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Selective representation of relevant information by neurons in the primate prefrontal cortex

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The severe limitation of the capacity of working memory, the ability to store temporarily and manipulate information[1], necessitates mechanisms that restrict access to it. Here we report tests to discover whether the activity of neurons in the prefrontal (PF) cortex, the putative neural correlate of working memory[2–8], might reflect these mechanisms and preferentially represent behaviourally relevant information. Monkeys performed a 'delayed-matching-to-sample' task with an array of three objects. Only one of the objects in the array was relevant for task performance and the monkeys needed to find that object (the target) and remember its location. For many PF neurons, activity to physically identical arrays varied with the target location; the location of the non-target objects had little or no influence on activity. Information about the target location was present in activity as early as 140 ms after array onset. Also, information about which object was the target was reflected in the sustained activity of many PF neurons. These results suggest that the prefrontal cortex is involved in selecting and maintaining behaviourally relevant information.

In the 'array trials', a sample array of three objects was briefly presented while the monkeys maintained central gaze (Fig. 1a). Monkeys needed to find the target object in the array and remember its location. After a brief delay, a test array appeared and the monkeys had to release a lever if the target object appeared in the same location as it had in the sample array. Although each of the three objects was a target, in turn, for a block of trials, its location in the sample array was chosen randomly on each trial. Monkeys were cued to the target object with 'cue trials' (Fig. 1a) in which the target object appeared alone.

Table 1 Summary of neuronal selectivity in different task periods

	Sample period	Delay period	Both periods
$n = 97$ cells			
Number of cells selective for:			
Target object only	20	16	7
Target location only	22	30	13
Target object and location	24	15	8
Total selective for object	44	31	15
Total selective for location	46	45	21
Selectivity depth:			
Object	48%	44%	–
Location	48%	53%	–
Selectivity index:			
Object	0.24	0.24	–
Location	0.24	0.28	–

Cell counts are based on ANOVA (see Methods), evaluated at $P < 0.01$. Mean selectivity depths and selectivity indices were computed from delay activity on array trials for cells showing a significant ANOVA. For cells not showing significant effects, mean selectivity depths ranged from 12 to 15% and mean selectivity indices ranged from 0.08 to 0.09.

We recorded the activity of 97 neurons from the lateral prefrontal cortex of two monkeys (Fig. 1b). Based on analysis of variance (ANOVAs) (evaluated at $P < 0.01$), many PF neurons showed activity to physically identical sample arrays that varied depending on which of three array positions contained the target (46/97 or 47% during sample presentation, 45/97 or 46% during the delay, Fig. 2 and Table 1). Information about the target location appeared very early in neural activity, starting about 140 ms after array onset (Fig. 3a). The activity of these neurons after this time largely reflected the target location alone; information about the location of the irrelevant, non-target, objects had little or no influence. Although almost half of PF cells showed activity that varied with the location of the target object, only a few cells (sample period: 10/97 or 10%; delay period: 5/97 or 5%) showed activity that varied with the location of non-target objects (*t*-tests, evaluated at $P < 0.01$). In fact, many cells showed similar activity on array trials and on cue trials in which the target object appeared alone (Fig. 2).

The task also required monkeys to remember which object was currently the target. This was also reflected in PF activity. On array trials, many PF neurons showed activity during the sample period

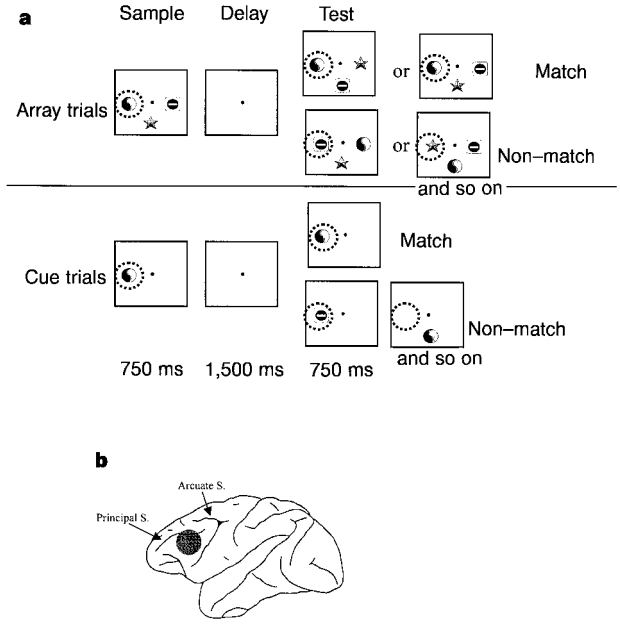

Figure 1 The behavioural task and recording sites. **a**, Sequence of trial events. Each trial began when the monkey grasped a lever and fixated a small fixation target at the centre of a computer screen. The location of the target object is indicated by the dotted circle on this figure. Examples of array trials (top) and cue trials (bottom) are illustrated. **b**, Location of recording sites: Arcuate S, arcuate sulcus; Principal S, principal sulcus.

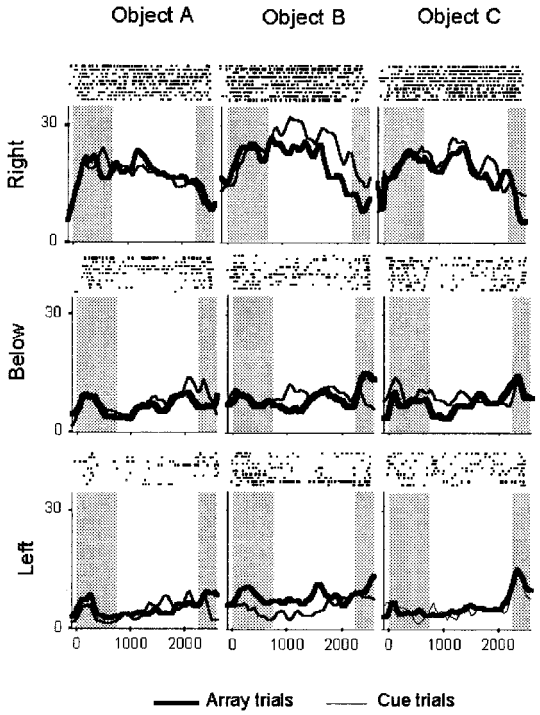

Figure 2 Delay activity from a single PF neuron that varied with the location of the target object. The grey bar on the left of each histogram indicates time of sample presentation and the grey bar on the right indicates presentation of the test array. The column labels refer to the object that was the target in the array trials or which single object was used as the sample on cue trials. The row labels refer to the location of the target. Bin width, 40 ms. Above each histogram are rasters from 10 trials of array presentation. Each dot represents an action potential from the neuron and each row corresponds to a different trial.

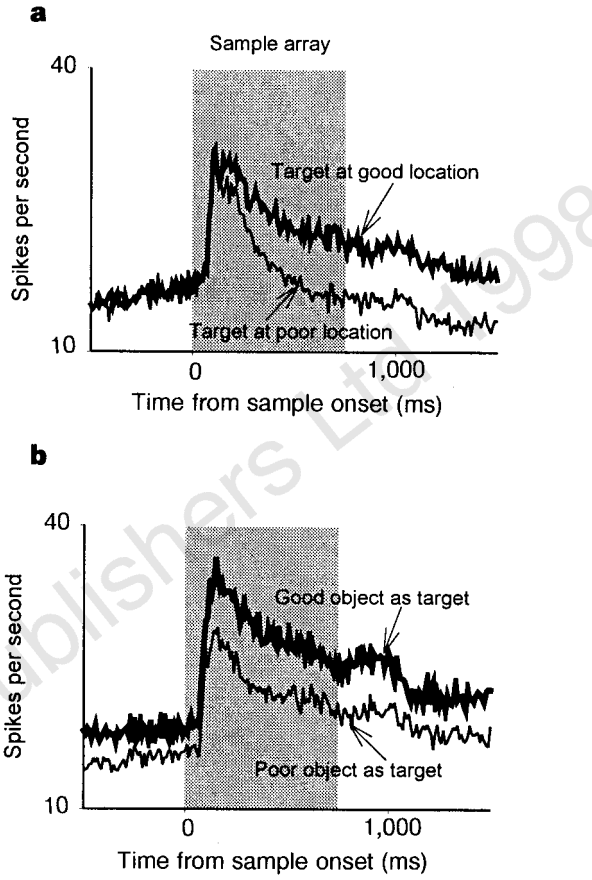

Figure 3 Time course of location and object effects for cells showing those effects. **a**, Average activity of 45 PF neurons selective for the target location. **b**, Average activity of 31 PF neurons selective for the target object. Neurons were selected for this figure if they showed a significant effect in the last 1,000 ms of the 1,500 ms delay. The grey bar shows the time of sample array presentation. The difference in activity when a good object was the target and when a poor object was the target was evident in the baseline activity before sample onset (*t*-test, $P < 0.001$).

(44/97 or 45%) and during the delay (31/97 or 32%) that varied depending on which object was relevant. However, unlike activity selective for the target location, information about the target object was reflected in activity even before the appearance of the sample array on each trial (Fig. 3b). Because a given object was the target for an entire block of trials, this chronic change in activity presumably reflects its maintained memory across trials.

These results suggested that monkeys were focusing their attention on the target location. In behavioural experiments, we confirmed that the target was 'capturing' the attention of the monkeys. The monkeys were allowed to look freely at the sample array. Under free gaze, eye movements and attention are closely coupled[9,10]. During the 750 ms of the sample array, the monkeys spent, on average, 529 ms looking at the target and only 82 ms looking at each of the non-targets. Thus, the requirement to remember the target resulted in the monkeys directing attention to it.

It is well established that working memory is severely limited in capacity[11–13]. Our results suggest that focal attention can play a major role in regulating access to working memory. Information about the location of an attended target dominated the activity of many PF neurons, the putative neural correlate of working memory. Thus, we hold in working memory that to which we attend. But the converse is also true. That is, working memory can direct attention: PF activity provided a representation of the to-be-attended target object that could have been used to select it from the array[14]. Psychological studies and models of selective attention have suggested this intimate relationship between attention and working memory[14,15]. These results illustrate it in prefrontal activity and provide support for the notion that the attentional effects observed in regions such as the parietal cortex[16] and the inferior temporal cortex[17] arise from bias signals originating in the PF cortex[15]. □

Methods

Behavioural task. The task is illustrated in Fig. 1. The objects were colour pictures of objects 2° by 2° in size. 'Real world' stimuli such as these have been shown to elicit selective responses readily from prefrontal neurons[6]. For each day of recording, three objects were chosen at random from a large pool of objects. We did not determine which features of the object elicited activity from the neurons under study; for the purposes of this study all that mattered was whether different objects or their locations elicited different levels of activity.

Monkeys were required to maintain central gaze throughout the trial. Each of the objects appeared 4° to the right, to the left, and below fixation. The location of each of the three objects in the sample array was chosen randomly on each trial. Each object was the target, in turn, for a block of about 80 trials. Each block began with 10 cue trials in which the target object was used alone as the sample. The cue trials were then randomly intermixed with array trials throughout the blocks. Blocks in which a given object was the target were interleaved with blocks with another target object until there were about 9–12 blocks (3–4 repetitions of each block of each object as a target). This interleaved design compensates for any changes in neural activity across blocks that were unrelated to the task. Monkeys were well trained for this task, averaging over 85% correct.

Electrophysiological recording. Recordings were made in the lateral prefrontal cortex (see Fig. 1b) of two adult rhesus monkeys (*Macaca mulatta*) using standard electrophysiological techniques. Recording sites were

localized using magnetic resonance imaging. We advanced the electrode until neuronal activity was well isolated. Then, data collection commenced. To ensure an unbiased assessment of PF activity, we made no attempt to select neurons based on task-related activity.

Data analysis. Visual responses to the samples were analysed over an interval from 100 ms to 750 ms after sample onset. Delay activity was analysed over the last 1,000 ms of the 1,500 ms delay after the sample array.

Activity was appraised using ANOVA, evaluated at $P < 0.01$. To determine whether activity on array trials reflected the target object, its location, or both, a two-factor ANOVA was used. One factor was which object was the target and the other was its location. To assess the influence of the location of non-target objects, we computed nine *t*-tests for each cell (evaluated at $P < 0.01$, with Bonferroni correction for multiple tests). Each *t*-test compared responses to the two possible arrays that contained a given target at a given location. For example, one array might contain object A as a target at position 1, B at position 2, and C at position 3 (that is, A1 B2 C3) whereas the other would contain target A at 1, C at 2, and B at 3 (A1 C2 B3). Thus, the arrays differed in the position of the non-target objects only. This allowed us to determine whether activity to an array with a given target (such as A) at a given location (such as 1) was influenced by the positions of the two non-targets (such as B and C).

'Good' and 'poor' refer to the object or location that elicited the most or least activity, respectively. Selectivity depth measured the difference in activity on array trials when a good versus poor object or location was relevant. It was computed by dividing the difference in their activity by their sum and then converting this value to per cent difference[18]. The selectivity index[19], by contrast, takes into account changes in activity to each of the three relevant objects or locations. It was computed on array trial activity and was defined as:

$$S = \frac{n - \left(\frac{\Sigma r_i}{r_{\max}}\right)}{n - 1}$$

n = number of objects or locations; r_i = activity to a target object or location i; $r_{\max}$ is the maximum r_i. A value of zero indicates identical responses to all stimuli; a value of 1 indicates activation by one stimulus and silence to other stimuli. Values typically do not approach 1 because PF neurons are rarely silent and have some baseline firing.

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Gli/Zic factors pattern the neural plate by defining domains of cell differentiation

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Three cell types differentiate in the early frog neural plate: neural crest at the lateral edges, floorplate at the midline and primary neurons in three bilateral stripes. Floorplate cells and ventral neurons are induced by Sonic hedgehog[1,2] (Shh) and neural crest and dorsal neurons are induced by epidermal factors such as bone morphogenetic proteins (BMPs)[3]. Neurogenesis in a subset of cells within the stripes involves lateral inhibition[4]. However, the process by which pools of precursors are defined in stereotypic domains in response to inductive signals is unknown. Here we show that frog *Zic2* encodes a zinc-finger transcription factor of the Gli superfamily which is expressed in stripes that alternate with those in which primary neurons differentiate and overlap the domains of floorplate and neural crest progenitors. Zic2 inhibits neurogenesis and induces neural crest differentiation. Conversely, Gli proteins are widely expressed, induce neurogenesis and inhibit neural crest differentiation. *Zic2* is therefore a vertebrate pre-pattern gene, encoding anti-neurogenic and crest-inducing functions that counteract the neurogenic but not the floorplate-inducing activity of Gli proteins. We propose that the combined function of Gli/Zic genes responds to inductive signals and induces patterned neural cell differentiation.

Frog *Zic2* encodes a nuclear protein (Fig. 1a, inset) homologous to mouse Zic2 (refs 5, 6) and all Zic proteins seem to recognize Gli binding sites[7]. *Zic2* messenger RNA is detected in the dorsal ectoderm of the early gastrulae and early neurulae (Fig. 1a). *Zic2* is then expressed in stripes alternating and non-overlapping with those of primary *N-tubulin*$^+$ (*N-tub*$^+$) neurons, although not all *N-tub*-negative areas express *Zic2* (Figs 1b, c, e and 2a, b). Midline and, later, floorplate cells show low levels of *Zic2* expression (Fig. 1d, top). The most medial stripe of *N-tub*$^+$ primary motor neurons is located in between the midline, which expresses *Shh*[2], and a medial *Zic2* stripe (Fig. 1d, e). The intermediate stripes of *N-tub*$^+$ interneurons in anterior regions is in between the medial *Zic2* stripe and its expression in the neural folds (Figs 1b, c and 2a, b). Posteriorly, the outer stripe of *N-tub*$^+$ neurons is lateral to *Zic2* in the neural folds (Figs 1b–d and 2a, b). Trigeminal ganglion neurons are also adjacent to the anterior domain of *Zic2* (Fig. 2a, inset). At later stages, *Zic2* is expressed at high levels in the anterior edge, the future anterior brain being marked by delayed *N-tub* expression (Fig. 1b, c). *Zic2* is also detected at high levels in the lateral neural folds, which contain the presumptive cranial neural crest (Fig. 1b), and later in early migrating hindbrain crest (Fig. 1f). In tadpoles, *Zic2* is expressed in distinct regions of the brain (Fig. 1f, g) and in the spinal cord, it is detected dorsally (Fig. 1g).

To test whether Zic2 participates in defining neurogenic domains, we analysed the expression patterns of *N-tub* (Fig. 2b) and *Neurogenin1* (ref. 8) (*Ngnr1*: Fig. 2e) in injected embryos. Unilateral Zic2 misexpression resulted in the unilateral loss of *N-tub*$^+$ and *Ngnr1*$^+$ cells (Fig. 2c, f) that inherited Myc–Zic2 protein or β-galactosidase from injected RNAs. Suppression of neurogenesis is not due to inhibition of neural development, as the neural plate of most of the injected embyros displayed normal expression of neural cell adhesion molecule (NCAM) (Fig. 2d: stage ~14, 66% normal, $n = 18$; and data not shown). We also analysed the ability of Zic2 to inhibit ectopic neurogenesis, downstream of neural induction,