PASUPATHY: SUPPLEMENTARY FIGURE 3
(2004-08-23602)
Correct trials vs. Average direction selectivity (300 - 600 ms)

PFC and Cd regions are indicated on the graph.

PASUPATHY: SUPPLEMENTARY FIGURE 4
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**Supplementary Figure Legends**

**Supplementary Figure 1.** Peri-cue saccade-direction selectivity in single cells.
A, B: Mean (and standard errors of the mean (SEM)) of firing rate across all correct trials for a PFC (A) and Cd (B) neuron. Line colour indicates saccade direction (right; left); line style indicates cue identity. X axis shows time relative to cue onset. 0-500 ms : cue epoch; 1500 ms : onset of target spots. Neurons show transient (A: PFC) and sustained (B: Cd) selectivity for rightward saccade starting in the late-cue epoch. Transient and sustained selectivities were observed in both areas. C, D: Average activity (and SEM) during peri-cue period (300-600 ms; grey hatches in A & B), as a function of correct trial number, for cells in A & B. Y axis plots 5-trial moving average (from trial 1 to 30) for rightward and leftward saccade trials, averaged across cue objects and blocks. Cue epoch activity is initially similar but separation between right and left saccade trials increases with learning: activity for rightward saccades becomes significantly greater (T-test, P < 0.01) from trials 15 (PFC) and 5 (Cd), respectively.

**Supplementary Figure 2.** Rapid change in activity during the peri-cue epoch in a Cd neuron. Rightward (top) and leftward (bottom) saccade trials are shown. Darkness of lines indicates trial number (as per legend). Grey lines show SEM. X axis as in Supplementary Figure 1A, B. On the first three rightward but not leftward saccade trials, activity peaked during the delay period (↓). This peak shifted back to the cue period after 4-6 trials (↓) and remained there on subsequent trials (7-12). On leftward saccade trials, delay period activity became progressively weaker.
**Supplementary Figure 3.** Comparison of direction selectivity on correct and error trials. Average direction selectivity ($\text{PEV}_{\text{dir}}$) for the PFC (left) and Cd (right) populations based on error (dashed lines) and correct (solid lines) trials. Grey lines represent SEM. Cue (0 - 500 ms), delay and peri-saccadic epochs are shown. In the PFC, $\text{PEV}_{\text{dir}}$ is significantly weaker (T-test, P < 0.01) on error trials than on correct trials from 430-620 ms and again 730-840 ms after cue onset and at no other time. In the Cd, $\text{PEV}_{\text{dir}}$ based on error trials is not significantly less than $\text{PEV}_{\text{dir}}$ based on correct trials.

**Supplementary Figure 4.** Comparison of average strength of peri-cue epoch direction selectivity in PFC and Cd populations. Average direction selectivity ($\text{PEV}_{\text{dir}}$) for the PFC and Cd populations during the peri-cue epoch (300-600 ms after cue onset) as a function of correct trials. As with risetimes (Figure 2C) Cd activity undergoes a rapid change in strength of selectivity early in learning and the changes are best described by a sigmoidal function (dashed red line). PFC activity undergoes a slow gradual change over a longer timecourse and the trend is linear (blue dashed line).
Supplementary Notes

1. Six to nine months were required to train each initially naïve monkey to reliably complete a minimum of 3 reversals (or 4 blocks) during a 2 hour training session. Associations were reversed after monkeys reached criterion performance (>=90% correct over 10 trials/cue) and at least 30 correct trials per cue had been completed.

2. The fixed memory delay (1000 ms) allowed monkeys to anticipate the time of target onset and likely resulted in overall shorter reaction times than an unpredictable delay or no delay.

3. We performed a two-way ANOVA on the average activity across each trial epoch (cue, delay, saccade, reward), with cue object and saccade direction as factors, evaluated at P < 0.01 (see Methods). PFC: Activity of 15.3% (66/432) of neurons showed a significant main effect of cue object, 38.9% (168/432) of saccade direction, and 20.1% (87/432) for both factors and/or their interaction in one or more trial epochs. Cd: Activity of 18.6% (52/279) of neurons showed a significant main effect of cue object, 36.2% (101/279) of saccade direction and 36.2% (101/279) of both and/or their interaction in one or more trial epochs. Here, we focus our analyses on changes in the neural activity reflecting the direction of the saccade the monkeys learned to make in response to each cue.

4. Figure 2 is based only on correct trials and does not depict the first few trials after reversals, which were exclusively errors (behaviour at or near 0%, Figure 1b) because the animals still followed the previous associations. Not surprisingly, on those trials, neural activity was similar to that on trials immediately preceding
reversal. Then, when performance jumped up to chance (50% correct, Figure 1b), early-trial activity in the PFC and Cd reverted to the weak saccade-direction selectivity evident on the first few correct trials illustrated in Figure 2. Unlike correct trials, error trials have opposite instructed and executed saccade directions, making their activity more ambiguous. Therefore, in evaluating direction selectivity, only correct trials were included. Figure 2 is collapsed across blocks, but behavioural learning rates were similar across blocks and, correspondingly, plots of direction selectivity based on the “early” blocks (i.e. the first two blocks) were similar to those based on the “late” blocks (i.e. the last two blocks of each recording session).

5. Note that because we found a learning-related increase in prospective information about a specific response (right versus left saccades), these results cannot be explained by a generalized increase in the anticipation of reward (because both behavioural responses were rewarded) nor can they be explained by a generalized increase in motor system activity in preparation for initiating action.

6. Asymptote was the trial number when 95% of the change in risetime was attained. Trend in risetime (y) was quantified by fitting sigmoidal functions of trial number (x) using the four parameter ($x_0 - x_3$) sigmoidal equation shown below.

$$y = x_0 - \frac{x_1}{1 + e^{x_2(x-x_3)}}$$
7. Indeed, performance of conditional learning tasks is disrupted following PFC damage (see review by Passingham 1993, The Frontal Lobes and Voluntary Action, Oxford University Press).

8. Analyses of error trials revealed that peri-cue saccade-direction selectivity (sorted by the executed direction) tended to be weaker than on correct trials in both areas, but this difference was significant only for the PFC (Supplementary Figure 3). Saccade-direction selectivity around the time of saccade execution was similar on error and correct trials in both areas.


10. This task required monkeys to learn which saccade (right versus left) would be rewarded following a given cue. It should be noted, however, that other types of learning can also contribute to task performance. For example, as monkeys improved their performance and were increasingly rewarded, the cues and responses became increasingly predictive of reward.

11. Because this measures effect strength in terms of signal variance, other variance measures such as that based on Receiver Operating Characteristics (ROC) yielded similar results. Population analyses based on square root and log transformed neural data produced similar results. Bias in $R^2$ due to sample size was adjusted based on estimates derived from baseline activity, i.e. average $R^2$ during 200 ms to 500 ms after fixation onset was subtracted from $R^2$ at all time points.
12. On a very few trials early in learning, \( \text{PEV}_{dir} \) failed to reach the half-maximum across trials. Then risetime was defined as the time to the maximum \( \text{PEV}_{dir} \) on that trial. When we assessed learning trends by computing average \( \text{PEV}_{dir} \) (instead of risetime) during the peri-cue epoch (300-600 ms after cue onset), similar results were obtained (see Supplementary Figure 4).