

Supplementary Methods

We reanalyzed fMRI data that were collected for a previous study (Reeder et al., 2017). The raw data are publicly available to view and download on OpenNeuro (<https://openneuro.org/datasets/ds001306>). Subjects, stimuli, and experimental design were the same as in the previous study. fMRI preprocessing and analyses were unique to the current study. All experimental measures conformed to the Declaration of Helsinki and were approved by the research ethics committee of Otto-von-Guericke University Magdeburg.

Subjects

Seventeen right-handed healthy adults with normal or corrected-to-normal vision were recruited from the Universitätsklinikum fMRI subject pool in Magdeburg, Germany (age range=20-33 years, mean age=26.9 years, 8 women). They were all native German speakers and had participated in previous fMRI experiments unrelated to the current study. Prior to taking part in our experiment, they completed an fMRI screening questionnaire and provided written, informed consent to take part in the study. All received monetary reimbursement for their participation.

Stimuli

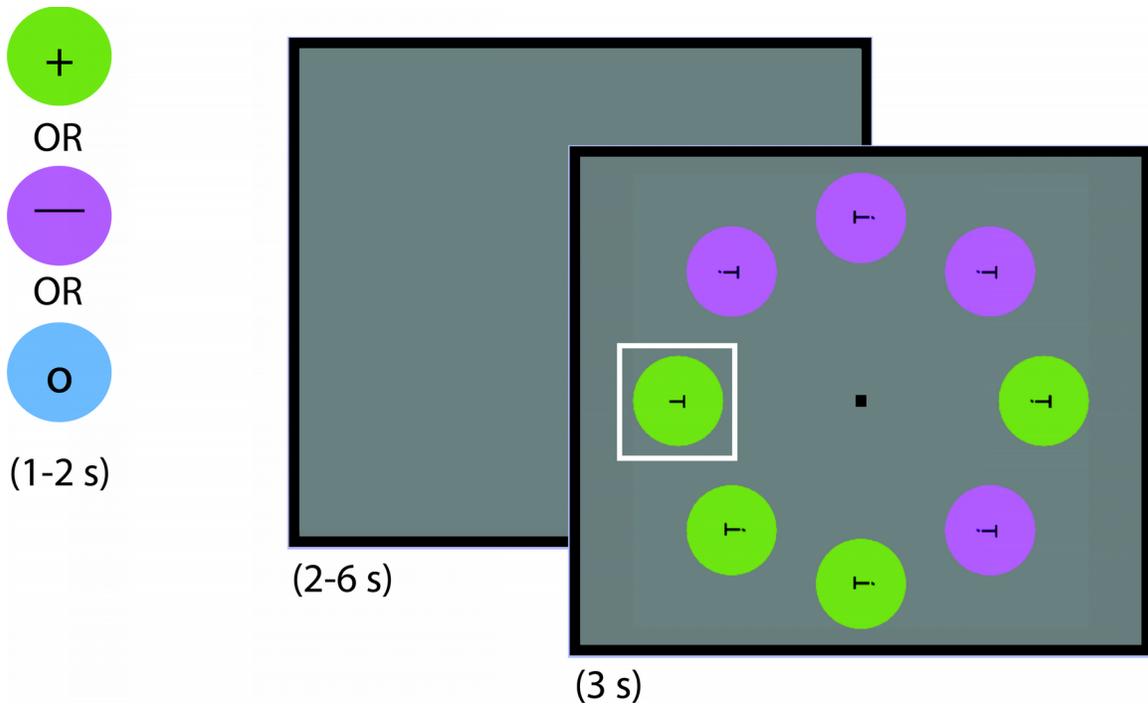
Stimuli were presented on a grey background back-projected onto an 18-inch display with a screen resolution of 1920×1080 pixels and 60 Hz refresh frequency. The distance from observers' eyes to the screen was 35 cm. The experiment was coded in Python using functions from the PsychoPy psychophysics toolbox (Peirce, 2007).

Cues, probes, and search stimuli were all colored discs. Discs presented as cues and search stimuli each had a radius of two degrees of visual angle, whereas discs presented as probe stimuli had a radius of four degrees of visual angle (probes were simply larger versions of the cues). Discs could appear in one of five possible colors: pink (RGB hex code: #FFB6C1), orange (#FFA500), chartreuse (#7FFF00), cyan (#00EEEE), or purple (#E066FF). Colors were matched for luminance with a photometer (Chroma Meter CS-200, Konica Minolta Sencing, 2005) prior to each fMRI session ($L_v = 90\text{-}100 \text{ cd/m}^2$). A search array was composed of 8 discs equally spaced around an imaginary circle with a radius of eight degrees of visual angle. Two colors appeared in a single search array, with four discs of each color randomly distributed in the array. Task instructions were black alphanumeric characters in Arial font “+”, “-”, or “o”. The characters were generated within an imaginary bounding box of a height of three degrees of visual angle, and each character appeared within the boundaries of the cue disc. For the characters that appeared in the search array, the longest line of the target letter “T” and distractor letters “Ṭ” (a T with a comma attached) appeared with a length of one degree of visual angle and were rotated 90 or 270 degrees. A black fixation point appeared centrally with a size of 0.5 degrees of visual angle.

Experimental Procedure

Subjects performed a visual search task (see Figure S1) in which they responded as fast and accurately as possible whether a letter T among Ts in a search array was rotated 90 (left button) or 270 degrees (right button). The distractors were almost identical to the target to ensure that pop-out did not occur for the target. Subjects were given the same task instructions: “The cues will help you to perform this task quickly. Please try to use the cues to the best of your ability during this task”. Subjects were monitored during practice trials to further ensure that they were able to use the cues (particularly the negative cues) correctly prior to entering the scanner.

Figure S1. The experimental paradigm. Cues could be positive (+), neutral (o), or negative (–) on a given trial. A jittered delay period followed the cue, during which subjects fixated a gray screen. Search arrays reflected the identity of the cue: if the cue was positive, the target appeared in the same colored disc as the cue; if the cue was neutral, the cued color did not appear in the search array; if the cue was negative, only distractors appeared in the same colored disc as the cue. A white box is shown around the target for visualization purposes in the figure, but this did not appear in the actual experiment. Stimuli are not to scale.



At the beginning of each experimental run, an instruction message appeared on screen indicating that subjects should press the left key when the letter T appears rotated to the left, and the right key when the letter T appears rotated to the right. All text appeared black against a grey screen and was presented at a height of one degree of visual angle. Following the block instructions, an fMRI trigger initiated the trial sequence.

Each trial started with a central fixation point for one second, followed by a central cue that appeared in one of the five possible colors. Each color appeared an equal number of times within a run but the order of the appearance of each color was randomized. One of three task instructions appeared overlaid on the cue color: “+” meant that the target “T” would appear within a disc of the same color in the search array with 100% validity; “-” meant that four of the “T” distractors would appear within a disc of the same color with 100% validity; and “0” meant that discs of the same color would not

appear in the search array. Each of the three task instructions appeared with each of the five cue colors twice within a run, for a total of 30 trials per run (10 trials per task condition). The cue/task instruction appeared for a duration restricted to values between one and two seconds, sampled pseudo-randomly from a list of 10 values following a logarithmic distribution.

Following the cue/task instruction, a central fixation point was presented on screen for a duration between one and two seconds, also sampled pseudo-randomly from a list of 10 values following a logarithmic distribution. These values were chosen independently of cue duration, to maximally separate BOLD signal elicited during the cue period and search period.

After fixation, a search display appeared for three seconds. If the task instruction was “+” or “-”, four discs in the search array appeared in the cued color and four appeared in a different color, chosen pseudo-randomly from a list of the remaining four colors. If the task instruction was “0”, one color was chosen pseudo-randomly from a list of the remaining four colors that were not the cued color, and then a second color was chosen pseudo-randomly from a list of the remaining three colors. This ensured that the cue color did not appear in the search array on these trials. If the task instruction was “+”, a rotated “T” appeared randomly within one of the four discs matching the cue color. If the task instruction was “-”, a rotated “T” appeared randomly within one of the four discs of the uncued color, and only “T”’s appeared in the discs corresponding to the cue color. If the task instruction was “o”, a rotated “T” appeared randomly in one of the eight discs in the search display.

Subjects were instructed to make a response on a two-button pad with their right

hand as fast and accurately as possible following the onset of the search array. Responses did not terminate a trial. Subjects completed one practice run outside the scanner to ensure they understood task instructions. Each run lasted five minutes and four seconds. All subjects performed six runs within the scanner, with approximately 30 minutes and 24 seconds total experiment time with 60 total trials per task condition.

fMRI data acquisition

Subjects were scanned on a 3 Tesla MAGNETOM Prisma (Siemens) with a 64-channel head coil (Head/Neck 64 Siemens) at the Universitätsklinikum in Magdeburg, Germany. fMRI data were collected using a simultaneous multislice sequence (TR, 2000 ms; TE, 30 ms; flip angle, 80°; matrix size, 106 × 106; FOV, 212 mm; 64 slices with interleaved acquisition, 32 shots, and multi-band acceleration factor of 2; 2 mm isotropic voxels; 10% interslice gap). A scanning session consisted of six runs of 310 seconds each. We also acquired T1-weighted MPRAGE scans (TR, 2500 ms; TE, 2.82 ms; flip angle, 7°; matrix size, 256 x 256; FOV, 256 mm; 192 slices, 1 mm isotropic resolution) from each subject.

fMRI preprocessing

fMRI preprocessing was performed anew in the current study to generate datasets in a format suited to performing RSA in PyMVPA. Raw functional images were motion corrected using FSL's mcflirt function (Jenkinson et al., 2002). Motion-corrected

functional data were then transformed into MNI standard space using FSL's `applywarp` function. Data were fitted with a canonical event-related hemodynamic response function (HRF) model with AR1 enabled using the PyMVPA function `fit_event_hrf_model` (Hanke et al., 2009). Each dataset was modeled with both cue period and search period events, with 15 conditions of interest for each period (5 colors x 3 cue types) across 6 experimental runs, for a total of 30 regressors. Model-fitted datasets were then detrended and z-scored per voxel for each run. Only cue period events were analyzed further with RSA.

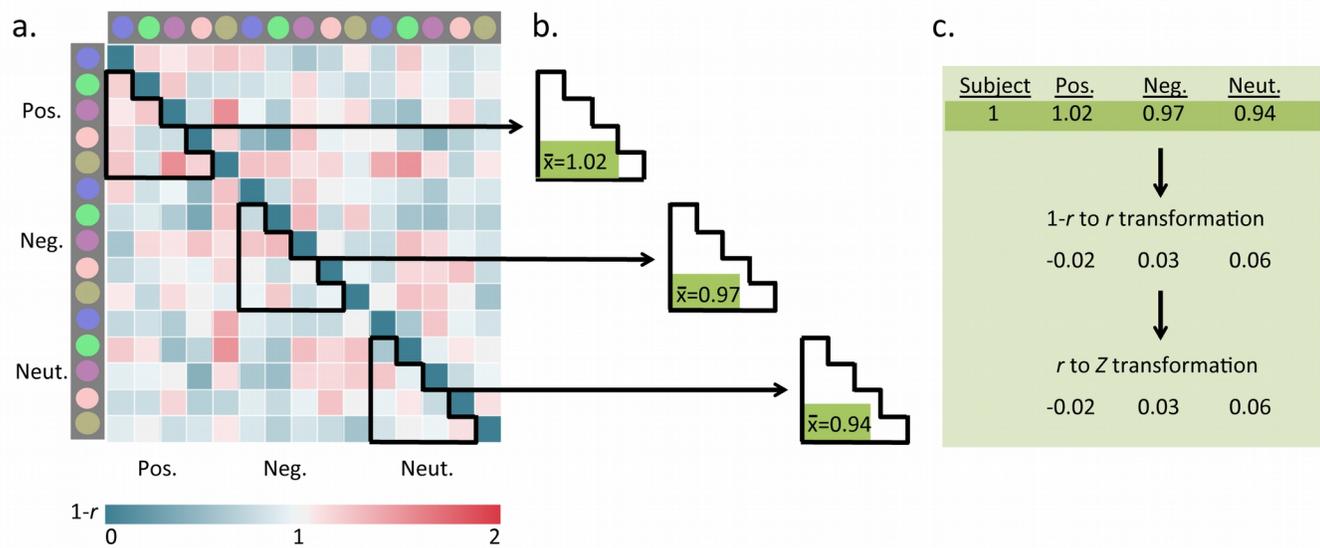
The EVC mask was reused from Reeder et al. (2017). The EVC mask was created by combining the bilateral masks for V1, V2, and V3v as defined by the Juelich Histological Atlas implemented in FSL (Jenkinson et al., 2012). The EVC mask was then thresholded to include only areas with >40% probability.

Representational Similarity Analysis (RSA)

RSA (Kriegeskorte et al., 2008) is a pattern analysis method that can determine similarities between patterns of activity for different conditions of interest. Conditions are input into a representational dissimilarity matrix (RDM), which encodes the correlation distance between all pairwise combinations of conditions. Correlation distance (1 minus the correlation, r) was used as the representational distance (RD) metric here. Therefore, values closer to 0 represented lower dissimilarity, and values closer to 2 represented higher dissimilarity. All functions used for the current analysis can be found in the PyMVPA toolbox (Hanke et al., 2009).

For each subject, we performed a sphere-based searchlight (2 mm³ voxels) on cue period data masked to EVC for every run to find all observed pairwise distances between conditions of interest (5 colors x 3 cue types = 15 conditions) for each sphere, using the `rsa.PDist` function with correlation distance set as the metric. We then calculated the mean RDM across runs for each sphere (the 2mm³ volume surrounding each voxel) using the `mean_group_sample` function. We then determined the most consistent RDM across runs using the `rsa.PDistConsistency` function, which was the final RDM used for further analysis (note that this RDM is not necessarily indicative of the most widespread pattern within EVC, but is rather the most stable pattern found within the region; Figure S2a). To obtain a single “color dissimilarity” value for each cue type from these matrices, we calculated the mean RD value from the lower triangle of each portion of the RDM corresponding to within-cue type comparisons (Figure S2b). We then obtained 3 color dissimilarity values (one for each cue type) for each subject that we could then input into statistical hypothesis tests after Fisher Z transformation (Figure S2c).

Figure S2. A schematic of the RSA implementation. a.) A sample representational dissimilarity matrix depicting the correlation distance ($1-r$) between the 15 conditions of interest in EVC. The diagonal depicts a $1-r$ of 0 (e.g., the correlation distance between condition 1 and condition 1 = 0). b.) The mean $1-r$ for the 5 colors presented within each cue condition. c.) The transformation from $1-r$ to r to Z .



Supplementary References

Hanke, M., Halchenko, Y. O., Sederberg, P. B., Hanson, S. J., Haxby, J. V., & Pollmann, S. (2009). PyMVPA: a python toolbox for multivariate pattern analysis of fMRI data. *Neuroinformatics*, 7(1), 37-53.

Jenkinson, M., Bannister, P., Brady, J. M., & Smith, S. M. (2002). Improved optimisation for the robust and accurate linear registration and motion correction of brain images. *NeuroImage*, 17(2), 825-841.

Jenkinson, M., Beckmann, C.F., Behrens, T.E., Woolrich, M.W., Smith, S.M. (2012). FSL. *NeuroImage*, 62, 782-790.

Kriegeskorte, N., Mur, M., & Bandettini, P. (2008). Representational similarity analysis—connecting the branches of systems neuroscience. *Frontiers in Systems Neuroscience*, 2.

Peirce, J. W. (2007). PsychoPy—psychophysics software in Python. *Journal of Neuroscience Methods*, 162(1), 8-13.

Reeder, R. R., Olivers, C. N., & Pollmann, S. (2017). Cortical evidence for negative search templates. *Visual Cognition*, 25(1-3).