair-wise comparisons revealed that the BOLD response was greatest for the big circle presented at the furthest viewing distance in the biggest Eccentricity ROI. Although this experiment showed a similar spatial pattern of V1 activation as in the afterimage experiment, the results were not as robust in terms of levels of statistical significance (corrections using the more conservative Bonferroni method yielded only one significant pair-wise contrast in this experiment; see Suppl. Fig. 6). This could be related to a number of factors: 1) ROIs were defined on a separate day for some subjects, 2) the sample size was smaller, and 3) the afterimages are arguably better suited to examine size-constancy mechanisms than real stimuli given that they allow a number of extraneous variables to be more equally matched (e.g. luminance and retinal images).

Suppl. Fig. 6. BOLD extracted from the simulated afterimage experiment. The panels show the time course of the mean (±SEM) BOLD percent signal change extracted from each of our Eccentricity ROIs at different viewing distances. Note that each distance corresponds to a different physical size of the stimulus (i.e. a small circle at Distance 1, a medium circle at Distance 3, and a big circle at Distance 5) and that the retinal size was constant across all viewing distances (i.e. 4.1 degrees of visual angle). One asterisk (*) denotes significant pair-wise comparisons using the Tukey's HSD method for correcting for multiple comparisons and two asterisks (**) denote significant pair-wise comparisons using both the Tukey's HSD and Bonferroni methods for correcting for multiple comparisons.