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LETTERS

Categorization of behavioural sequences in the prefrontal cortex

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Although it has long been thought that the prefrontal cortex of primates is involved in the integrative regulation of behaviours[1–4], the neural architecture underlying specific aspects of cognitive behavioural planning has yet to be clarified[5–8]. If subjects are required to remember a large number of complex motor sequences and plan to execute each of them individually, categorization of the sequences according to the specific temporal structure inherent in each subset of sequences serves to facilitate higher-order planning based on memory. Here we show, using these requirements, that cells in the lateral prefrontal cortex selectively exhibit activity for a specific category of behavioural sequences, and that categories of behaviours, embodied by different types of movement sequences, are represented in prefrontal cells during the process of planning. This cellular activity implies the generation of neural representations capable of storing structured event complexes at an abstract level, exemplifying the development of macro-structured action knowledge in the lateral prefrontal cortex[9].

We trained two monkeys to perform a series of four movements in 11 different temporal orders[10] (Supplementary Methods). The temporal structures of the 11 motor sequences were designed as follows: four were 'paired' sequences, which included two pairs of movements (for example, turn–turn–push–push), four were 'alternate' sequences, which were composed of an alternation of two movements (for example, pull–turn–pull–turn), and the remaining three were 'four-repeat' sequences, which required the same movement to be repeated four times. After a series of learning trials under visual-signal guidance, the monkeys prepared to perform a sequence of four movements based on memorized information about temporal sequences that fell into the three categories. We recorded from the lateral prefrontal cortex above and below the principal sulcus in the left hemisphere[11]. Among the 376 prefrontal (PF) cells that were task-related, 165 cells exhibited modulation of their activity during the waiting period preceding the first movement-trigger signal; these were the focus of the present study.

Our most striking finding was that in more than half of the cells ($n = 85$) that showed modulated activity during the waiting period, the activity was selectively induced as the monkey prepared to perform sequences from one of the three sequence categories. Figure 1 shows three examples of PF cells that showed category-selective activity. The cell shown in Fig. 1a was preferentially active as the monkey prepared to perform the sequences of motor activities categorized as paired. The activity of this neuron was not significantly different during the periods preceding each of the four paired sequences. In contrast, the activity of the cell shown in Fig. 1b significantly increased when the forthcoming sequences of motor activities were categorized as alternate. The activity of the cell shown in Fig. 1c was selectively elevated when the sequence belonged to the four-repeat category. Using linear regression analysis, we found that 31 cells were selective for the alternate sequences, 32 cells were selective for the paired sequences, and the remaining 22 cells were selective for the four-repeat sequences. In contrast, a minority of the PF cells examined ($n = 30$) was found to be selectively active before the execution of an individual sequence of motor activities (that is, selectivity for any one of the 11 sequences). These 30 cells were not classified as category selective. The remaining 50 cells were not selective either for the category or the sequence.

To what extent do the 85 category-selective cells differentiate between the different categories of sequence during the preparatory period? To answer this question, we plotted a time-varying discharge density function for the populations of cells that responded in preparation for each of the three different categories of sequences. As can be seen in the population data presented in Fig. 2a, activity in cells selective for each of the three sequence categories steadily increased during the preparatory period, clearly showing whether or not the forthcoming sequence belonged to the preferred category of sequences for those cells. Further analysis of the activity of individual cells revealed that differences in the preparatory activity for sequences belonging to one category (within-category variations) were small, whereas across-category variations were large (Supplementary Figs 3 and 4).

Although the monkeys did not make many mistakes, there was unusual cellular activity during error trials. Figure 2b illustrates a typical example of activity that accompanied errors. As long as the performance was correct, the activity of the PF cell shown here increased selectively when the monkey was preparing to perform alternate sequences but not others (black traces). The increase in activity was not observed when the monkey made the mistake of preparing a sequence that belonged to the paired or four-repeat categories (trials labelled 'across category'). In contrast, even when the monkey was preparing to perform an incorrect sequence the activity of the cell was greater if the forthcoming sequence belonged to the alternate category (trials labelled 'within category'). In addition, Fig. 2b (middle panel) illustrates that the activity of the same cell increased when the monkey made the mistake of preparing to perform an alternate sequence when a paired sequence was required. This example is typical in showing that the activity of a cell during the preparatory period faithfully reflected whether or not a sequence from a particular category was planned (see Supplementary Information for other cells).

Subsequently, we examined the extent to which the activity of PF cells during the preparatory period predicted the sequence category that the animals performed. For this purpose, we determined an index of predictive activity (the receiver operating characteristic index)[12,13] using the cellular activity measured during the early, middle and late portions of the preparatory period. This index estimates

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the probability that an observer can predict the category that will be performed by the monkey on the basis of the spike rate. As presented in Fig. 3, the activity of PF cells during the early part of the delay period was not a good indicator of the sequence category. However, during the last 0.5 s of the preparatory period the activity of PF cells classified on the basis of their selectivity for one of the three sequence categories served as indicators of whether the monkey would perform a paired (green triangles) or four-repeat (red circles) sequence, or an alternate sequence (orange diamonds). The category selectivity started to grow already during early visually guided trials, and developed steadily during memorized trials (Supplementary Fig. 5).

Histological examination revealed that our recording sites were located in the lateral prefrontal cortex in front of the frontal eye field. Category-selective cells were identified more frequently at penetrations that were dorsal to the principal sulcus than at ventrally located penetrations (Fig. 1d; $P < 0.01$ with the χ^2 test), although no spatially selective distribution of the cells classified as belonging to any of the three categories was found (see Supplementary Information).

Previous reports have shown that cells in the medial motor areas of monkeys are selectively activated on the basis of the sequence of multiple movements[14–19]. Neural coding of the sequence of movements has also been reported in the prefrontal cortex[20,21], suggesting prefrontal involvement in representing subjective knowledge of the action sequence[22]. In line with these reports, we found cellular activity that was selective for the sequence of movements in the present study. However, the cells that were selective for a sequence during the preparatory period were not abundant. The novel finding in this study is the identification of PF cells that are selective for the category of the motor sequence to be performed, rather than the individual sequence itself. The information about the category was not obtained from signals in the external world but was generated internally as a neural representation. In particular, knowledge of a category represented an assembly of information about a set of sequences. In that sense, the representation of the category is of a higher order than the representation of the sequences. We therefore propose that the development of category information represented in PF cells implies the generation of a unit of knowledge that specifies the macrostructure of an event series at an advanced level of unification. This view is consistent with a functional magnetic resonance imaging study[23] reporting the involvement of the lateral PF in action selection on the basis of

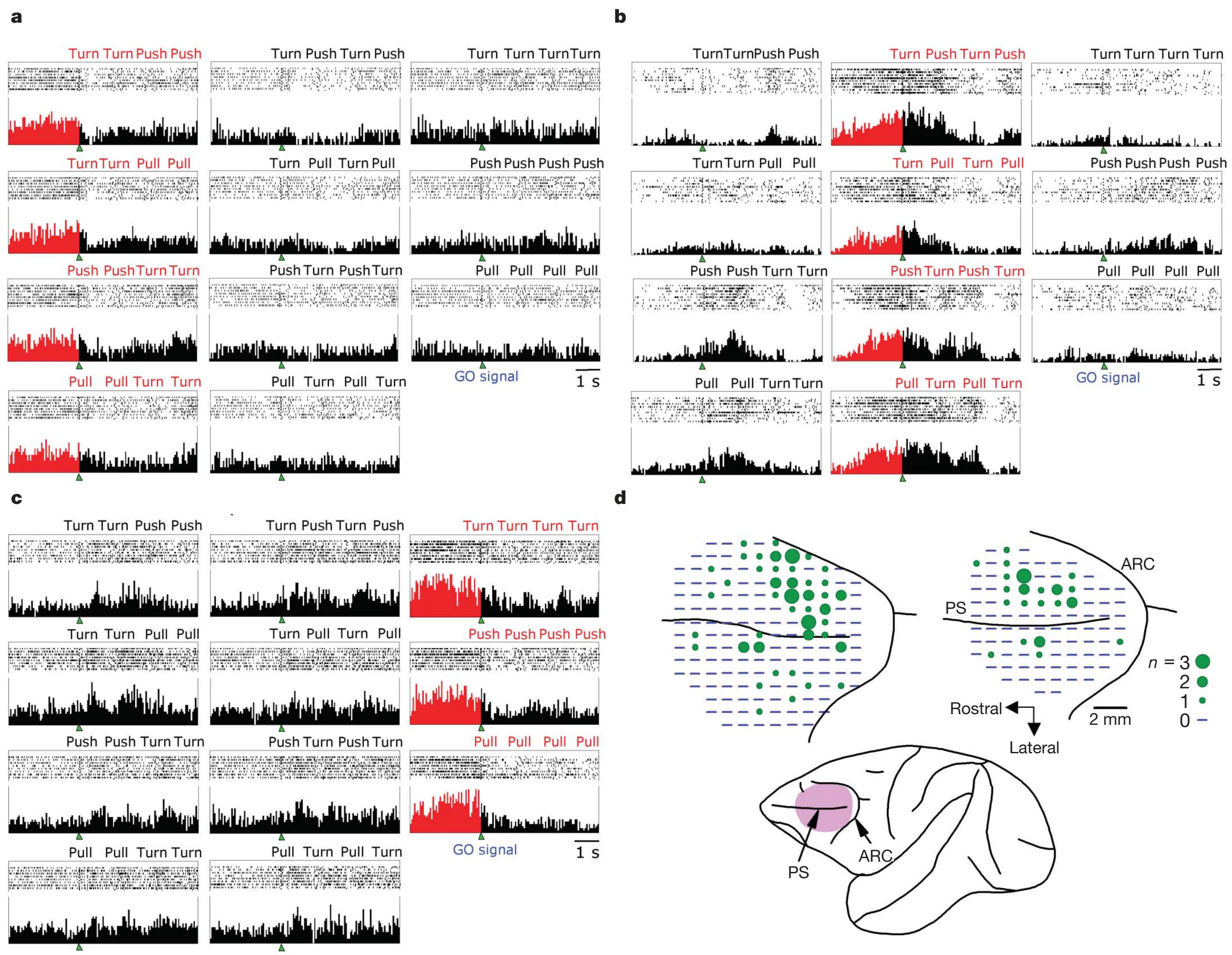

Figure 1 | Activity of PF cells selective for a category of sequences during planning. a, Raster displays and peri-event histograms illustrating the cellular activity selective for the 'paired' category. The displays are aligned on the appearance of the GO signal (green-filled triangle) for the first of the memorized movements. **b**, Activity selective for the 'alternate' category. **c**, Activity selective for the 'four-repeat' category. **d**, Top: recording sites of category-selective cells. The size of the circle is proportional to the number of selective cells at each site. Bottom: a cortical surface map showing the surveyed area. PS, principal sulcus; ARC, arcuate sulcus.

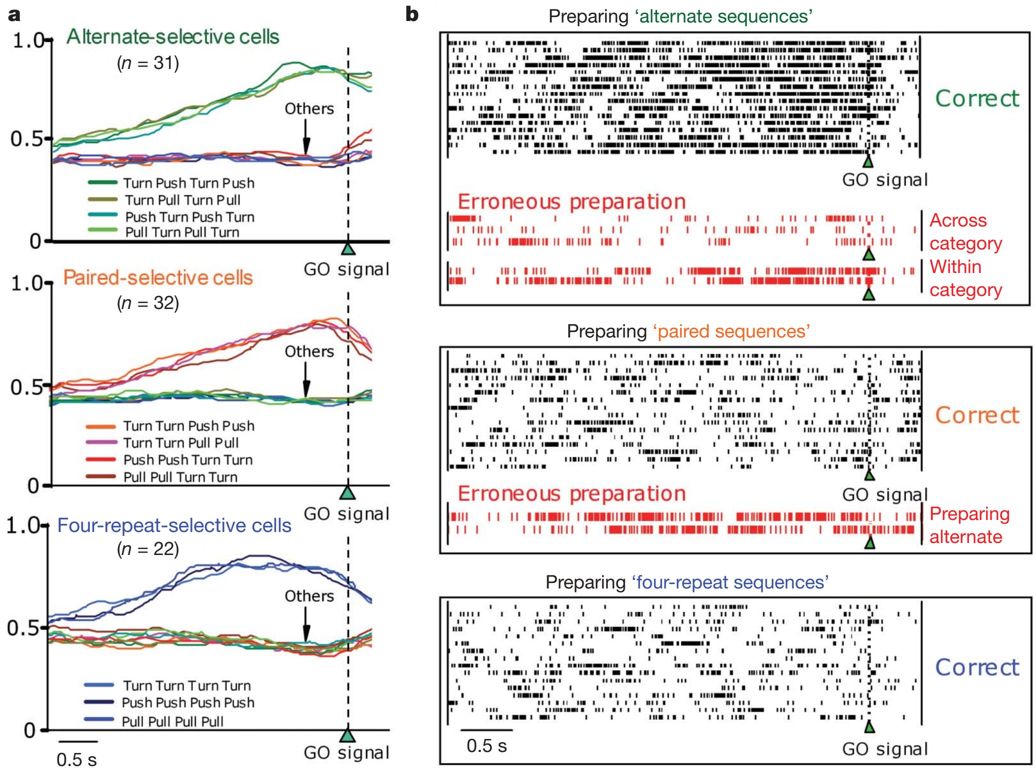

Figure 2 | Time courses of the activity of cell populations showing category selectivity and a comparison of cellular activity before the monkey executed the correct or an incorrect sequence. a, Spike density function for populations of PF cells that were selectively active as the monkey prepared behavioural sequences from one of the three categories. The traces compare the activity during preparation for alternate (top), paired (middle) and four-repeat (bottom) sequences with the activity during preparation for sequences of other categories. **b,** Activity of a PF cell when the monkey was preparing to perform the correct or an incorrect behavioural sequence.

hierarchical structures of behavioural plans, and is also consistent with clinical reports that patients with prefrontal lesions are impaired in analysing clusters of action sequences[24] or in formulating action plans that accord with managerial knowledge[25,26]. It is remarkable that about half of the cells modulated during the preparatory period were tuned to a category of sequences. This result is in line with an adaptive coding hypothesis[27] that PF cells adapt their properties to carry information that is relevant to current concerns. It is conjectured that the monkeys used the category information for task performance because they made more within-category than between-category errors, and also because cellular activity during the preparatory period predicted the types of errors that the monkeys would make. This implies that our monkeys were likely to have learned abstract as well as surface structure of movement sequences[28] during extensive training. The responses of PF cells in monkeys trained to categorize animal forms as either doglike or catlike were examined with the use of the technique of 'morphing' the basic form of one animal into the other[29]. The authors found that PF cells selectively responded to visual stimuli that belonged to either the cat or the dog category, showing that category information in the perceptual domain is represented in the prefrontal cortex[30]. Our present results extend these findings on the representation of categories into the domain of behavioural guidance with respect to macro-level planning. Taken together, category-selective cellular activity is useful for

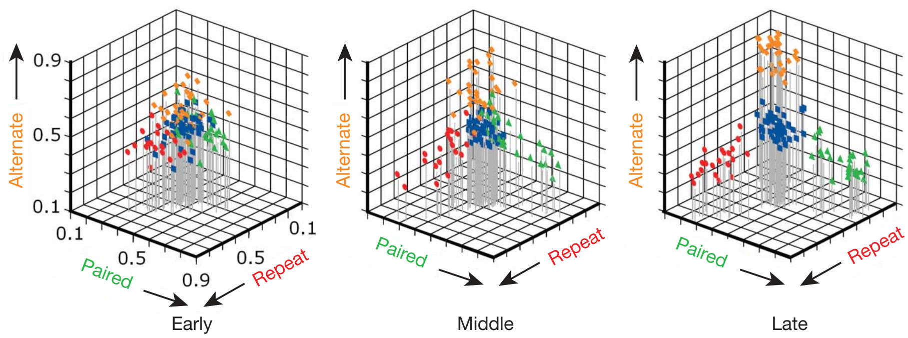

Figure 3 | Plots of the predictive activity of 135 PF cells. The predictive index approximated the accuracy with which an observer could estimate the category of the sequence that the monkey was preparing to execute on the basis of cellular activity during the early (first 500 ms, left), middle (1,000–1,500 ms, middle) and late (last 500 ms, right) phases of the preparatory period. The indices plotted along the *x*, *y* and *z* axes predict the preparation to perform sequences belonging to the three categories shown. Orange diamonds, data for alternate-selective cells; green triangles, paired-selective cells; red circles, four-repeat-selective cells; blue rectangles, non-selective cells.

generalizing perceived or internally generated information, for the purpose of guiding behaviour by integrated knowledge represented in the prefrontal cortex.

METHODS

Behavioural procedures. We trained two monkeys (*Macaca fuscata*) to perform a series of four movements in 11 different temporal orders (see Supplementary Fig. 2). Each movement was a push, a pull, or a turn of a handle with the right hand. The task began when the monkey held the handle in a neutral position and waited until a tone signal triggered the first of the four movements. After completion of an individual movement the animal had to wait for the next movement-trigger signal. Initially, during the learning phase, green, yellow and red light-emitting diodes indicated that the monkey was required to push, pull and turn the handle, respectively. The animals underwent five learning trials and subsequently performed the sequential motor task without the visual signals. After five trials based on memory, the sequence of the movements was changed for the next set of trials. For the temporal structures of the 11 motor sequences, the four paired sequences consisted of two movement pairs, four alternate sequences consisted of the alternation of two movements, and the three remaining four-repeat sequences required the one movement to be repeated four times. Thus, after the learning trials, monkeys had to prepare to perform a sequence of four movements on the basis of memorized information about the temporal patterns that fell into one of three categories. When five trials were accomplished under memory, a new sequence began for the next visually guided trials. We used conventional electrophysiological techniques to obtain *in vivo* single-cell recordings[10] from the lateral prefrontal cortex above and below the principal sulcus in the left hemisphere, rostral to the frontal eye field.

Data analysis. We focused on studying cells that had their activity modulated during the preparatory period. To assess statistically how the three categories of motor sequence were related to the activity of the cells, we performed linear regression analysis with the use of the following regression model: firing rate = $\beta_0 + \beta_1 \times$ (categories), where β_0 is the intercept and β_1 the regression coefficient. The categorical factor was the sequence categories (paired, alternate or four-repeat). Subsequently, for cells satisfying this criterion, we examined whether the cellular activity was related to an individual sequence of movements. For this purpose we performed a second round of linear regression analysis with the following equation: firing rate = $\beta_0 + \beta_2 \times$ (sequences), where β_2 is the regression coefficient, and the categorical factors for sequences were the 11 different movement sequences. In this study we defined the activity of a cell to be selectively reflecting a category if it was found to be related to the category but not to an individual sequence within this category. Furthermore, we determined an index that described the association between cellular activity during the preparatory period and the monkey's subsequent selection of a sequence from one of the three sequence categories. This index approximated the ability of an observer to predict the monkey's behaviour from the cellular activity. We performed a receiver operating characteristic analysis[12,13] to obtain this predictive index.

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