TITLE Cholinergic innervation is necessary to shift reward timing activity in rodent visual cortex

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SUMMARY While the biological analogue of prediction error has been well characterized in the midbrain dopaminergic system, the possibility of other neuromodulatory systems acting as global reinforcers is a topic of much debate. Reward timing, the phenomenon by which single unit responses in primary visual cortex (V1) reflect an operantly learned stimulus-reward interval, offers a tractable preparation to investigate reinforcement learning *in vivo*: theoretical work suggests that reward timing results from the interaction of stimulus-evoked recurrent network activity and a global reinforcement signal that indicates the time of received reward. We hypothesized that this signal is conveyed by cholinergic neurons arising from the basal forebrain (BF), a strong candidate system that projects globally to most cortical regions, has a known role in plasticity, and is involved in attention and the representation of salience. To test the necessity of such a signal in entraining reward timing in V1, rats were trained on an initial stimulus-reward contingency, received a neurotoxin in V1 that eliminated BF cholinergic terminals, and subsequently trained on a second contingency. We found that extracellular single unit recordings from V1 of lesioned animals, but not saline-infused controls, failed to show shifted neural reports of reward that matched the new contingency. Importantly, neurons of lesioned animals continued to display intervals associated with the initial contingency, arguing that cholinergic input is required to learn, but not to express, reward timing activity.

DESCRIPTION We hypothesized that single unit responses in animals lacking BF cholinergic innervation in V1 would show perseverant reward timing activity under a novel cue-reward contingency. To test this, animals were chronically implanted with microelectrode arrays in V1 and trained to lick a fixed number of times to receive reward after right or left eye stimulation. Following the initial training period, either saline or 192-IgG-saporin – a neurotoxin selective for BF cholinergic neurons and their terminals – was infused into the immediate vicinity of the recording site. After a three day recovery period, animals were given a final session under the initial contingency and then trained under a new contingency. Since the reward timing activity of individual neurons is specific to one eye or the other, average firing rates following stimuli to each eye were evaluated with receiver operator characteristic (ROC) analysis. The neural report of reward was defined as the first moment when the area under the ROC curve fell back to chance with 95% confidence. Figure 1 shows example neurons following the contingency change from a control (A) and a lesioned animal (B). While the control unit reports a time that accords well with the new cue-reward interval, the lesioned unit continues to approximate the previous interval (average reward times before/after contingency change: 1.17s/1.79s for control; 1.49s/0.89s for lesioned). Comparing the distributions of reported reward times from before and after the contingency change revealed a significant difference in control but not lesioned animals (Wilcoxon rank sum test, $p < 0.05$). These results support the assertion that BF cholinergic modulation in V1 is necessary to form new reward timing but is not required for the expression of previously learned intervals.

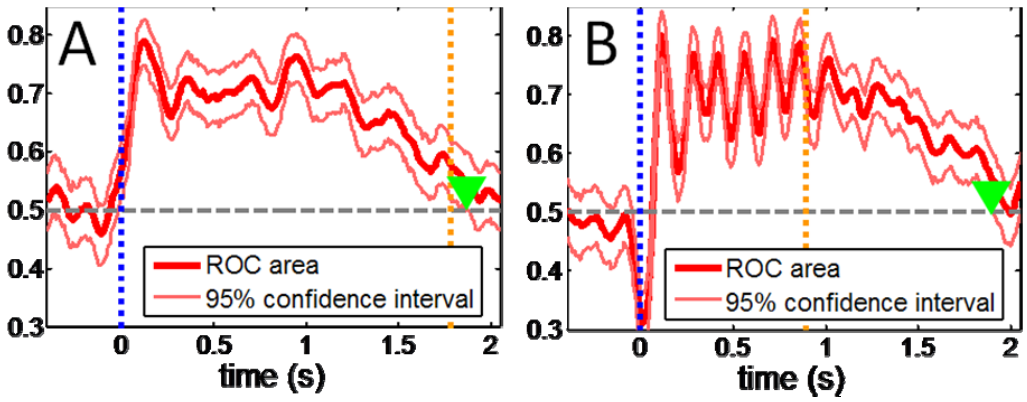

Figure 1. ROC analysis comparing average firing rate after left and right eye stimulation. A) Control neuron. Dashed blue line marks onset of cue, dashed orange line is average reward time, and green triangle indicates the first moment confidence crosses chance. B) Lesioned neuron fails to update and shows reward timing to initial contingency.