

# Beyond the salience network: threat-induced brain activation unfolds from salience to default mode regions

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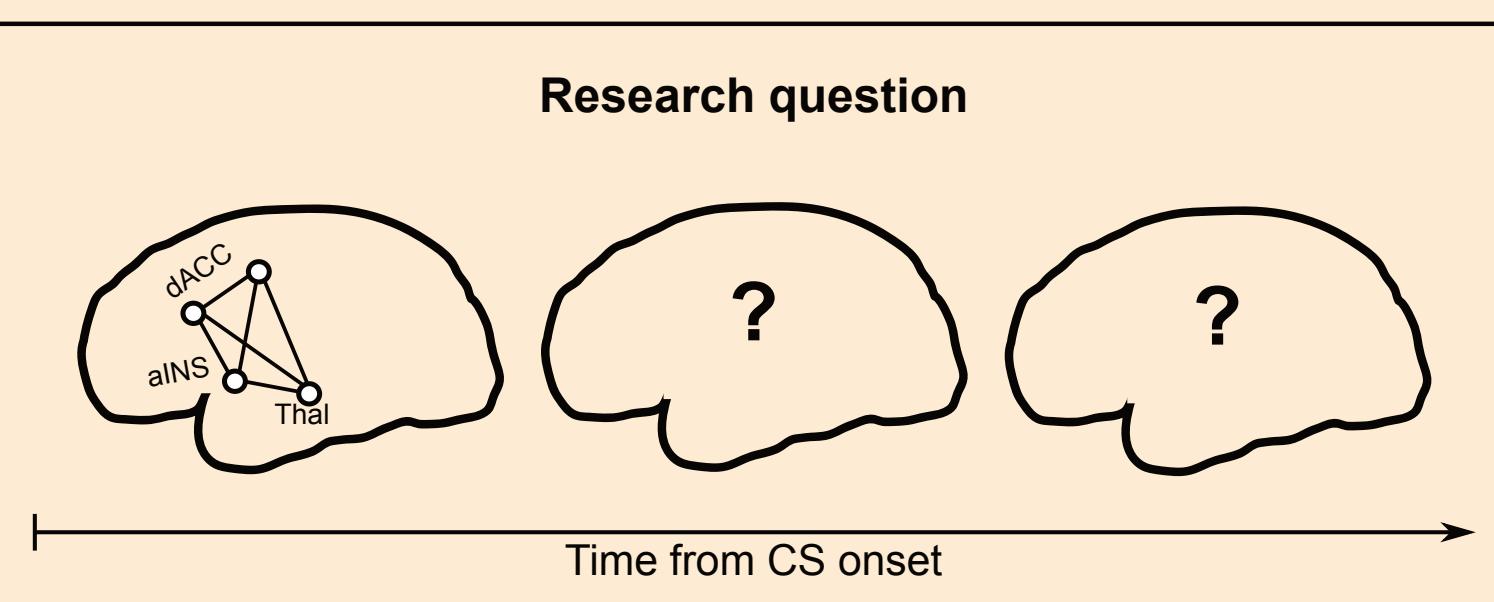
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## Introduction

Fear conditioning is a type of learning that involves pairing an aversive stimulus (the unconditioned stimulus, UCS) with a neutral stimulus (the conditioned stimulus, CS). As a result, CS alone elicits a fear response.

fMRI research has shown that CS co-activates subcortical and cingulo-opercular regions, comprising the salience network [1]. Here, we explore whether CS elicits subsequent activity beyond the salience network, indicating multiple levels of CS representation expressed in a sequence of co-activation patterns.

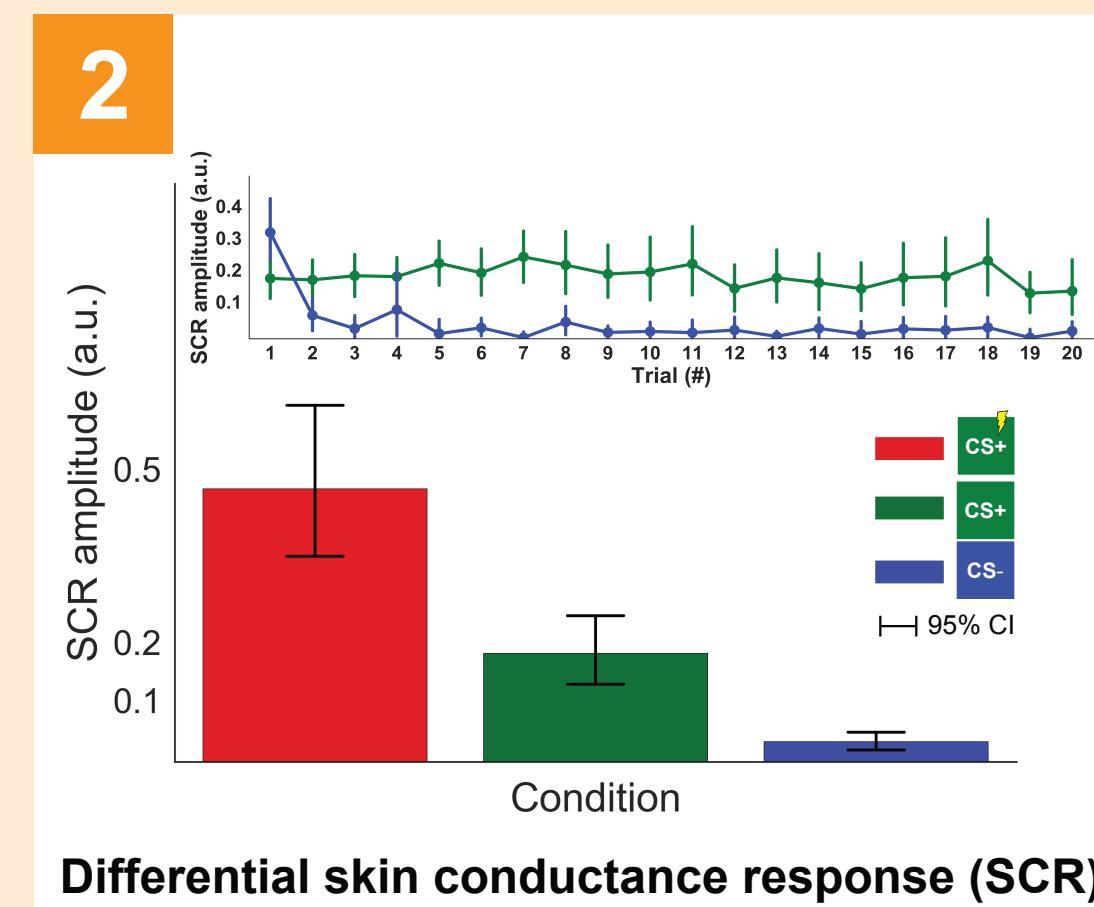
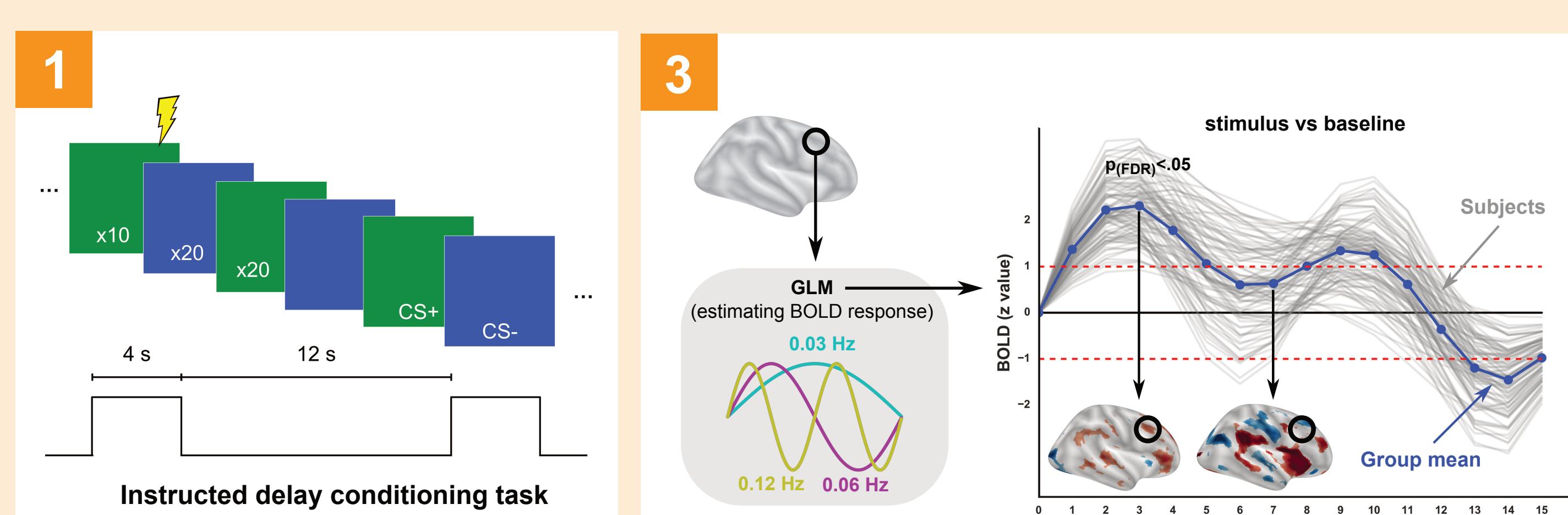


## Methods

An instructed delay conditioning task with partial reinforcement [2,3] was used to induce learning associations between a visual cue and an electric shock in 29 healthy participants (14 female, median age 24 y/o), during which fMRI and skin conductance data were collected.

Second-by-second whole-brain BOLD activation [4] was estimated over 15 seconds after a visual cue onset.

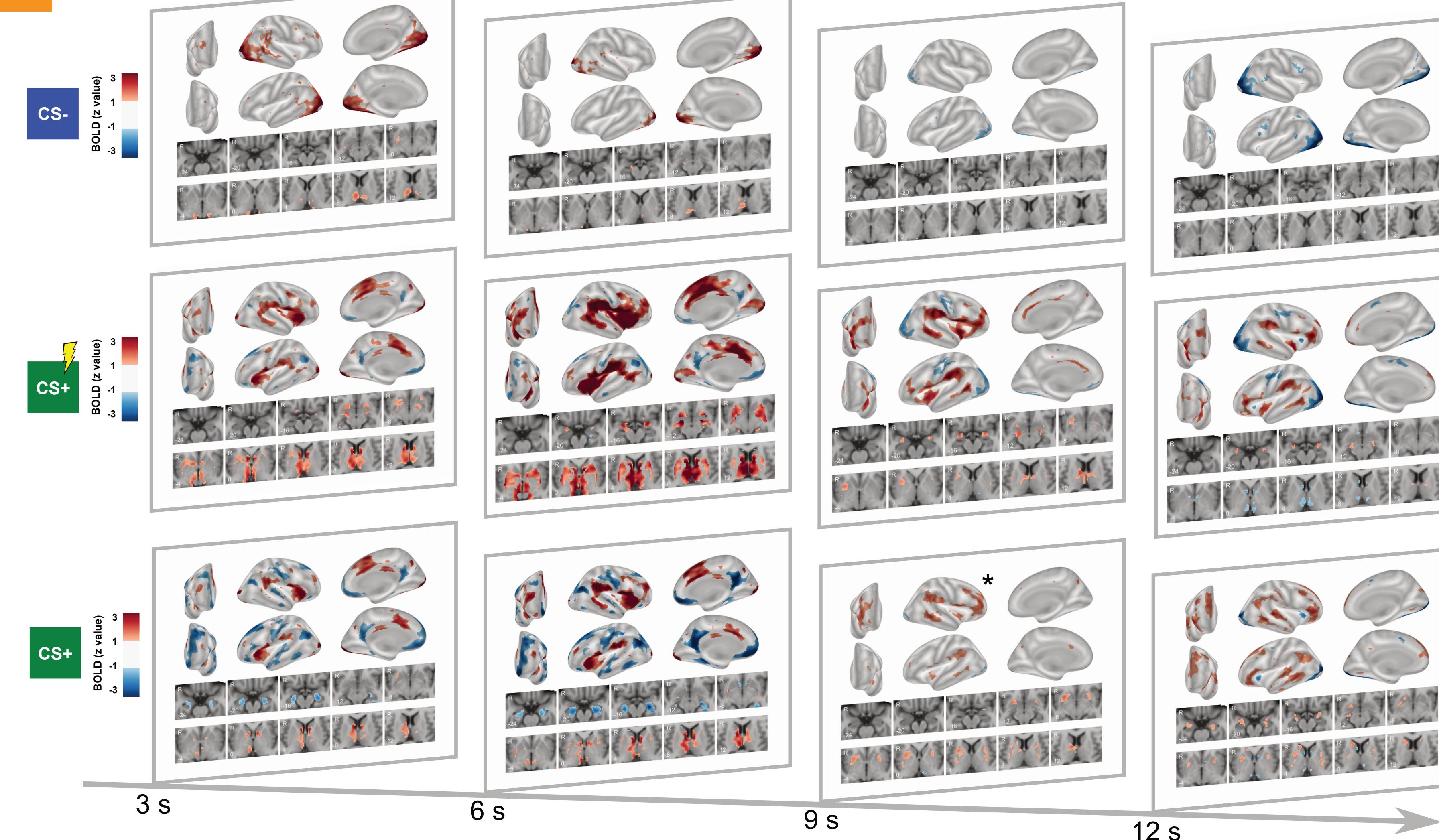
**fMRI data**  
Acquisition: Philips 3T scanner, 39 axial slices (sequential ascending), flip angle=80°, 3x3x2.4mm (0.6mm slice gap), 413 volumes, TR=1960ms, TE=30ms  
Quality control: motion, tSNR, co-registration  
Preprocessing (FSL, ANTs, FreeSurfer): slice timing and motion correction, denoising with aCompCor [5], highpass temporal filtering (0.01 Hz), 6 mm FWHM smoothing, normalization to fsaverage template (cortex) [6] and 2mm MNI (amygdala, hippocampus, basal ganglia, thalamus) [7].



**Individual level:** To estimate BOLD response per condition, a GLM-based deconvolution with three orthogonal sine waves (FSL FILM) was used.  
**Group level:** To obtain whole-brain second-by-second activation maps per condition, a non-parametric t-test (FSL PALM [8,9,10], sign-flips, 2000 permutations, tail approx.) was used to test whether vertex/voxel-wise BOLD amplitude is sig. different than baseline at each time point. To assure reliability of the deconvolution model and alleviate BOLD overshoot/undershoot considered as subsequent activation/deactivation, only vertices/voxels whose F value (converted to Z dist.) was sig. (non-parametric t-test) and group mean amplitude was greater/less than +/- 1 (Z value) were included in the final maps. To correct for multiple comparisons over space, contrasts (pos/neg), and time points (or conditions), FDR-adjusted p-values were calculated and thresholded at  $p=0.05$ .

## Results

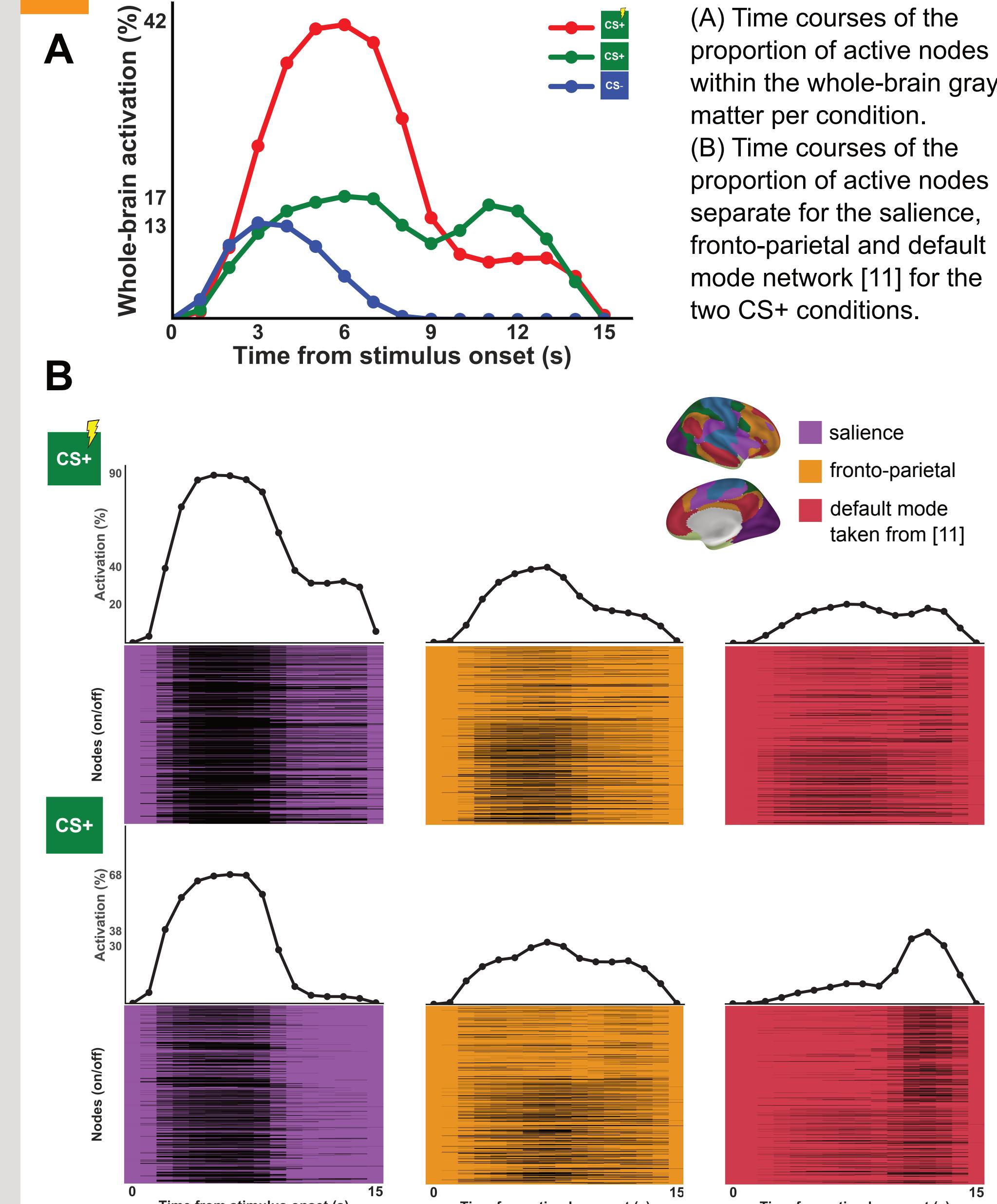
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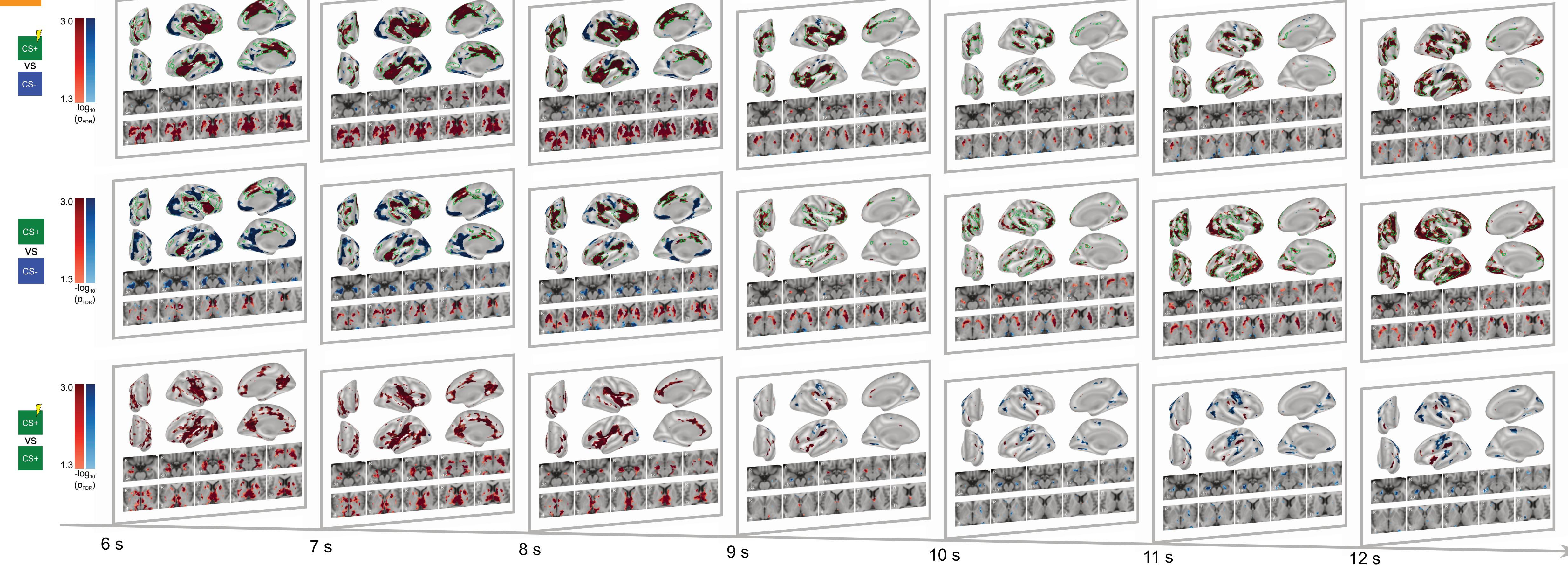
Sequences of activation maps per condition for representative time points.

While neutral cues (CS-) induced an early activation restricted primarily to visual areas of the occipital lobe, threat-related cues (CS+) induced complex whole-brain spatio-temporal patterns. These patterns comprised an early activation in cingulo-opercular regions of the salience network and late activation in lateral regions of the default mode network in the prefrontal, temporal, and parietal cortex. The transient shift between the two networks was accompanied by the intermediate activation of the right dorso-lateral prefrontal cortex\*, which belongs to the cognitive control network.

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Sequences of differential activation maps.

Directly comparing BOLD amplitudes at each time point between conditions (non-parametric t-test) confirmed the observed qualitative differences in the spatio-temporal patterns between neutral and threat-related cues (Fig. 4). The green outline indicates activation based on the stimulus > baseline contrast for the two CS+ conditions respectively. Directly comparing BOLD amplitudes at each time point between CS+ with a shock and CS+ without a shock revealed increased activation mainly located in the salience network (cingulate cortex, insula, supramarginal gyrus), extending to the amygdala, putamen, and thalamus as well as increased late deactivation in hippocampus and somatosensory cortex.

## Discussion

We found that the conditioned stimulus, which elicited a fear response, triggered coordinated brain activity that unfolded over a 15-second period from salience to default mode regions, particularly those implicated in conceptual processing [12]. We speculate that the observed sequence of co-activation patterns may reflect continual elaboration of threat-related information beyond low-level automatic associations.

## References

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