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# Chapter 4

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## **Learning to select visual stimuli in area FEF of the macaque monkey**

Liviu Stănişor, Chris van der Togt, Arezoo Pooresmaeili and Pieter R. Roelfsema

## ABSTRACT

In daily life, ongoing learning is an essential aspect of our conscious behavior and underlies our ability to integrate past memories and present experiences. In this study we investigated how learning influences the process of decision making by recording the activity of single neurons in the frontal eye fields (FEF). The responses of FEF neurons to visual stimuli are modulated by attentional selection. In a two-choice saccadic reaction-time task, we used two clearly visible icons and the monkeys had to learn by trial and error which one was rewarded and we added an extra positional cue so that we could vary task difficulty. For the difficult trials without a positional cue, we observed initially very rapid decisions with an accuracy that was close to chance level but that improved with learning. In area FEF, neuronal responses evoked by the target of the saccade were stronger than responses evoked by the stimuli that were not selected. Learning also influenced the latency of the target selection signal. Before learning, the monkeys reached a fast, uninformed decision that was associated with an early FEF selection signal. After learning, the selection signal took more time to develop, and performance improved. We conclude that neurons in area FEF initially participate in fast and random decisions, and that the neuronal activity builds up more gradually after learning, causing slower but more accurate decisions.

## INTRODUCTION

Decision making and learning are intimately linked. Learning can be described as a trial and error process in which subjects obtain an estimate of what is the most rewarding choice given a set of possible actions (selecting different targets or stimuli).

When a subject is required to choose one of two possible outcomes in a dual choice task, areas of the parietal and frontal cortex are critically implicated. Neurons in frontal eye field (FEF) are involved in the generation of goal-oriented saccades (Schall 1991). The activity of these neurons changes when a target, which is presented in the neuron's receptive field (RF), is selected among an array of distracters (Schall et al. 1995). FEF neurons also show increased activity when a cue informs the monkeys that one of the stimuli will appear in their RF, before any stimulus is actually presented (Zhou and

Thompson 2008). It therefore appears that this area is an integral part of the circuitries which mediate both attentional and decision-making processes if the task requires an eye movement response. The roles of area FEF and the principal sulcus (PS) in decision making have been investigated in a task in which randomly moving dots indicated to the monkey which of the two targets was to be selected for an eye movement (Kim and Shadlen 1999; Roitman and Shadlen 2002). These studies investigated saccade target selection (decisions) in the presence of stochastic and unreliable sensory evidence. In these situations the optimal strategy is to integrate sensory evidence over time and neurons in frontal and parietal cortex involved in the generation of eye movements carry signals that are related to this evidence integration. Their activity increases when the evidence favors an eye movement towards their receptive field and decreases if the evidence favors a saccade to a different direction (Yang & Shadlen 2007). The increase in this putative decision signal depends on the strength of sensory evidence so that the accumulation is faster if the motion signal is unambiguous and slower if it is unreliable. Similar results have been obtained in a visual search task, where the latency of the target selection signal also depends on the quality of the sensory evidence and on the number of distracters (Sato et al. 2001). These previous studies have provided important insights into the processes responsible for decision making in the presence of stochastic sensory evidence (Gold & Shadlen 2007).

Here we ask how decisions are made if the sensory evidence is unambiguous, but the stimulus-response mapping is unknown and has to be learned by trial and error. Some neurons in the frontal cortex and striatum are particularly active during the learning of new associations between visual stimuli and motor responses (Chen and Wise 1995a, 1995b; Brasted and Wise 2004). Other cells in these structures change their tuning during the learning process (Chen and Wise, 1995a, 1995b; Brasted and Wise 2004; Tremblay et al. 1998) and comparable changes in the tuning of cells have also been observed in the hippocampus (Wirth et al. 2003). Moreover, in tasks where the associations between stimuli and responses are continually reversed, neurons in prefrontal cortex and striatum change their tuning in a comparable way (Asaad et al. 1998; Pasupathy & Miller 2005).

Previous studies therefore agree that the learning of new stimulus-response mappings is associated with changes in the tuning of neurons in a widespread network of brain regions that includes the frontal cortex and the striatum. It is not well understood how

these changes in tuning influence decision making signals in structures related to the selection of an eye movement. In the present study we therefore developed a new paradigm where the monkeys had to learn to select one of two objects ('icons') that were connected by a curve to the target of an eye-movement. We recorded neuronal activity in the frontal eye fields and recorded from neurons that responded to the target of the impending eye movement. We took care to place the icons outside the receptive fields of the neurons, because a substantial fraction of FEF neurons is selective for the shape of visual stimuli (Peng et al. 2008). Moreover, we varied the difficulty of the task by adding positional cues so that we could compare the effects of sensory ambiguity to the effects of learning a new stimulus-response association.

With this new paradigm we can investigate how learning of new stimulus-response mappings influences target selection in FEF, and how the effects of learning compare to the effects of uncertain sensory evidence. We find that area FEF neurons carry strong signals that reflect the upcoming decision (see also Hanes & Schall 1996). More difficult decisions are associated with a later target selection signal. Remarkably, the reduction of this ambiguity by learning caused an *increase* rather than a decrease in the latency of this decision signal. We found that monkeys initially make fast and random decisions that are predicted by neurons in FEF and that learning causes a delay in this decision signal so that it builds up slower and starts to reflect the newly learned stimulus-response mapping.

## METHODS

Standard surgical and electrophysiological techniques were used to record single-unit activity in area FEF. Two rhesus monkeys, A and J (*Macaca mulatta*), participated in this study. In a first operation, a head holder was implanted and a gold ring was inserted under the conjunctiva of one eye for the measurement of eye position. In a second operation, a trepanation was performed over area FEF and a recording chamber was placed. The surgical procedures were performed under aseptic conditions and general anesthesia. The FEF was localized with a magnetic resonance imaging scan. Details of the surgical procedures and the postoperative care have been described elsewhere (Khayat et al 2009). All procedures complied with the US National Institutes of Health Guidelines for the Care and Use of

Laboratory Animals and were approved by the institutional animal care and use committee of the Royal Netherlands Academy of Arts and Sciences of the Netherlands.

### **Behavioral task**

The animals performed a forced choice task where they had to select one of two icons and then make an eye movement to a circular disc that was connected to this icon by a curve (Fig. 1). A trial started as soon as the monkey's eye position was within a  $3^{\circ} \times 3^{\circ}$  square window centered on a red fixation point (FP). After an interval of 500 ms, two objects appeared on the screen (Fig. 1) that consisted of an icon at one end and a red disc at the other end, connected by a curve. The monkey had to choose one of the icons by making a saccade to the corresponding red disc. There were three types of stimuli: easy, intermediate and difficult. In the case of the easy and intermediate stimuli, we introduced a positional cue. We placed the target icon on top of the FP (easy) or closer to it (intermediate) and increased the length of the connecting curve accordingly, ensuring the same sensory stimulation inside the response field of the recorded neurons. In the difficult condition the relevant and irrelevant icon were at the same distance from the FP so that the monkey had to evaluate icon identity for a correct response. The three types of trials, easy, intermediate and difficult were randomly interleaved. Care was taken to use different icons (combinations of shapes and colors) so that the relevant icon was perceptually distinct from the irrelevant one and every pair of icons was different from the next (see Fig. 1, inset on the right). The monkey was allowed to initiate its response as soon as the stimulus was presented (reaction-time task). For each correct choice, the monkey was rewarded with a drop of apple juice.

We imposed a criterion for successful learning, which was that behavioral performance for the difficult stimuli reached at least 75% correct at the end of the recording session. At least 75 correct trials for the difficult condition were recorded in each recording session.

## Recording and data analysis

We obtained extracellular recordings from single neurons with tungsten microelectrodes which were lowered through the dura mater with a hydraulic microdrive (Narishige). Action potentials were amplified, filtered, discriminated and recorded on-line using spike-sorter software (Tucker Davis). Each single unit was confirmed to be in FEF by using the recording electrode for intracortical microstimulation (biphasic current pulses, 70-ms train duration, 400 Hz). The penetration was considered in FEF if a saccade could be triggered using currents that had an intensity of  $<100\ \mu\text{A}$  (generally  $<50\ \mu\text{A}$ ) (Bruce et al. 1985). The eye position was measured using either the double magnetic induction technique (Bour et al. 1984, sampling rate 1 kHz) or an infrared camera system (sampling rate 250 Hz).

We constructed learning curves to investigate behavioral performance. For each condition (easy/intermediate/difficult) we assigned 0 to an error trial and 1 to a correct trial and then averaged over days and smoothed with a box-car of 15 data points. Each point of the learning curve was calculated based on at least 8 learning sessions. Reaction times were analyzed equivalently, but in this case only the correct trials contributed to the analysis. We found that the animals' accuracy tended to decline towards the end of the sessions, and therefore we excluded these trials (typically 20% of the trials at the end of the session).

We recorded the activity of 35 FEF single units from two monkeys (10 from monkey J and 25 from monkey A). For each isolated neuron, we first mapped its response field (RF) by presenting a single saccade target at various eccentricities and directions. Then separate blocks of trials were recorded with mapping tasks for eccentricity and radial tuning, as well as the main learning paradigm. We also used a memory-guided saccade task to classify the cells according to Bruce and Goldberg (1985). In this study we recorded 7 visual neurons, 27 visuomotor neurons and 1 motor neuron. One of the two target discs was placed in the RF of the recorded neuron and the other disc at an angle of 90 or 135 deg at a similar eccentricity (ranging from 8 to 18°) (Fig. 1). The stimuli were chosen such that one of the discs (saccade endpoints) fell well within the RF. The icons were presented well outside the response fields to avoid differences in activity due to different visual stimulation, since it has been shown that FEF neurons can be modulated by various sensory cues (Peng et al 2008).

We thereby ensured that differences in FEF activity between conditions were caused by target selection.

We obtained a quantitative estimate of location and spatial extent of the RFs, on the basis of the eccentricity and radial tuning profile. To make the plots, we assumed that the effects of eccentricity and direction on a neuron's response are separable, i.e. the overall response can be described as a multiplication of the effect of these two factors (Khayat et al 2009). We fitted a Gaussian to the radial tuning profile and a spline to the eccentricity profile, respectively.

Peristimulus or perisaccade time histograms of single-unit responses were computed and aligned to stimulus appearance and saccade onset. Only correct trials were included in the analyses, unless otherwise specified. To compute the population responses, we first normalized the responses of individual neurons to the maximum response, and then averaged across cells. We determined the significance of differences in response strength between stimuli in various time windows, applying paired *t*-tests for the population data.

We defined the target selection activity (attentional modulation) of the FEF neurons as the difference between responses evoked by target and distracter curve. This difference is expected to be zero at response onset followed by an increase to some maximum level at a certain latency reflecting the effect of attention on activity. Based on these assumptions, we fitted a piecewise polynomial function of the 3<sup>rd</sup> degree (cubic spline) with three uniform intervals in the case of modulation and in case of its cumulative sum a cubic spline with 4 uniform intervals, both constrained to be zero at onset, using the shape prescriptive modeling toolbox implemented in MATLAB by John D'Ericco (Mathworks 2009). Using this toolbox, a least squares estimation was obtained from the fit of the splines, from which latencies were derived (see also de Boor & Rice 1968, Wright & Wegman 1980). We estimated modulation onset time as the time point at which the fitted function reached 50% of its own maximum.

When comparing the latencies or differences in latencies between conditions we used a bootstrapping method. A random sample of *n* cases were selected with replacement from our number of *n* recorded cells (*n* = 26 which allowed fitting) and then the latency of the average target selection activity between 100 ms before stimulus onset and 500 ms



afterwards was estimated using the curve-fitting algorithm described above. The algorithm was repeated 10,000 times to create distributions of latencies for the easy, intermediate and difficult conditions and to estimate the 95% confidence intervals. This bootstrapping procedure was used to test the significance of both the modulatory activity and its cumulative sum.

## RESULTS

The aim of our paradigm was to investigate the time course of learning in a task where monkeys had to select one of two icons and to measure the influence of learning on neuronal activity in area FEF. The two icons were connected to discs that were targets for an eye movement, one of these icons (target icon, yellow in Fig. 1) had to be selected by the monkey and the other icon was a distractor (green in Fig. 1). The monkeys indicated their choice by making a saccade towards one of the discs (Fig. 1), but they were only rewarded if they chose the disc that was connected to the target icon. There were three levels of difficulty. In easy trials, the relevant icon overlapped with the fixation point and the monkey could trace the target curve from the fixation point to its other end to identify the target disc. In the intermediate condition, the target icon was closer to the fixation point than the distractor icon. In the difficult condition the target and distractor icon were at the same distance from the fixation point so that the monkey could only rely on icon identity for a correct response. The six conditions shown in Fig. 1 were randomly interleaved.

### Behavioral measures of learning

Figure 2 presents the progression of accuracy and reaction time of the two monkeys across a total of 61 sessions for monkey J and 104 sessions for monkey A. In case of easy stimuli, the monkeys hardly made any errors (Fig. 2A), performance in the intermediate condition started at 80-85% and soon improved to almost 100%. The behavioral improvement for the difficult stimuli was more gradual, starting at an average of 55-60% and increasing to higher than 80% in both animals. To further analyze these differences, the proportion of correct trials was calculated for the first 30 (early) trials and trial 101 to 130 (late) from all training

sets. We found significant differences between accuracy in the early and late trials (intermediate condition; both monkeys,  $P < 10^{-6}$ ; difficult condition,  $P < 10^{-6}$ ). The improvement in performance was accompanied by a gradual increase in overall reaction time in all three conditions. Using a two way ANOVA we tested the effects of learning (first-last) and condition (easy-intermediate-difficult) on reaction times. Reaction time averaged across the conditions increased from the early to the late trials in both monkeys (from 213 ms to 238 ms in monkey A and from 284 ms to 303 ms in monkey J) and these effects were highly significant (Monkey A:  $F_{1,11922}=1021$ ,  $P < 10^{-6}$ ; Monkey J:  $F_{1,10614}=200$ ,  $P < 10^{-6}$ ). We also observed a significant effect of task difficulty on reaction time (Monkey A:  $F_{2,11922}=31.8$ ,  $P < 10^{-6}$ ; Monkey J:  $F_{2,10614}=12.3$ ,  $P < 10^{-6}$ ). In both monkeys reaction times for the difficult task were longer than those in the other two conditions (Monkey A: easy:  $229 \pm 1.0$ ms, intermediate:  $224 \pm 1.1$ ms, difficult:  $233 \pm 1.3$ ms; Monkey J: easy:  $293 \pm 1.9$ ms, intermediate:  $298 \pm 2.2$ ms, difficult:  $303 \pm 2.2$ ms).

We also compared the average reaction time on error trials to the reaction times on correct trials in the difficult condition. Because error trials were most abundant at the start of the task when reaction times were comparatively short, we chose for every error trial a matching correct trial that occurred within 2 trials of the error trial. We found that the reaction times for the correct and erroneous trials were similar in the intermediate condition (t-test, Monkey A:  $n=1042$ ,  $P > 0.05$ , Monkey J:  $n=344$ ,  $P > 0.2$ ) but significantly different for the difficult condition in both monkeys (t-test, Monkey A:  $n=2096$ ,  $P < 10^{-6}$ ; Monkey J:  $n=1205$ ,  $P < 0.05$ ). Reaction times were shorter for error trials than for correct trials in the difficult condition (213 vs. 220 ms in monkey A and 284 vs. 290 ms in monkey J).

### **Neuronal correlates of target selection in area FEF**

Figure 3A shows example stimuli of the difficult condition, with either the target or distractor disk in the receptive field (RF) of a single FEF neuron. The RF of the neuron fell on the circular disc and the more eccentric segment of one of the curves, but not on the parts of the stimuli that were more central, thus ensuring that the visual stimulation of the RF was identical across the conditions. Yet, the neuron was activated more strongly when the target disc fell in the RF than if the distractor disc fell in the RF (Figure 3B). We attribute this

additional activity to the process of saccade target selection, in accordance with previous studies. This activity is defined as modulation and calculated as the difference between target and distractor response (grey area in Figure 3C). It can be seen in Figure 3 that the latency of this response difference increased with difficulty.

We observed similar effects when we pooled data across the population of neurons. Figure 4A shows the average activity evoked by the target and distractor disc across the various levels of difficulty. At all three difficulty levels, the activity evoked by the target disc was significantly stronger than the activity evoked by the distractor (paired t-test,  $p < 10^{-5}$  for all conditions, in an interval from 50-250 ms after stimulus onset).

Interestingly, the activity evoked by the target disc was similar across the difficulty conditions whereas the response evoked by the distracter was stronger in the more difficult conditions. We tested this effect with a paired t-test in a time window between 50 and 250 ms after stimulus onset. We found that there was no difference between easy and difficult targets ( $p = 0.27$ ) but that the distracter activity was significantly higher in the difficult than in the easy condition ( $p < 0.001$ ). This finding suggests that target discrimination in FEF is weaker in the difficult condition due to a decreased suppression of the representation of the distracter object.

We then investigated how the timing of the target-selection signal, i.e. the difference between target and distracter activity, depended on task difficulty (figure 3B). To this aim we fitted a curve to the target selection signal, at every level of difficulty. The latency was 144 ms in the easy condition, it increased to 162 ms in the intermediate condition and to 170 ms in the difficult condition. The latency in the intermediate condition was significantly longer than the latency in the easy condition, and the latency in the difficult condition, in turn, was longer than the latency in the intermediate condition (bootstrap-test,  $N = 26$  cells, both  $p < 0.05$ ).

To investigate if there were differences in pre-saccadic activity, we investigated the response evoked by the target curve in a 50 ms time window before the saccade but found no difference between conditions (one-way ANOVA, d.f. = 104,  $F = 0.51$ ,  $p = 0.6$ ), which implies that target selection signal is delayed in the difficult condition compared to the easy condition, but that the target response reaches the same level before the saccade. We

conclude that our task reproduces previous work on the neuronal correlates of decision making: (1) an accumulation of neuronal activity towards a threshold, (2) this threshold is reached around the time of the saccade and (3) a slower accumulation of neuronal activity if the task is more difficult.

### **The effects of learning on neuronal activity in area FEF**

We next investigated the changes in the activity of FEF neurons that occurred in the course of learning. Will neuronal activity accumulate faster when the monkeys learn the meaning of the icons? We expected to see the clearest effects in the difficult condition where a correct response depended in the identity of the target icon. To investigate the time course of FEF activity, we looked at the first and last 20 trials of the difficult condition (10 trials with the target curve in the RF and 10 with the distractor). We found that the total amount of activity evoked by the target and distracter disc did not vary greatly with learning (Figure 5A). However, we found a difference in the time point where the response evoked by the target and distractor disc started to diverge. The estimated target selection time was 163 ms at the start of the session and it increased to 185 ms after learning (figure 5B), a difference that was significant (N=26 cells,  $P < 0.05$ , bootstrap test). We also computed the latency of the target selection signal with the cumulative sums method and also observed a significant increase in the latency which increased from 248 ms to 285 ms with learning (figure 5C,  $P < 0.05$ , bootstrap test). Thus, unlike previous studies (Asaad et al 1998) the target discrimination time in our task *increased* with learning.

We considered the possibility that this increase in target-discrimination time was caused by a general slowing of the monkeys (Fig. 2), for instance due to fatigue or because they had consumed part of their daily fluid intake. We therefore conducted the same analysis in the easy condition where the response time also increased in the course of the learning task, but where the monkeys were always performing close to perfect and no learning took place. We found that the target discrimination time was 127 ms in the first 20 trials and 125 ms in the last 20 trials, a difference in target discrimination time that was not significant ( $p > 0.2$ , bootstrap test), which suggests that the increase in modulation latency in the difficult condition is related to learning.

To further characterize the time course of the change in response modulation we distinguished between early (100-170 ms after stimulus onset) and late response modulation (180-250 ms) and tested how early and late modulation changed with learning. Experience with the new set of stimuli did not change the activity in either window for the easy stimuli (paired t-test,  $p > 0.2$  in both windows). For the difficult stimuli however, early response modulation (100-170 ms) decreased in the last blocks of trials ( $p = 0.02$ ), whereas late modulation (180-250 ms) was relatively constant ( $p > 0.1$ ). The strength of the response modulation in the difficult condition was also constant across the blocks when we analyzed the response modulation in the last 50 ms before the saccade ( $p > 0.2$ ). These results, taken together, indicate that the onset of the response modulation is delayed during the course of learning.

### **The representation of erroneous decisions in area FEF during learning**

The findings described so far suggest that decisions become slower when the identity of the target icon is learned. We note however, that we only included the correct trials when we determined the target discrimination time. The monkeys started with random performance and then gradually improved in the task (Fig. 2). It is therefore conceivable that the short target discrimination time early in the task reflects decisions that are fast, but random.

We next compared the average FEF activity between correct and erroneous trials (Fig. 6A) for stimuli with the RF on the target or distractor disk. In this analysis we only included cells if there were at least five erroneous trials in one of the conditions ( $n = 30$  cells if the RF was on the distractor disc and  $n = 29$  cells if it was on the target). The activity of the FEF cells was largely determined by the planned eye movement. If the RF fell on the distractor disk but the monkey made an eye-movement to it, activity was similar to that on correct trials with the RF on the target disc (compare red continuous and blue dashed curves in Fig. 6A). Conversely, if the RF was on the target but the monkey made an erroneous saccade to the distractor, activity was similar to that in correct trials with the distractor disc in the RF (red dashed and blue continuous curves). Thus, neuronal activity in FEF in correct and erroneous trials is similar and mainly determined by the endpoint of the saccade (Heitz et al 2010).

These results suggest that decisions are taken quickly but randomly in the beginning of the task. In the early phase neurons in area FEF could rapidly select either disc as target for an eye movement so that the main effect of learning is to increase the probability that the correct disc is selected in FEF. To investigate this possibility, we averaged across correct and erroneous trials of the difficult condition and investigated how the average activity evoked by the target and distractor disc evolved during learning for the difficult condition (Fig. 6B, N=34 cells). Early in the task (first 20 trials), the average activity evoked by the target and distractor disc was similar ( $p > 0.2$ , paired t test), an effect that is due to the large proportion of error trials (42%, i.e. the accuracy was 58%). At the end of the session (last 20 trials), accuracy has improved to 84% and now the target disc evoked stronger responses in FEF neurons than the distractor disc ( $p < 0.01$ , paired t test). Thus, FEF activity in the difficult condition is mainly determined by the selected eye movement and the mixing of correct and erroneous trials in the beginning of the task causes on average a weak target selection signal. Although the monkeys did not have evidence for either decision in the difficult condition at the beginning of the task, they could nevertheless reach fast decisions and these decisions were reflected by a rapid increase of activity of neurons in area FEF.

At first sight, these results appear to indicate that the saccadic decisions are not formed in FEF, but that this area simply executes a motor program once the decision has been reached. However, this conclusion is contradicted by our finding that target discrimination time is even shorter in the easy and intermediate conditions. In the easy condition performance was close to perfect and the FEF activity increased faster than in the difficult condition. FEF apparently carries neuronal correlates of decision making in this task and is not only informed by time the decision has been made elsewhere, in accordance with earlier work (Hanes & Schall 1996).

## DISCUSSION

Here we have investigated the effects of learning on the activity of neurons in area FEF in a task where monkeys had to select one of two icons to obtain a reward. We focused on the neuronal responses evoked by the target of an eye movement, thereby linking previous work on the neuronal correlates of learning in the frontal cortex (Chen & Wise 1995a,

1995b; Asaad et al 1998; Pasupathy & Miller 2005) to studies on decision making in the same structures (Kim & Shadlen 1999; Roitman & Shadlen 2002; Schall 1991; Schall et al 1995; Law & Gold 2008; Stanford et al 2010).

The monkeys could rely on two types of cues to solve the task. In easy and intermediate trials, they could use the distance between the icons and the fixation point as well as icon identity for the selection of a correct response. In the difficult trials they could only rely on icon identity because both icons were at the same distance from the fixation point. Accordingly, the monkeys always had a high accuracy on the easy trials and learning improved performance only in the intermediate and difficult conditions.

Neurons in area FEF showed an initial visual response and then increased their activity if an eye movement was planned in the direction of their response field, in accordance with previous studies (Bruce & Goldberg 1985; Schall et al 1995). The eye movement occurred when the activity had reached a relatively fixed threshold (Hanes & Schall 1996). The accumulation of neuronal activity was more gradual in the more difficult conditions, in line with previous studies showing that the increase in activity of neurons in areas LIP (Law & Gold 2008), FEF (Sato et al 2001), prefrontal cortex (Kim & Shadlen 1999) and the superior colliculus (Horwitz & Newsome 1999) depends on the quality of the sensory signal.

We found that the error trials were also associated with a gradual increase of neuronal activity, but now for FEF neurons coding the erroneous eye movement. Before learning took place, neurons in area FEF had similar responses to the target and distractor disc and their activity only depended on the eye movement that the monkey selected. After learning, the target disc consistently evoked more activity in FEF than the distractor, and now the target disc had a higher probability to be selected. These results suggest that learning caused a more efficient selection of the target icon and that this selection signal was transferred to the representation of the corresponding target disc in area FEF.

Learning caused an unexpected increase in the target discrimination time in area FEF in the difficult conditions, i.e. an additional delay in the moment in time at which the neurons started to code the impending eye movement. The increase in target discrimination time was associated with a comparable increase in the reaction time, although this increase

in reaction time occurred at all levels of difficulty. Interestingly, the increase in reaction times in the easy condition was not accompanied by an increase in target discrimination time, indicating that the additional delay in the neuronal selection signals was specific for the difficult condition. In the difficult conditions, the monkeys started with fast and inaccurate responses and they then gradually delayed their responses (by 30-40ms) when they learned the meaning of the icons, perhaps allowing for more time for the newly learned information about the icons to influence the decision process. These results are in accordance with a recent study showing that an addition delay of only 30ms can change random performance into accurate performance in a task that requires sensory decisions (Stanford et al 2010).

This additional delay was not necessary for the easy condition where the monkeys could rely on the distance cue. It is likely that this cue was represented more efficiently than the icon meaning because it had been available consistently, across many sessions. The distance cues started to influence neuronal activity at an earlier point in time than icon identity, and the monkeys could always respond accurately in the easy condition with comparatively short reaction times. A previous study demonstrated that consistent mapping across many sessions can indeed cause very early target selection signals in area FEF (Bichot et al 1996).

### **Sequential processing in the icon-selection task**

The easy condition of the present study is comparable to previous curve tracing studies where monkeys traced a target curve that started at the fixation point in the presence of a distracting curve not connected to the fixation point. In this task, the neuronal representation of the target curve is enhanced in the primary visual cortex (Roelfsema et al 1998 and 2004). Initially the V1 cells code the presence of the contours in their receptive field and after a delay of approximately 140ms, neuronal responses evoked by the target curve are enhanced over responses evoked by the distractor. This attentional selection signal is first observed for contour elements of the target curve that are relatively close to the fixation point and it then spreads along the representation of the curve until the target disc is reached (Pooremaeili & Roelfsema, in preparation). In a recent study we compared



V1 activity to that in area FEF during curve tracing and found that the enhancement of neuronal responses to the target curve in area V1 occurs at approximately the same time as target selection in area FEF (Khayat et al 2009). It is therefore likely that the easy condition of the present study is solved by the neuronal selection of the entire target curve across areas of the visual cortex including areas V1 and FEF.

An additional process is required to solve the difficult condition of the present task, because accurate performance relies on the selection of the appropriate icon. We have previously investigated the neuronal activity in area V1 in a comparable task where the monkey had to first search for a marker with a specific color and then trace a curve starting at that marker (Roelfsema et al 2003, Moro et al 2010). In this task neuronal responses evoked by the colored marker at the start of the curve are enhanced first, and the enhanced neuronal activity then spreads along the target curve. Successful performance in the difficult condition of the present task required the initial selection of the target icon, which was presumably followed by the spread of enhanced activity along the target curve and onto the target disc. Our finding that the accumulation of activity in area FEF in the difficult condition occurs later in the course of learning may therefore reflect the insertion of an additional visual search operation for the newly learned target icon before the start of tracing, which then delays the accumulation of activity in FEF and at the same time improves the monkey's accuracy.

### **Changes in the neuronal tuning as the result of learning**

A number of previous studies investigated the consequences of learning on neuronal activity in various brain regions. In an early study on the neuronal correlates of learning on eye movement selection in monkeys, Chen & Wise (1995) recorded single unit activity in areas FEF and SEF (supplementary eye fields) in a task where monkeys had to associate the identity of an icon with the direction of a saccade. Some neurons were activated while the monkeys learned the new associations whereas other cells changed their tuning in the course of learning (Chen and Wise, 1995a, 1995b; Brasted and Wise, 2004). Neurons coding eye movements in a particular direction started to respond to those objects that were associated with the preferred eye movement, and similar effects of learning on neuronal

activity have been observed in the hippocampus (Wirth et al., 2003), the striatum (Tremblay et al., 1998) and in the orbitofrontal cortex (Tremblay & Schultz, 2000). These studies typically did not map the response field of the cells as we did in area FEF, and it was therefore more difficult to dissociate changes in the tuning to the sensory stimuli from changes in motor preparation.

Asaad et al. (1998) dissociated tuning to visual stimuli from tuning to saccade direction by training monkeys to map visual stimuli onto eye movement responses, but reversing these associations from time to time. After a reversal the target selection signal in prefrontal cortex occurred progressively earlier with learning. This contrasts with our finding that the target selection signal was delayed during learning. The apparent inconsistency is presumably related to a difference between tasks. In the study of Asaad et al. (1998) the monkeys had to withhold their response for 1.5s after stimulus presentation whereas the monkeys could make a saccade immediately after stimulus presentation in our task, and the animals indeed made fast but random decisions.

### **The effects of learning on the neuronal mechanisms of decision making**

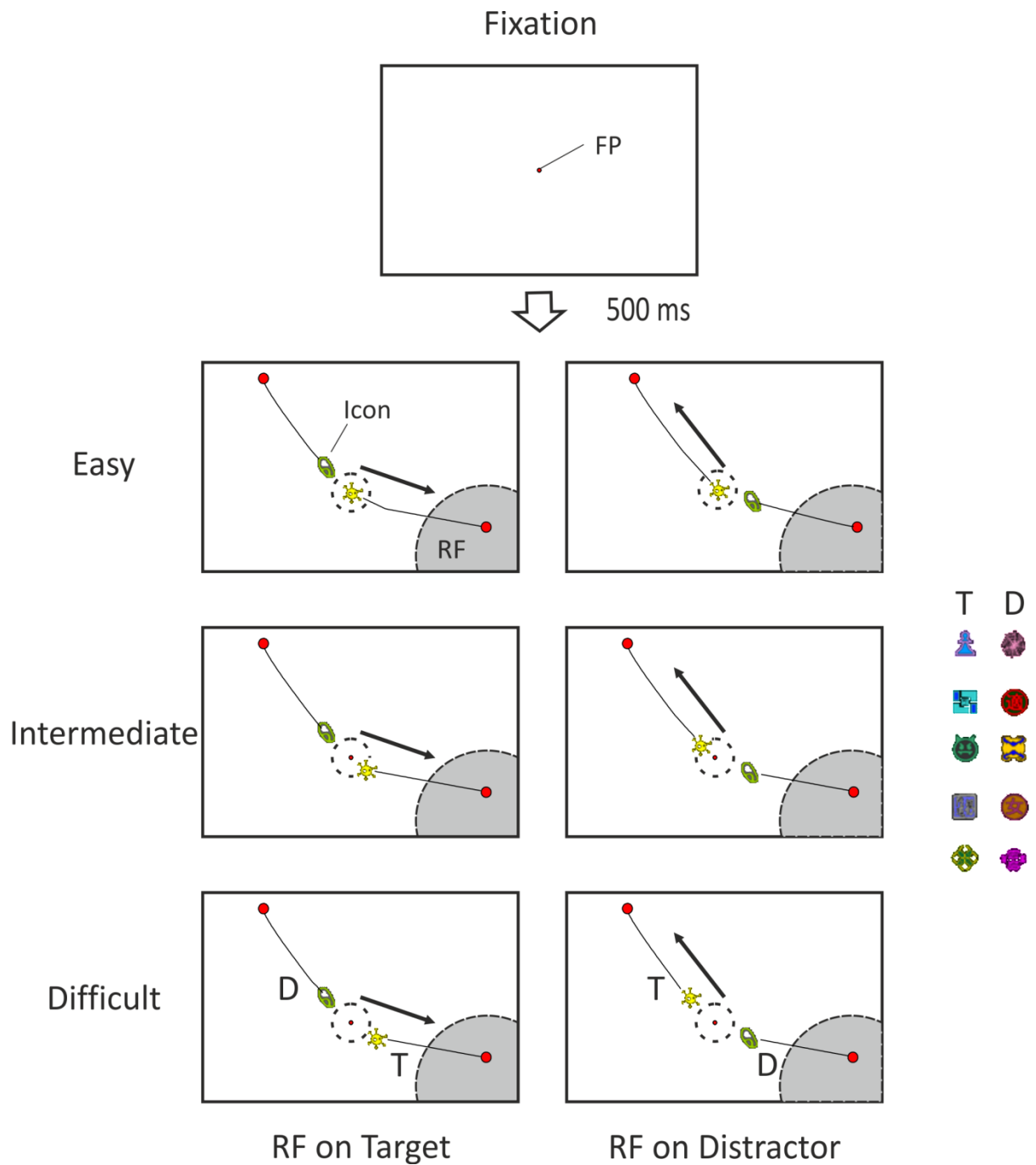
Because we placed the target or distractor disc in the RF of the recorded neurons, we can relate our results to previous studies on the neuronal correlates of decision making in area LIP and FEF. These previous studies investigated the integration of sensory evidence over time with a task where monkeys had to judge the direction of motion of a visual stimulus at various motion strengths and indicated their decision with an eye movement to one of two targets (e.g. Roitman & Shadlen 2002; Churchland et al 2008). The motion signal influenced the activity of neurons that coded eye movements to these targets. Motion signals increased the activity of neurons that implemented the corresponding eye movement and decreased the activity of the cells coding the other eye movement. Interestingly, the neurons the structures coding for eye movements gradually increased their activity until a relatively fixed threshold was reached and then the eye movement followed with a short delay. In this task, the accumulation of activity towards the threshold occurs fast for strong motion signals and is slower if the motion signal is weaker.

One might therefore have anticipated that the accumulation of activity in favor of one of the two eye movements becomes faster with learning, as learning icon identity increased the quality of the evidence. We obtained the opposite result, because accumulation was fast and decisions were taken rapidly and randomly before learning, and learning caused a more gradual accumulation of activity with more accurate decisions. There is, however, an important difference between taking a decision in the presence of unreliable sensory evidence and taking a decision without knowledge of the meaning of the sensory stimuli. The icons of our task were clearly visible and delaying the decision would not have improved the reliability of the sensory evidence. If the sensory stimulus is noisy, however, it can be advantageous to wait for additional evidence. But not too long, because it is better to make a fast choice if the stimulus is truly random. Previous studies suggested that the total waiting time in these situations is curtailed by an ‘urgency signal’ that adds activity to the neurons coding for different decisions, so that eventually one of them crosses the threshold for a decision, even if the evidence is ambiguous (Churchland et al 2008). In our task, fast decisions were taken before learning, but these were rather associated with less activity in area FEF. Thus, in our paradigm the urgency evident in the fast and random responses is associated with a fast accumulation of activity for one of the objects that is accompanied by an efficient suppression of the other, non-selected object.

In conclusion, we have shown that learning by trial and error is associated with the transition of a fast and random decision process into a more gradual and accurate decision process. Future studies could investigate if there are changes in the representation of the newly learned icon and how this changed representation causes the delay in the decision and the increase in accuracy which presumably takes place as an altered interaction between neurons in visual and frontal cortex.

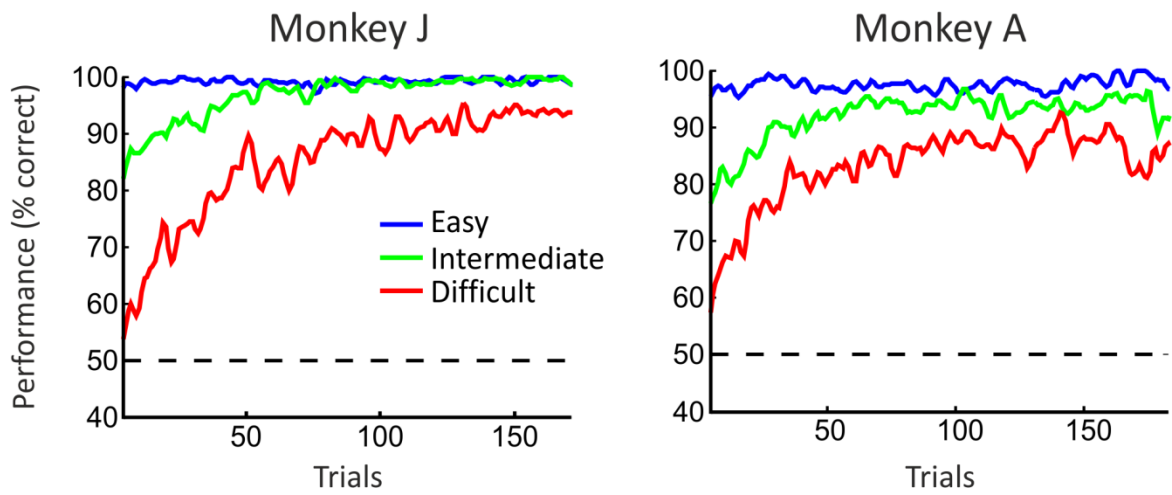
## ACKNOWLEDGEMENTS

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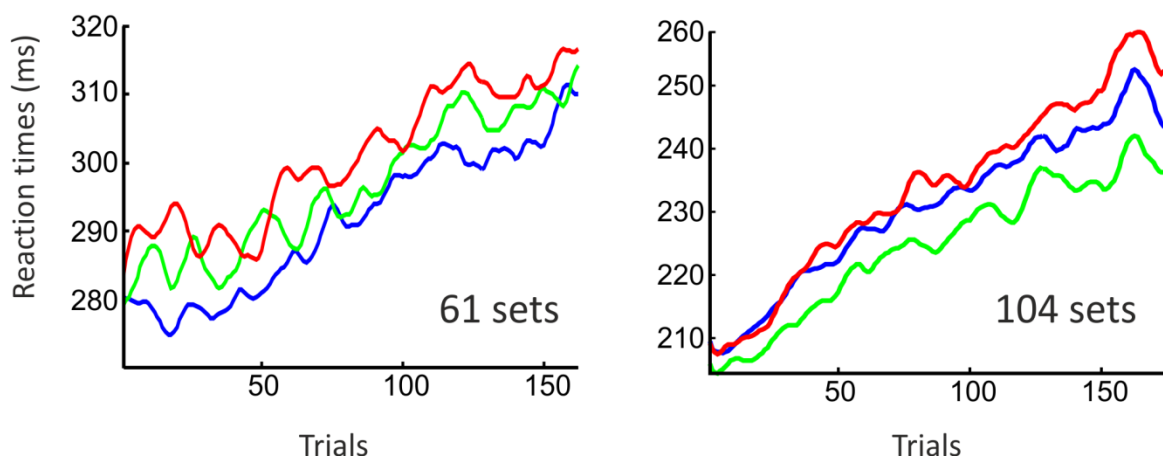


**Figure 1. Icon selection task.** The monkeys fixated a central dot for 500 ms, and then two icons appeared that were connected by curves to two circular discs. The monkey had to make a saccade to the target disc (arrow) that was connected to the relevant icon by a curve. In easy and intermediate stimuli the target icon (T) was nearer to the fixation point than the distractor icon (D). The distance between the fixation point and two icons was identical in the difficult condition, so that the monkeys had to rely on icon identity to make a correct choice. RF, receptive field of single unit in area FEF. FP, fixation point.

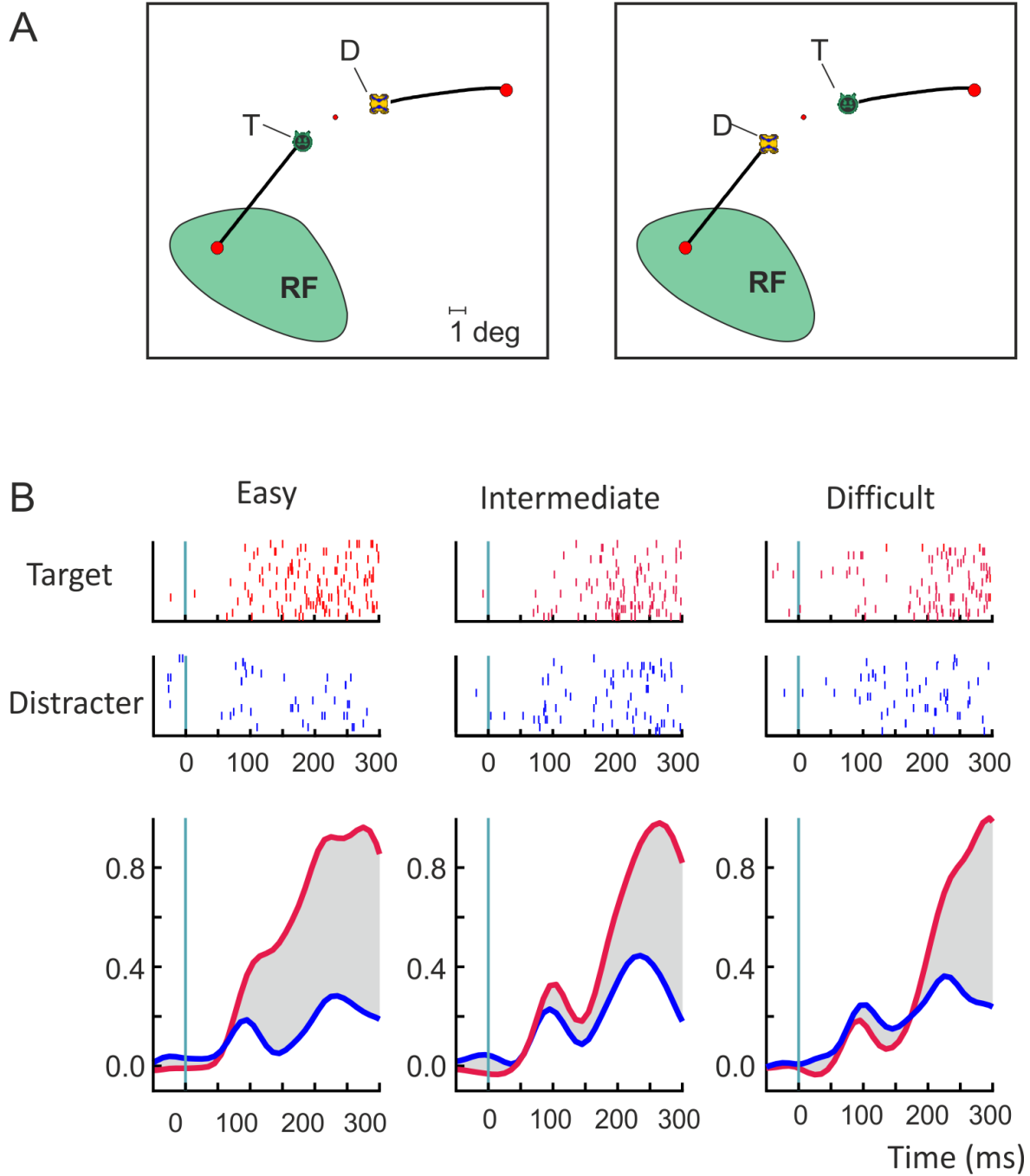
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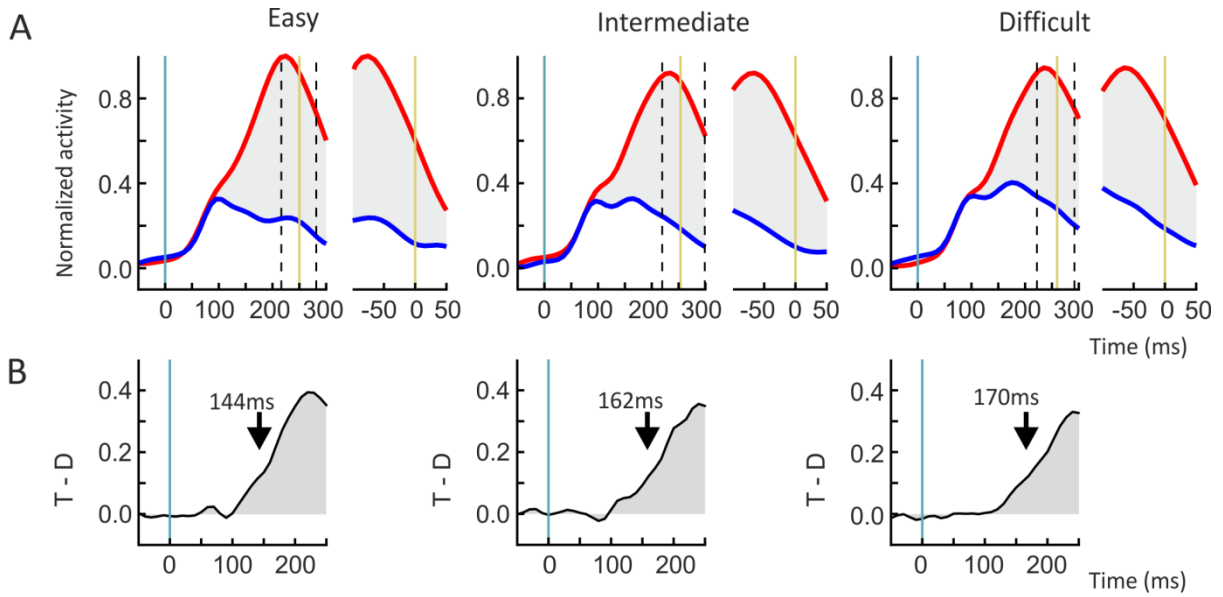
B



**Figure 2. Accuracy and reaction time during learning.** **A**, Accuracy improved in the intermediate (green) and the difficult condition (red), and was always near 100% in the easy condition (blue). **B**, The average reaction time tended to increase with the progression of trials.

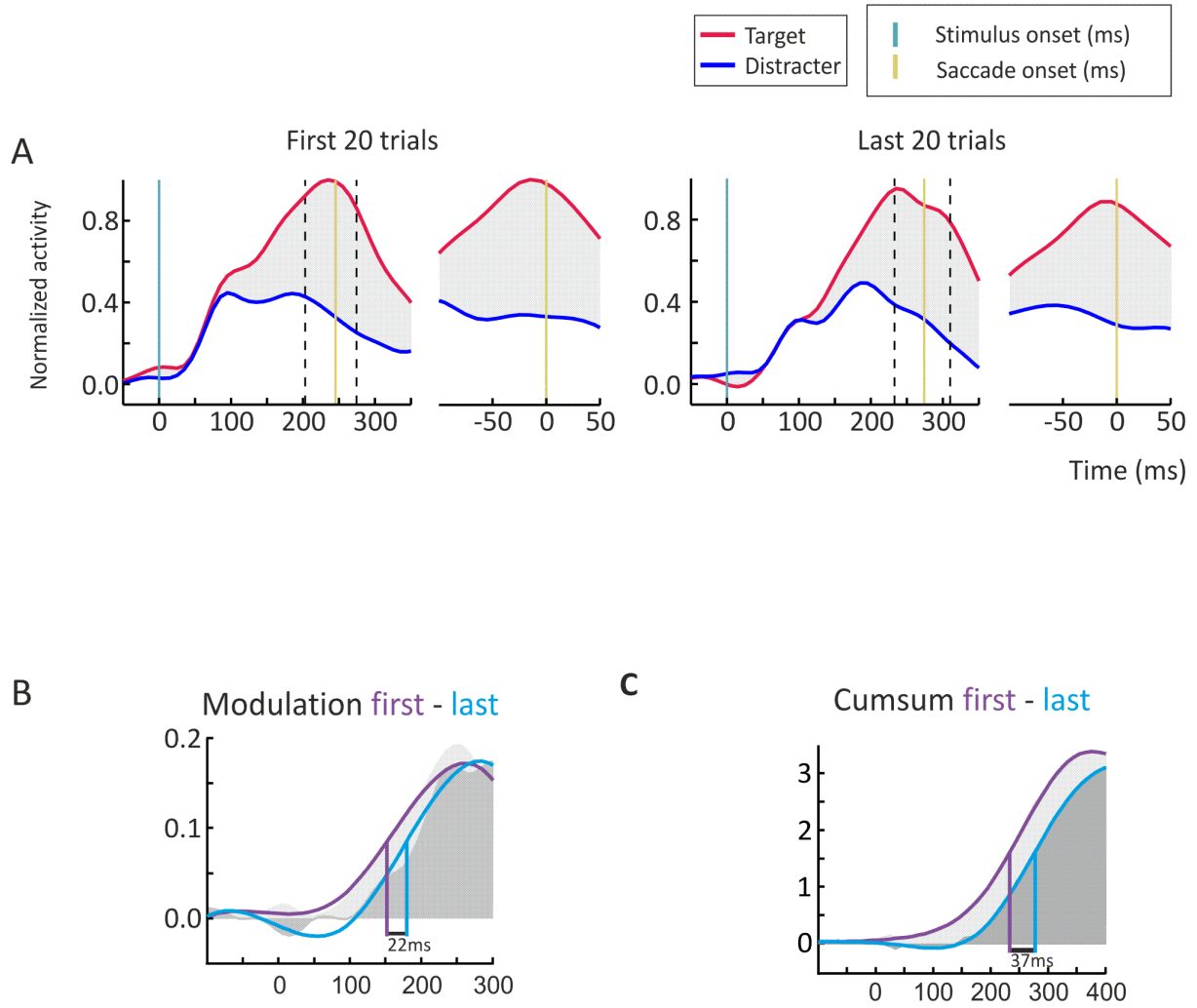


**Figure 3. Example FEF neuron in the icon learning task.** **A**, Stimuli of the difficult condition. The receptive of the FEF cell is shown in green. **B**, Activity of the FEF cell. The upper panels show raster plots of single trials in easy, intermediate and difficult condition with the target (red) or distracter disk (blue) in the RF. The lower panels show the average responses. The grey region denotes the difference in activity between target and distracter disk.



**Figure 4. Average FEF responses in correct trials of the easy, intermediate and difficult conditions.**

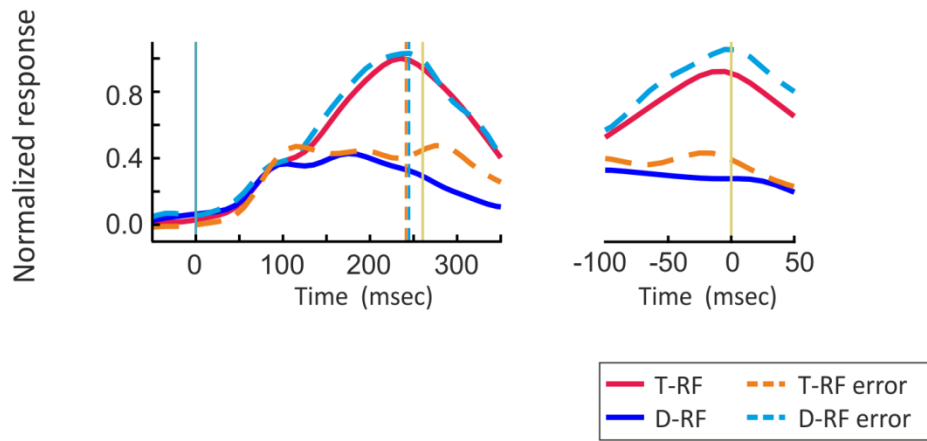
**A**, Normalized activity for targets and distracters, centered on stimulus presentation (left panels) and saccade onset (right panels). Dotted vertical lines depict the 25th and 75th percentile of the reaction times. **B**, Difference between the neuronal activity evoked by the target and distracter (modulation). The latency of target discrimination was measured as the time point where the response difference reached 50% of its maximal value.



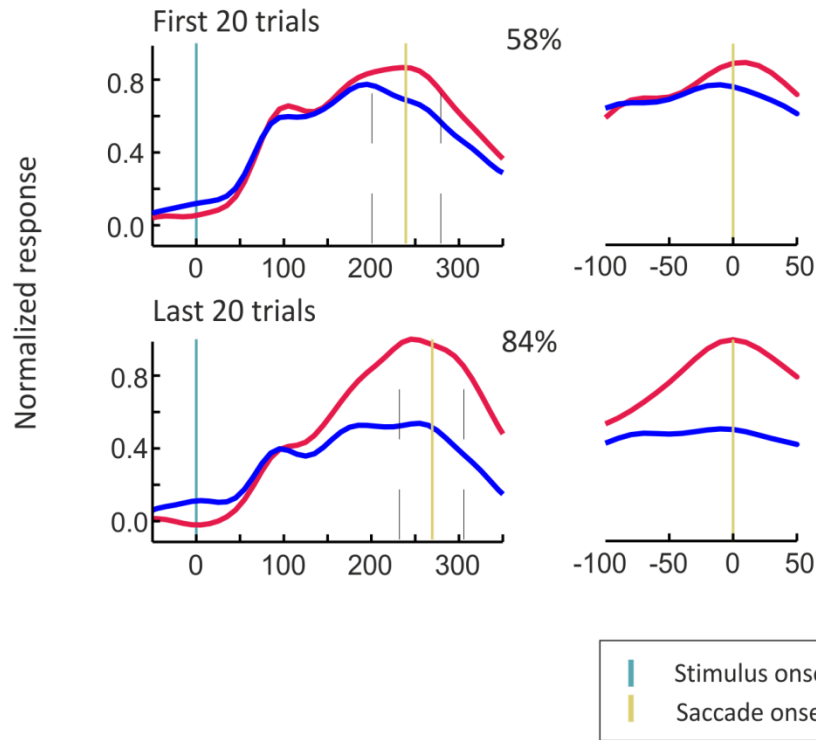
**Figure 5. Time course of learning in area FEF.** **A**, Averaged activity of target and distracter for the first and last 20 trials, centered on stimulus and saccade onset. Color codes are the same as in figure 4. **B**, Target-distracter modulation across time with red trace representing first ten trials and blue trace – last ten trials. The time intervals are differences between the first and last trials when activity reached half of its maximum (latency). **C**, The same analysis for the cumulative sum of the modulation. Purple traces – first trials, magenta traces – last trials.



A



B



**Figure 6. Analysis of error trials.** **A**, Comparison of the FEF population response on correct trials (continuous traces) and error trials (dashed traces) with the target (red) or distractor disc in the RF (blue). **B**, Evolution of the population response during learning when error trials are included. The lower panels shows the average response during the first 20 trials and the lower panel shows activity in the last 20 trials. Numbers above the panels show average accuracy in these trials.

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