fMRI of delay and trace eyeblink conditioning in the visual cortex of the rabbit

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Introduction

Classical conditioning has long been used as a method to study learning and memory. This basic associative task pairs a behaviorally neutral conditioning stimulus (CS) with a salient unconditioned stimulus (US) that evokes a behavioral response. After repeated paired presentation the subject learns to associate the two stimuli and exhibits a conditioned response (CR) to the CS, before onset of the US. A major advantage of this technique is that simple manipulation of the timing of the stimulus presentation can engage different brain regions. Previously, we used fMRI to examine functional changes in the cerebellum during delay eyeblink conditioning [1]. This study uses fMRI in parallel with both delay and trace eyeblink conditioning to examine functional changes associated with learning in the primary visual cortex (V1). These paradigms are characterized by a difference in cognitive complexity and dependence upon midbrain and forebrain structures, and thus it is expected that V1, which mediates perception of the CS, should exhibit differential responses during delay and trace conditioning.

Methods

Ten Dutch belted female rabbits (2-3 kg) were used in this study (5 delay and 5 trace). The rabbits were stereotaxically implanted with restraining headbolts to restrict motion and allow accurate repositioning between experiments, as described previously [2]. All procedures were carried out under NIH, Evanston Northwestern Healthcare Research Institute and Northwestern University IACUC approved protocols. Images were acquired using a Bruker Avance 4.7 T imaging spectrometer. A flat, circular surface coil (60 mm diameter) was used for RF transmission and reception. A multi-slice, single-shot gradient echo EPI pulse sequence, with a TR of 2 s, and a TE of 21 ms, was used to map brain activation. Coronal images in a plane perpendicular to the surface coil were collected from eight slices (1.0 mm thickness) using a 64 × 80 matrix size reconstructed to 64 × 128, and a 48 × 48 mm FOV, corresponding to an in-plane resolution of 750 × 375 µm. 35 images were collected per trial. The continuous visual CS was delivered by a 2 × 2 array of green LEDs (2 × 2 mm, separated by 8 mm along each axis). The US consisted of a 3 psi airpuff supplied by compressed air and controlled by a regulator and solenoid valve, using a system described previously [3]. The durations of the CS and US were 850 ms and 100 ms, respectively, for the delay paradigm, and 250 ms and 100 ms, respectively, for the trace paradigm, with a 500 ms interval. Nictitating membrane movement was measured with a fiber optic-based IR reflectance sensor [4]. Each subject received two sessions (40 trials per session) of pseudoconditioning (unpaired control CS/US-alone trials). Subjects then received training sessions (CS-US paired trials) until they reached a learning level of 80% CRs. The animals then received one session of CS-alone ("memory") trials. The fMRI data were registered, detrended, and then averaged within each session. The data were then denoised using wavelets, and activated voxels were detected using a support vector machine-based clustering method [5]. The volume of activation within V1 contralateral to the stimulus presentation was measured for each session, and mean BOLD responses were calculated from active voxels.

Results

The rabbits adapted well to the restraint and imaging environment, and showed no obvious sign of movement or struggling during the experiments. All subjects successfully reached the criterion level of 80% CRs with their respective conditioning paradigms. Figure 1 shows images from representative animals trained with the delay (a) and trace (b) paradigms during pseudoconditioning CS trials and early learning (40% CRs). Note the visibly greater change in activated area in V1 (yellow arrow) with learning in response to the more cognitively demanding trace paradigm, in contrast to delay conditioning. The color scale in this case indicates the probability of activation, and does not reflect the BOLD magnitude. Figure 2 shows the BOLD response in V1 averaged across subjects for the delay (a) and trace (b) paradigms during both pseudoconditioning CS-alone trials and at 40% CRs. The dashed line indicates the stimulus presentation. Note that trace conditioning produces no significant change in maximum BOLD magnitude with training. In contrast, the magnitude of the BOLD response nearly doubles during delay conditioning.

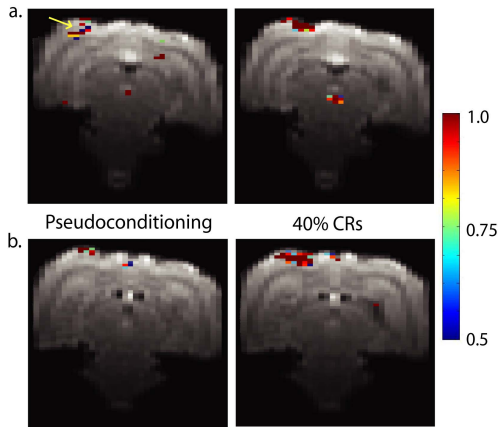

Figure 1: Activation in V1 during delay (a) and trace (b) conditioning

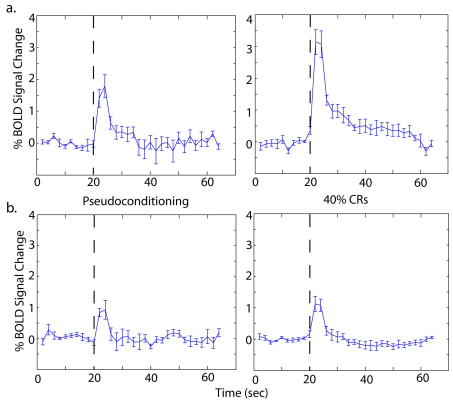

Figure 2: BOLD response in V1 during delay (a) and trace (b) conditioning

Discussion

These results indicate that the difference in cognitive demand between trace and delay conditioning is reflected in distinct patterns of functional activation in V1. The increase in area observed during trace but not delay conditioning suggests that recruitment of additional neurons is necessary to support the additional presence of the trace interval during the formation of the learned association. In contrast, the increased BOLD response during delay conditioning indicates a strengthening of neuronal responsiveness in this region, even though it is not necessary for the acquisition of the paradigm, although the longer visual CS duration during delay conditioning (850 ms compared to 250 ms) could also be a factor. Future investigation into other mid- and forebrain structures during eyeblink conditioning using fMRI should provide a more comprehensive picture of the circuitry involved in these basic forms of learning and memory.

References

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