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27. Tables of supplementary data are available at www.sciencemag.org/feature/data/986310.shl and at http://rex.nci.nih.gov/RESEARCH/basic/lgd/front_page.htm.
28. The following alleles were detected in seroconverters: *HLA-A*01, 02, 03, 11, 23, 24, 25, 26, 29, 30, 31, 32, 33, 34, 36, 66, 68, 69, 74, 80; HLA-B*07, 08, 13, 14, 15, 18, 27, 35, 37, 38, 39, 40, 41, 42, 44, 45, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 67, 78, 81*; and *HLA-Cw*01, 02, 03, 04, 05, 06, 07, 08, 12, 14, 15, 16, 17*. Associations with AIDS-1987 progression were not significant ($P > 0.05$, uncorrected) except for in Caucasians *B*35* (RH = 2.34, $P = 2 \times 10^{-6}$), *Cw*04* (2.41, 2×10^{-7}), and *Cw*12* (0.61, 0.03); and in African Americans *A*29* (3.96, 0.01), *B*27* (6.86, 0.01), and *B*41* (3.89, 0.03). All associations except *B*35* and *Cw*04* were not significant ($P > 0.3$) after correction for multiple comparisons. Neither *B*35* nor *Cw*04* displayed significant acceleration to AIDS endpoints among 144 African Americans. Although this failure may involve smaller sample size or the fact that the principal African American cohort, ALIVE, is younger and may not include sufficient AIDS cases ($N = 37$) (*21*), it is possible that the differential effect represents either different *B*35* or *Cw*04* alleles represented by African compared with Caucasian alleles or ethnic group differences in linkage disequilibrium for the B and C alleles. For example, *Cw*04* and *B*53* are associated by strong linkage disequilibrium in African Americans, whereas *Cw*04* and *B*35* are associated in Caucasians. Resolution of this discrepancy in examined African or African American AIDS cohorts may help resolve this question.
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31. Because two Caucasian cohorts, MHCS and SFCC, are nonrandomly depleted of rapid progressors [S. M. Donfield, H. S. Lynn, M. W. Hilgartner, *Science* **280**, 1819 (1998); M. W. Smith, M. Dean, M. Carrington, C. Winkler, S. J. O'Brien, *ibid.*, p. 1819; M. W. Smith *et al.*, *Nature Med.* **3**, 1052 (1997)] and because ALIVE is 94% African American (*15*, *21*), we also computed the attributable fraction (AF) for surviving 10 or more years AIDS free from the 197 Caucasian MACS seroconverters that have no such bias in ethnic or survival representation. This analysis indicated a higher attributable fraction for *HLA* class I homozygosity (AF = 70%, 47%, and 37% for AIDS-1993, AIDS-1987, and death, respectively), for the sum of *B*35/Cw*04* sensitive genotypes (AF = 61%, 62%, and 66%, respectively) and for HLA class I homozygosity plus *B*35/Cw*04* sensitivity combined (AF = 62%, 50%, and 50%, respectively).
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40. For *HLA* class I typing, genomic DNA was isolated from patients' lymphoblastoid B cell lines or from peripheral blood lymphocytes and amplified with a panel of 96 sequence specific primers (SSP-PCR) for *HLA-A*, *-B*, and *-C* [M. Bunce *et al.*, *Tissue Antigens* **46**, 355 (1995)]. Each reaction included positive control primers that amplify a 796–base pair fragment from the third intron of *HLA-DRB1*. *HLA* class I polymerase chain reaction (PCR) products were electrophoresed in 1.5% agarose gels containing ethidium bromide, and predicted size products were visualized under ultraviolet light. To resolve cryptic (to SSP technology) heterozygosity, all homozygotes were sequenced with the ABI Big Dye terminator cycle sequencing ready reaction kit. (Applied Biosystems Division/Perkin-Elmer, Foster City, CA). Primers in the first and third introns of *HLA-A*, *-B*, and *-C* [N. Cereb *et al.*, *ibid.* **45**, 1 (1995)] were used for locus-specific amplification of exons 2 and 3. The amplified product was purified in a Microcon-100 microconcentrator column (Amicon, Beverly, MA), subjected to cycle sequencing in both orientations, according to the manufacturer's protocol, followed by isopropanol precipitation. The samples were then run on an ABI 377 sequencer (Applied Biosystems Division/Perkin-Elmer), and the sequences were analyzed with the Match Tools and MT navigator allele identification software (Applied Biosystems Division/Perkin-Elmer). Sequence analysis of 125 homozygotes for class I loci revealed 17 individuals that were heterozygous for recognized nucleotide polymorphism subtypes within the type indicated by SSP typing. Sixteen of these were heterozygous at adjacent class I loci. Only homozygotes verified by sequence analysis were considered homozygous for all analyses in this report.
41. The class I alleles *B*35* and *Cw*04* were used as a covariable in the association analysis of homozygosity (Fig. 1 and Table 1), and homozygosity was used as a covariable in the analysis of allele association with progression to AIDS (Fig. 1 and Tables 1 and 3).
42. We would like to thank D. Marti, M. McNally, M. Weedon, and L. Main for technical assistance and M. Dean, M. Smith, and C. Winkler for discussions and critical review of the manuscript. The project was funded in part with Federal funds from the National Cancer Institute, NIH, under contract number N01-CO-56000.

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Motor Cortical Encoding of Serial Order in a Context-Recall Task

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The neural encoding of serial order was studied in the motor cortex of monkeys performing a context-recall memory scanning task. Up to five visual stimuli were presented successively on a circle (list presentation phase), and then one of them (test stimulus) changed color; the monkeys had to make a single motor response toward the stimulus that immediately followed the test stimulus in the list. Correct performance in this task depends on memorization of the serial order of the stimuli during their presentation. It was found that changes in neural activity during the list presentation phase reflected the serial order of the stimuli; the effect on cell activity of the serial order of stimuli during their presentation was at least as strong as the effect of motor direction on cell activity during the execution of the motor response. This establishes the serial order of stimuli in a motor task as an important determinant of motor cortical activity during stimulus presentation and in the absence of changes in peripheral motor events, in contrast to the commonly held view of the motor cortex as just an "upper motor neuron."

Ever since Lashley's famous paper in 1951 (*1*), the imagination of psychologists and neuroscientists alike has been captured by the problem of serial order in behavior. Accurate representation of temporal order is crucial for both perceptual and motor functions (for example, comprehending a sentence, playing a musical instrument). Moreover, serial order information must often be transiently kept in working memory before being translated to motor output, as, for exam-

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ple, when looking up a telephone number and dialing the individual digits in the proper order. Neurophysiological studies have commonly used sequential reaching movement tasks in which a series of targets is presented to the subject, who must then execute a series of movements to the targets in the same order, under visual guidance or from memory (*2*). By contrast, in the context-recall task (*3, 4*), the subject makes a single motor response dictated by the serial order of a test stimulus in a memorized list of stimuli. This task provides the requisite conditions for investigating the neural mechanisms of processing the serial order of stimuli uncontaminated by a confounding translation of this order into a series of motor responses, that is, in the absence of signals related to the planning and execution of sequential movements.

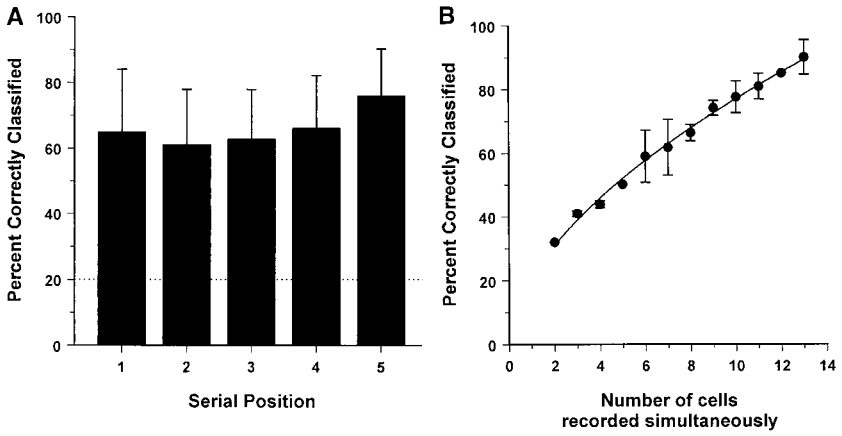

Fig. 2. Small ensembles of motor cortical neurons can classify stimulus items in a sequence. (**A**) Average correct classification rates of current serial position during sequences of five stimuli (data from monkey 2). A discriminant classification analysis was performed for each set of simultaneously recorded cells (*10*). The chance level of serial position classification for sequences of five stimuli is 20% (dotted line). The average level of correct classification obtained with sets of simultaneously recorded neurons was above 60%. Error bars are SDs across sets of cells recorded simultaneously ($N = 36$). (**B**) Average correct classification versus the number of cells recorded simultaneously. The level of correct classification increased with the number of cells in a set. A power function was fitted to the data and is shown as a continuous line in the plot (*12*).

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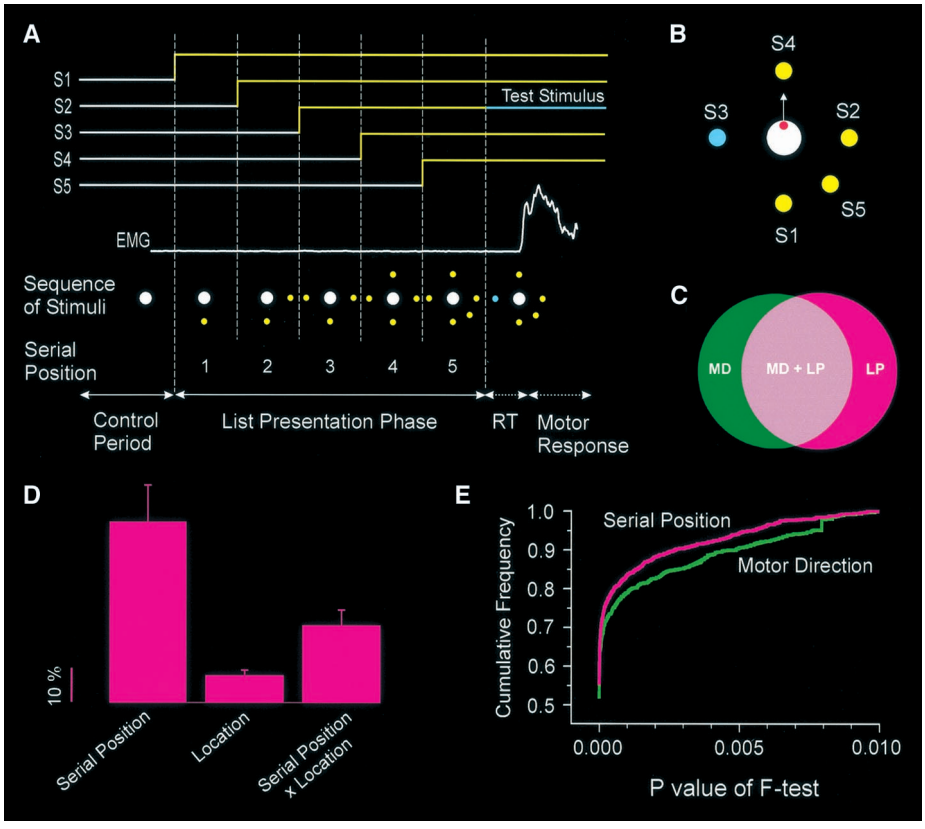

Fig. 1. (**A**) Schematic diagram of the context-recall task, illustrating a trial with a sequence of five stimuli. Time course of stimulus presentation and motor response (represented by EMG activity of anterior deltoid). After a 1000-ms control period where the monkey held the cursor in a center window, the stimuli appeared sequentially on the screen (S1 to S5). Therefore, each stimulus is defined jointly by its location and its serial position within the sequence. The periods between stimulus onsets (*5*) are referred to as epochs. Each epoch corresponds to a serial position. For example, epoch 1 represents the period from the onset of S1 in the downward position to the onset of S2 in the rightward position. At the end of the list presentation, the test stimulus consisted of a change in the color of one of the stimuli from yellow to blue. In this case, the third stimulus (S3) served as the test stimulus. The test stimulus serves as the go signal: The rule of the context-recall task is to move toward the stimulus that immediately followed the test stimulus in the sequence; therefore, in this example the correct response is a movement to the fourth stimulus in the sequence (S4). This report deals with the list presentation phase of the task, namely, from S1 onset until test stimulus onset. The locations of the list stimuli in this example are illustrated below the EMG trace by small dots on a circle. RT, reaction time. (**B**) Schematic diagram of the trial depicted in (A), as it actually appears on the screen during the recall phase. The third stimulus (S3) has changed from yellow to blue, instructing the monkey to move the red cursor from the center window toward the fourth stimulus (S4). (**C**) Venn diagram of the proportions of cells showing (i) a statistically significant effect of Motor Direction (*8*) only during the motor response period (green MD section), (ii) a statistically significant effect of stimulus Serial Position, Location, or their interaction only during the list presentation phase (hot pink LP section), and (iii) statistically significant effects during both the motor response period and the list presentation phase (light pink MD+LP section). The areas of sections are proportional to the actual percentages (see text). (**D**) Bar graph illustrating the proportions of cells in which statistically significant effects were obtained for the main effect of Serial Position, Location, and Serial Position × Location interaction. (**E**) Cumulative frequency distributions of the level of statistical significance obtained for Motor Direction during the motor response time (green) and Serial Position (main effect only) during the list presentation phase (pink).

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In a recent version of this task (*4*), several stimuli are presented successively on a screen, and then one of them changes color (the test stimulus); the subject is required to make a single motor response toward the stimulus that followed immediately the test stimulus in the list. In the present experiments, two monkeys were trained to perform the context-recall task shown in Fig. 1, A and B (*5*). They operated a semi-isometric joystick to control a force feedback cursor on a video screen. A trial began by turning on a white circle in the center of the screen, which the monkey captured with the force feedback cursor. After 1 s (the control period), three to five yellow stimuli were shown successively on a circle and stayed on (the list presentation phase); during both of these periods, the monkey had to keep the force feedback cursor within the white circle at the center of the screen (*6*). Then one of the stimuli (except the last) changed color from yellow to blue (the test stimulus), and this instructed the monkey to exert force to move the cursor from the center of the screen toward the stimulus that immediately followed the test stimulus during the list presentation phase. The reaction time was defined as the time from the onset of the test stimulus until the initiation of the motor response; the motor response period was defined as the time from the onset of the motor response until the threshold force was exceeded (*5*). In this task, each series of list stimuli was defined uniquely by the location of the stimuli on the screen and by their serial order in the series. We recorded the activity of 925 cells in the motor cortex during task performance (*7*).

As expected from the known role of the motor cortex in the initiation and control of movement, the activity of many cells during the motor response period was related to the direction of the response (*8*) ("motor direction" cells, 624/925 = 67.5%; green and light pink sections in Fig. 1C); a smaller proportion of cells (177/925 = 19.1%) showed relations only to motor direction (green section in Fig. 1C). Interestingly, a large proportion (447/624 = 71.6%) of these cells also changed activity during the list presentation phase in relation to stimulus parameters (serial position, location, or both), even though there was no overt motor response during that period ("motor direction + list presentation" cells, 447/925 = 48.3%; light pink section in Fig. 1C). In addition, 190/925 = 20.5% of cells showed such modulation of activity during the list presentation phase in the absence of a motor directional effect ("list presentation" cells, hot pink section in Fig. 1C); this brings the total number of cells engaged during the list presentation phase to 637/925 = 68.9% (hot pink and light pink sections in Fig. 1C). Finally, 111/925 (12%) of the cells did not show any significant effect.

Analyses of covariance (ANCOVA) tested the effects of the following factors that were varied during the list presentation phase: Serial Position (of a stimulus in the list) and Location (of the stimulus on the screen). The main effect of Serial Position was significant in 52.8 ± 10.67% of cells [mean ± SEM, $N = 5$ combinations of monkey and sequence size (*5*)] (Fig. 1D); the main effect of Location was significant in 7.8 ± 1.64% of cells, and the effect of the Serial Position × Location interaction was significant in 22.4 ± 4.61% of cells (*9*). To compare the Serial Position effect during the list presentation phase with a commonly assessed motor effect, such as the effect of Motor Direction on cell activity during the motor response period, we compared the level of statistical significance obtained for these two effects within the same sets of trials (*8*). The statistical significance of the Serial Position effect above was higher than that of the effect of Motor Direction during the motor response period [Fig. 1E; $P < 0.0001$, Kolmogorov-Smirnov test; $N = 1012$ and 978 cases for Motor Direction and Serial Position effects, respectively, out of a total of 1812 cases analyzed from the same trials (*8*)].

These results underscore the major impact

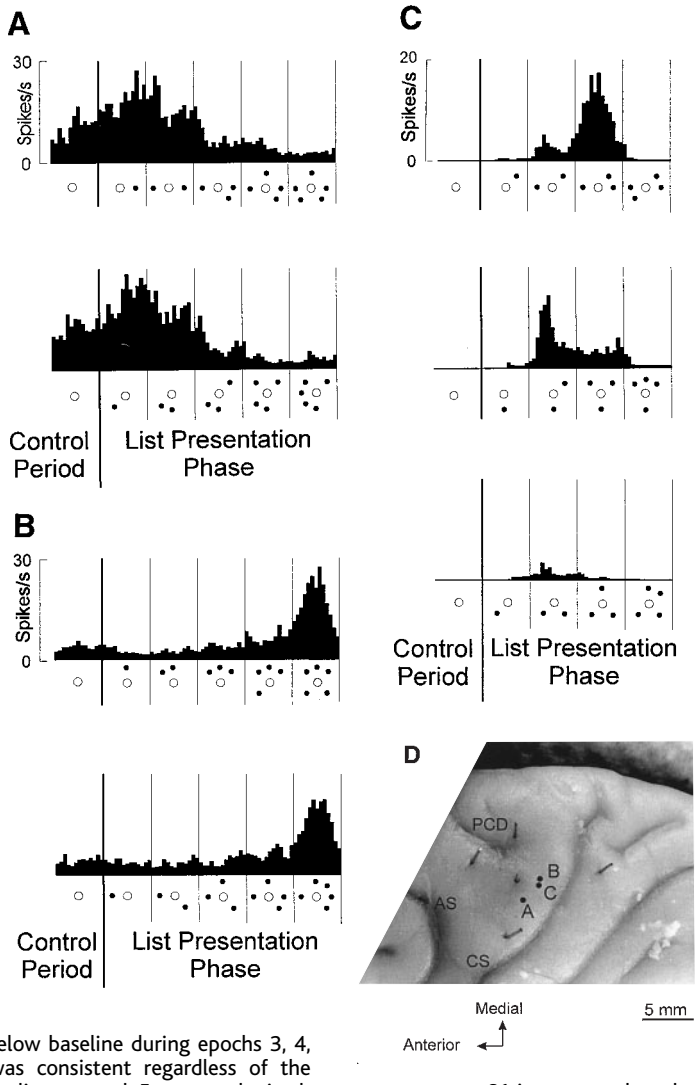

Fig. 3. (**A** to **C**) Peristimulus time histograms of cell activity during the list presentation phase of the context-recall task (bin = 50 ms; data from monkey 2). Histograms represent the average cell discharge during all correct trials of the depicted sequence. The sequence is illustrated below each histogram by small dots on a circle. Vertical lines represent the boundaries between epochs (at intervals of 650 ms). Each histogram ends at test stimulus presentation (the end of the list presentation phase), hence no motor response occurs during the periods illustrated. An example of a neuron that changes activity according to the current serial position within the sequence, irrespective of the location of the stimuli, is shown in (A). Two different sequences of five stimuli are depicted (upper and lower panels). For both sequences, activity increases when the first stimulus is presented (epoch 1), returns to the baseline (control period) rate during epoch 2, and then falls below baseline during epochs 3, 4, and 5. This pattern was consistent regardless of the actual sequence of stimuli presented. For example, in the upper sequence, S1 is presented to the right, whereas in the lower sequence S1 is presented in the lower left position. However, the cell response was similar during the presentation of the two sequences. An example of another neuron whose activity reflects the serial position of the current stimulus, regardless of its location, is shown in (B). As in (A), two different sequences of five stimuli are shown. This neuron maintained its baseline activity while the first four stimuli were presented, but responded with a burst of activity during epoch 5, irrespective of the sequence of stimuli. An example of another motor cortical neuron that was influenced by the interaction of the serial position and the location of the stimuli is shown in (C). Cell activity was modulated mostly during the second and third epochs of list presentation, but this depended on the particular sequence of stimuli presented. For example, clear increases in activity were present during some sequences (upper two panels) but not for others (lower panel). (**D**) Photograph of the peri-Rolandic cortex of the left hemisphere of monkey 2 showing the entry points of the microelectrode penetrations during which the neurons in (A) to (C) were recorded in the primary motor cortex. CS, central sulcus; AS, arcuate sulcus; PCD, precentral dimple.

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of serial order of the stimuli on cell activity during the list presentation phase, and of the interaction of the serial order with stimulus location, which defines the direction of a potential motor response. These two findings, taken together, indicate that the changes in neuronal activity observed during the list presentation phase truly reflect aspects of the sequence itself. We tested this hypothesis by analyzing ensembles of simultaneously recorded neurons to evaluate how well the combined patterns of activity could classify items in the sequence (*10*), namely stimuli defined jointly by their serial position in the sequence and their location on the screen. Indeed, high rates of correct classification were obtained (*11*) (Fig. 2). The mean correct classification rate for each serial position in sequences of five stimuli was greater than 60% (Fig. 2A). The correct classification rate increased as a function of the number of cells in the ensemble (Fig. 2B), which suggests, in turn, that individual cells provide largely independent information about the items in the sequence. Together, these results demonstrate that during different epochs of presentation of the stimuli, the patterns of distributed activity in even small ensembles of motor cortical cells (*12*) are sufficiently distinct and robust to provide a basis for encoding the sequence.

Representative examples of single-cell activity during the list presentation phase are illustrated in Fig. 3. The histograms in Fig. 3, A and B, illustrate consistent changes in the activity of two cells in association with certain serial positions. The visual stimuli displayed during a specific serial position epoch differed for different sequences (Fig. 3, A and B). Other neurons were influenced by both the serial position of the stimuli in the sequence and their location on the screen (Fig. 3C). Changes in neural activity were not related to eye position (Fig. 4 and Fig. 5, left side) nor to the associated retinal location of the most recently presented stimulus (Fig. 5, right side). Concerning the latter point, it is conceivable that the serial position of this stimulus could be associated with a particular retinal location when it appeared on the screen, which then could account for the serial position–related activity. For example, it could be that the monkey fixated its eyes such that when the fifth stimulus appeared, it would always fall in the same retinotopic position. However, this was not the case. As shown in Fig. 5 (top right), the retinal location of the fifth stimulus for the cell illustrated in Fig. 3B was indeed distributed throughout the retinotopic space; that is, it was not confined to any unique location. Similarly, stimuli during the other four epochs were also distributed throughout the retinotopic space (Fig. 5, middle right). The broad distributions of stimuli on the retina shown in Fig. 5 (top right and middle right) allow the comparison of neural activity during the presentation of stimuli with different serial position but with the same, or closely similar, retinal locations. These stimuli are shown as the overlapping points in Fig. 5 (bottom right) and the corresponding neural activity levels shown in the bar graph in Fig. 5 (bottom right): The activity was much higher for the stimuli at serial position 5 than for those at serial positions 1 to 4, even though they were matched for retinal location. The same considerations apply for eye position (Fig. 5, left side). We conclude that the serial position of the stimuli is the important determinant for cell activity, and not their retinal location or eye position (*13*). This is not surprising because these recordings were from the arm area of the motor cortex (Fig. 3D).

Together, these results document a strong effect of serial order on cell activity: In 34.4% of the cells, Serial Position was the only significant factor (*9*), whereas in 52.8% of the cells it was a significant factor alone or together with other factors (Fig. 1D). In addition, the level of significance of this effect was even higher than that of Motor Direction (Fig. 1E). These findings establish serial order as an important factor for motor cortical cell activity. In contrast, stimulus Location, denoting the direction of a potential motor response, had a slight effect alone (*9*) but interacted frequently with Serial Position (Fig. 1D). This suggests that serial order had a strong, pure effect on cell activity, whereas stimulus location was engaged within the context of serial order.

These results can be interpreted with respect to three key aspects of the task performed: (i) Unlike other tasks (*2*), in the present task just a single, one-directional motor response was made in a trial, that is, no sequence of motor responses to each stimulus was performed; this

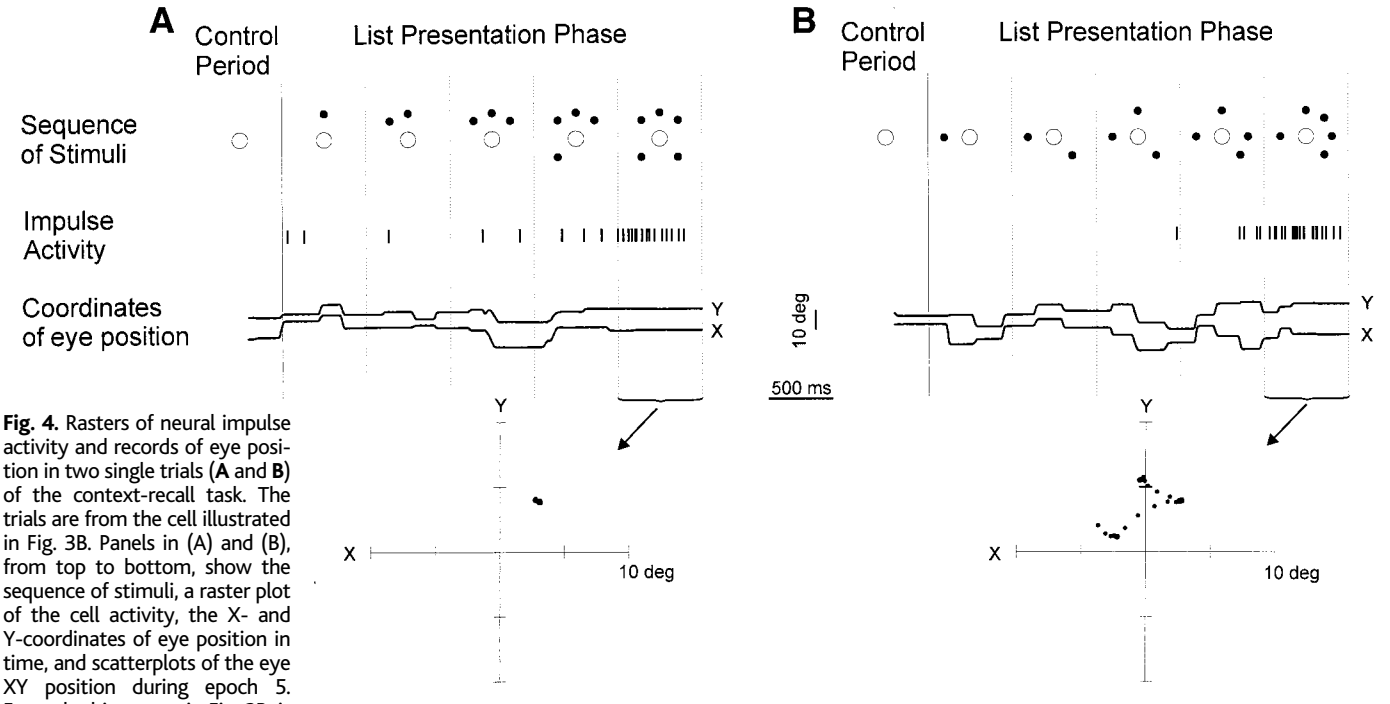

Fig. 4. Rasters of neural impulse activity and records of eye position in two single trials (**A** and **B**) of the context-recall task. The trials are from the cell illustrated in Fig. 3B. Panels in (A) and (B), from top to bottom, show the sequence of stimuli, a raster plot of the cell activity, the X- and Y-coordinates of eye position in time, and scatterplots of the eye XY position during epoch 5. From the histogram in Fig. 3B, it is apparent that this cell increased activity during epoch 5 of the list presentation phase; this can also be seen in the single-trial impulse activity here: The cell is relatively quiet during epochs 1 to 4, and increases its discharge rate during epoch 5. Even though the eye position varied widely during the list presentation phase, both with regard to fixation and saccadic eye movements, the increase in neuronal activity was restricted to epoch 5. This example shows that cell activity was not related to eye movement or eye position, but rather to the serial position epoch.

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could explain why stimulus Location alone was not a frequent effect. (ii) The required single, correct response could be arrived at only by taking into account the serial order of the stimuli, which means that information about serial order was indispensable; this could explain why Serial Position was such a frequent and strong (relative to Motor Direction) effect. (iii) A crucial step in the task was the identification of the location of the stimulus that appeared immediately after the test stimulus during the list presentation, which means that stimulus Location was tied to Serial Position; this could explain why the Serial Position × Location interaction was a frequent effect. It is remarkable that all of these effects were documented in the motor cortex, an area traditionally regarded as composed exclusively of "upper motor neurons." Our results add to a substantial body of evidence documenting the involvement of the motor cortex in other complex functions (*14*).

The neuronal responses described here were commonly phasic; that is, a change in neuronal activity, once evoked, was typically not maintained throughout the remainder of the list presentation phase (Fig. 3) (*15*). This suggests that the information about the sequence is processed in the motor cortex, which most likely participates as a component in a distributed network (*2, 16*) that collectively encodes, stores, and recalls the sequence. A prominent node in that network is the dorsolateral prefrontal cortex, which has been shown to play a key role in the capacity to act on the basis of serial order (*17*). Our results show that the motor cortex also participates in the processing of serial order information within the context of a motor task, that is, the serial order of stimuli on which the selection of a motor response must be based in the task used (*18*). This serial order information, once encoded and held in memory, is used after the presentation of the test stimulus to search the sequence, identify the serial position of the test stimulus in the sequence, and retrieve the stimulus associated with the next serial position, which specifies the required motor response. The unitary principle of this search was identified as an abrupt shift in the discharge of motor cortical neurons from that associated with the direction of a specific stimulus to that appropriate for the next one (*4*). The repeated application of this rapid-shift process from item to item would constitute memory scanning. Because the encoded sequence information can be accurately recovered from small ensembles of motor cortical neurons, this search could be monitored in time from the patterns of activity of these ensembles during the response time.

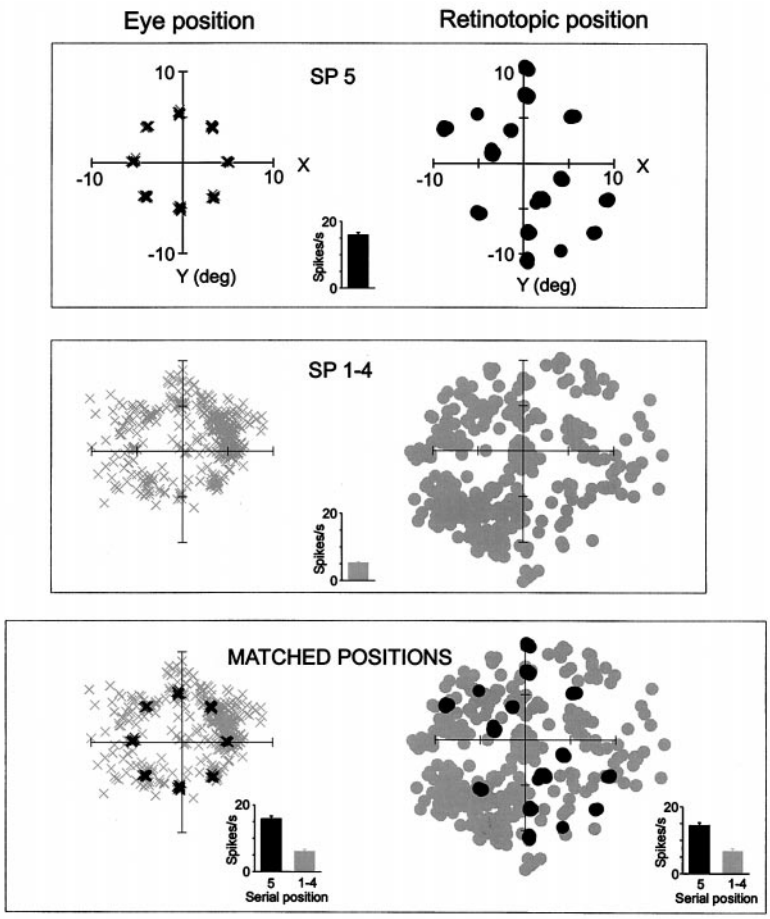

Fig. 5. Eye position, retinotopic location, and associated neural activity for the cell illustrated in Figs. 3B and 4. Upper panels (serial position 5): left, superimposed eye fixations (minimum of 100 ms duration) (N = 160 fixations); right, superimposed retinotopic positions of the fifth stimulus during these eye fixations; center, bar graph of mean (±SEM) neural activity during these fixations. Middle panels (serial positions 1 to 4): left, superimposed eye fixations (N = 420 fixations); right, superimposed retinotopic positions of the most recent stimulus (for each serial position) during these eye fixations; center, bar graph of mean (±SEM) neural activity during these fixations. Lower panels (superimposed records and neural activity for matched positions): left, eye positions for serial positions (SP) 1 to 4 (gray) and 5 (black); right, retinotopic positions for serial positions 1 to 4 (gray) and 5 (black); left bar graph, mean (±SEM) neural activity for the subset of eye positions that were similar (within 0.5°) between serial position 5 (black, N = 160) and serial positions 1 to 4 (gray, N = 116); right bar graph, mean (±SEM) neural activity for the subset of retinotopic positions that were similar (within 1.4°) between serial position 5 (black, N = 109) and serial positions 1 to 4 (gray, N = 73). Neural activity was consistently higher for serial position 5 than for serial positions 1 to 4. This difference was statistically highly significant when the overall rates were compared (bar graphs in upper versus middle panels) and when only the subsets of matched eye positions (bottom left) or retinotopic positions (bottom right) were compared (t test, $P <$ 0.00000001 for each of the three comparisons above).

References and Notes

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5. The monkeys were trained to exert a force pulse on a two-dimensional semi-isometric handle in eight different directions (at 45° intervals). The manipulandum was a vertical rigid metal rod, with a disc attached to the top, which was placed in front of the animal in the midsagittal plane and which the animal grasped with the hand pronated. A net force feedback cursor was displayed on a monitor in front of the monkey. This cursor was deflected constantly downward to simulate a bias force of 54*g* and reflected, at any given moment, the net force (the vector sum of this simulated force and the force exerted by the animal on the manipulandum). At the start of the trial, a white stimulus appeared in the center of the screen and the monkey had to place the force feedback cursor on the center stimulus by exerting a force of 54*g* in the upward direction and then keep it there within a 72*g*-radius circular window. After 1 s, three to five yellow stimuli were presented on a 270*g*-radius circle in different directions. During the presentation of the stimuli, the force feedback cursor had to stay within the central window. The response

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was considered correct if (i) the threshold of 270*g* was exceeded, and (ii) the direction of the force pulse stayed within ±22.5° from the correct direction, from the center to the stimulus. The monkey received a liquid reward after each correct trial. Monkey 1 performed the context-recall task using sequences of three and four stimuli, with 400 ms elapsing between stimuli (epoch duration = 400 ms). Monkey 2 performed with sequences of three, four, and five stimuli, with an epoch duration of 650 ms. Sequences of different lengths (three, four, or five stimuli) were presented in a randomized block design for both monkeys. Given eight possible stimulus locations (one every 45°), there are 8!/(8 – 3)! = 336 unique sequences of three stimuli. For each of these sequences, the test stimulus could be either the first or second stimulus (serial positions 1 and 2); thus, a total of 672 unique trials are possible for sequences of three stimuli. Likewise, there are 8!/(8 – 4)! = 1680 sequences with three different serial positions for a total of 5040 unique trials for sequences of four stimuli, and 8!/(8 – 5)! = 6720 sequences with four different serial positions for a total of 26,880 unique trials for sequences of five stimuli. During training, sequences were selected at random from this set of all possible trials, with the constraint that an equal number of correct responses be performed for each serial position of the test stimulus. During neurophysiological recording, a subset of sequences was selected so that five correct trials (repetitions) could be obtained for each combination of sequence and serial position. Monkey 1 worked with a fixed set of sequences (16 sequences of three stimuli and four sequences of four stimuli) during the neural recordings. In the recording sessions with monkey 2, each block consisted of seven randomly generated sequences and one sequence drawn from a pool of two fixed sequences. (We incorporated fixed sequences in the experimental design for the purpose of pursuing population analyses in the future.) No consistent differences in behavior or neural activity were observed between the fixed and random sequences. For both monkeys, the sequences were chosen such that (i) each of the eight possible stimulus locations was presented an equal number of times, (ii) each stimulus location was presented equally often in each serial position, (iii) the test stimulus was presented in each of the eight locations equally often, and (iv) the direction of correct response was equally distributed among the eight directions. Monkey 1 performed correctly in 85% of the trials with sequences of three stimuli and 50% of the trials with sequences of four stimuli. Monkey 2 performed correctly in 79%, 75%, and 47% of the trials with sequences of three, four, and five stimuli, respectively. An incorrect response could be due to moving in the wrong direction or to initiating a motor response outside prespecified temporal limits.

6. The monkey was required to maintain the force feedback cursor within a small center window, and electromyographic (EMG) recordings revealed no significant change of muscle activity during this time (Fig. 1A). EMG activity was recorded with intramuscular, multistranded, Teflon-coated wire electrodes [A. B. Schwartz, R. E. Kettner, A. P. Georgopoulos, *J. Neurosci.* **8**, 2913 (1988)] in separate sessions from the neural recordings. EMG activity was recorded in the following muscles: latissimus dorsi, rhomboids, paraspinal, infraspinatus, supraspinatus, trapezius (lower, middle, and upper), deltoid (anterior, middle, and posterior), pectoralis major, triceps (lateral and long heads), biceps, forearm flexor (unspecified), and forearm extensor (unspecified). Examination of EMG activity showed no change from the baseline level during the list presentation phase of the task; instead, when an increase of signal was observed, it began shortly before or during the motor response.
7. The electrical activity of single motor cortical neurons was recorded extracellularly with seven independently driven microelectrodes [V. B. Mountcastle, H. J. Reitboeck, G. F. Poggio, M. A. Steinmetz, *J. Neurosci. Methods* **36**, 77 (1991); D. Lee, N. P. Port, W. Kruse, A. P. Georgopoulos, in *Neuronal Ensembles: Strategies for Recording and Decoding,* H. Eichenbaum and J. Davis, Eds. (Wiley, New York, 1998), pp. 117–136]. The placement of the recording chamber was done aseptically under general pentobarbital anesthesia. In both monkeys, the neurons were recorded from the bank and crown of the precentral gyrus (contralateral to the performing arm), medial to the genu of the arcuate sulcus and posterolateral to the precentral dimple. All isolated neurons were recorded regardless of their activity during the task. The eye position was monitored with an infrared oculometer (Dr. Bouis, Karlsruhe, Germany) or a scleral search coil (CNC Engineering, Seattle, WA) [A. F. Fuchs and D. A, Robinson, *J. Appl. Physiol.* **21**, 1068 (1966); S. J. Judge, B. J. Richmond, F. C. Chu, *Vision Res.* **20**, 535 (1980)]. Eye position was recorded simultaneously with neural recordings in monkey 2 and in separate recording sessions in monkey 1. A personal computer controlled the task display and data collection. Care and treatment of the animals during all stages of the experiments conformed to NIH's Principles of Laboratory Animal Care (revised 1995). All experimental protocols were approved by the appropriate institutional review boards.
8. ANCOVAs were performed for each cell and each sequence size (three to five stimuli) as follows. The set of trials in a cell–sequence size combination was called a "case"; a total of 1812 cases were analyzed. For each period of interest (see below) in every correct trial, the firing rate was computed using fractional interspike intervals [M. Taira, J. Boline, N. Smyrnis, A. P. Georgopoulos, J. Ashe, *Exp. Brain Res.* **109**, 367 (1996)]. The square root transformation was applied to these firing rates to stabilize the variance [J. W. Tukey, *Exploratory Data Analysis* (Addison-Wesley, Reading, MA, 1977), p. 543; D. R. Cox and P. A. W. Lewis, *The Statistical Analysis of Series of Events* (Chapman & Hall, London, 1966); G. W. Snedecor and W. G. Cochran, *Statistical Methods* (Iowa State Univ. Press, Ames, IA, ed. 8, 1989)]. Cells were included in the analyses if they had a mean firing rate of at least 0.1 impulses per second during the task. Data from two periods were analyzed, namely from the list presentation phase and the motor response period, both from the same trials. The list presentation phase comprised three to five epochs corresponding to the stimuli presented (*5*); the factors in this ANCOVA were Serial Position (number of levels = number of stimuli in the sequence), Location of the most recently presented stimulus (eight levels), and their interaction. In addition, the factor Repetition (five levels) was included to control for the effect of repeated measurement of the same cell, and the control period activity was used as a covariate to adjust for the baseline activity of the cell. The factors in the motor response ANCOVA included Motor Direction (eight levels) and Repetition (five levels); the control period activity was used as a covariate. Effects at a conservative probability level ($P < 0.01$) were considered statistically significant. Cells were classified as list presentation–related when any of the factors of Serial Position, Location, or their interaction was significant, and as motor-related when the factor Motor Direction was statistically significant in any of the sequence sizes analyzed. The program 4V of the BMDP/Dynamic statistical package (BMDP Statistical Software Inc., Los Angeles, 1992) was used to perform the ANCOVA analysis.
9. The percentages shown in Fig. 1D denote the relative frequency of an effect irrespective of the effects of other factors. On the other hand, exclusive effects can be calculated such that, for example, a "Serial Position" effect will mean that only this factor (and no other factor or interaction term) was statistically significant. This is the most stringent criterion for evaluating the importance of a specific factor on cell activity. These percentages are as follows: the main effect of Serial Position was the only statistically significant effect in 34.4 ± 6.71% of cells [mean ± SEM, $N = 5$ combinations of monkey and sequence size (*5*)]; the main effect of stimulus Location was the only significant effect in 0.6 ± 0.18% of cells; and the Serial Position × Location interaction effect was the only significant effect in 4.95 ± 0.54% of cells. Thus, Serial Position was the most frequent statistically significant effect in this analysis too. The cumulative frequency curve of the statistical significance of Serial Position in this case was also significantly higher than the Motor Direction curve ($P < 0.0001$, Kolmogorov-Smirnov test).
10. The number of simultaneously recorded neurons ranged from 2 to 11 for monkey 1 and from 2 to 16 for monkey 2. A quadratic discriminant analysis was performed [P. A. Lachenbruch, *Discriminant Analysis* (Hafner, New York, 1975)] because cells in a simultaneously recorded set commonly did not have a common covariance structure, hence a linear discriminant analysis was not appropriate. Percentages of correct classification were computed for each sequence within a list length and averaged across sequences.
11. The average percent of correct classification of serial position for a sequence of three stimuli was 69% (range 45 to 99%), for a sequence of four stimuli it was 64% (range 35 to 99.8%), and for a sequence of five stimuli it was 66% (range 32 to 94%). The chance levels of correct classification were 33.3%, 25%, and 20% for sequences of three, four, and five stimuli, respectively.
12. A power function was fitted to the data using least squares. The fitted function was: Percent correct classification = $20.9N^{0.567}$ ($r^2 = 0.995$), where N is the number of cells in the ensemble. Extrapolating the power function indicates that an ensemble of as few as 16 motor cortical cells could perfectly classify all the items in a sequence of five stimuli.
13. Careful qualitative inspection of the data, using plots such as in Figs. 3 to 5, indicated that changes in cell activity could not, in general, be accounted for by eye movement, eye position, or stimulation of a specific part of the visual field. In addition, because the successively presented stimuli remained on the screen throughout the trial (Fig. 1A), there was a rich pattern of visual stimulation that differed from epoch to epoch and from sequence to sequence. Exhaustive quantitative analyses of these factors is beyond the scope of this report.
14. For reviews, see A. P. Georgopoulos, *Annu. Rev. Neurosci.* **14**, 361 (1991); ———, M. Taira, A. V. Lukashin, *Science* **260**, 47 (1993). The changes in activity during the list presentation phase were not related to concomitant motor events because no motor response occurred during this period (*6*). Instead, these changes in activity occurred while the monkeys were viewing a sequence of stimuli, whose order they had to transiently remember. It is possible that this activity may reflect intended, but not executed, limb movements. However, if this were the case, we would expect cell activity to reflect the location of the currently presented stimulus, which would determine the direction of a hypothetical motor response. Instead, we found that cell activity was mostly related to serial position in the sequence rather than location.
15. A similarly phasic encoding response has been described for inferotemporal neurons during an object recognition memory task [E. K. Miller, L. Lin, R. Desimone, *J. Neurosci.* **13**, 1460 (1993)].
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18. This study was not designed to address issues of neural coding of serial order per se, as an abstract entity (that is, the first-ness, second-ness, third-ness, . . . , *k*-ness of a series of stimuli). Rather, we focused on a specific motor task whose correct performance depended on identifying the serial order of stimuli. The motor cortex (this report) as well as premotor and prefrontal areas [A. F. Carpenter, G. Pellizzer, A. P. Georgopoulos, *Soc. Neurosci. Abstr.* **24**, 1426 (1998)] are involved in this task.

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