concentrations of inhibitors. Progeny virus was harvested from cell-free supernatants when the maximum cytopathic effect was observed, and was subsequently used to infect fresh MT-4 cells for further rounds (passages) of selection.
14. Wild-type virus was serially passaged in MT-4 cells (*25*) in the presence of 10 μM 3TC and increasing concentrations of AZT (*13*). During the first three passages (0.05 to 0.5 μM AZT), DNA sequence analysis (*19, 26*) of infected-cell DNA revealed mixtures of ATG (Met), GTG (Val), and ATA (Ile) at RT codon 184. By passage 4 (2 μM AZT), codon 184 was completely GTG (Val). During this time, RT codons 41, 67, 70, 215, and 219 remained wild type.
15. The AZT-resistant virus HIVRTMN (Leu^{41} and Tyr^{215} in RT) (*5*) was used to infect MT-4 cells (*25*) and passaged three times in the presence of 3TC (15, 50, and 100 μM). Rapid virus growth occurred during the second and third passages, and a high titer of virus was recovered after 3 days of culture in each instance. DNA sequence analysis (*19, 26*) revealed mixtures of ATG (Met), GTG (Val), and ATA (Ile) in infected-cell DNA samples from passages 2 and 3. The 3TC IC_{50} value increased from 0.2 μM (parental virus) to 200 μM (passage-3 virus), whereas the AZT IC_{50} value decreased from 0.7 μM (parental virus) to 0.08 μM (passage-3 virus).
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17. HIV-1–specific RNA copy number was determined as described (*18*) with a prototype quantitative PCR assay developed by Roche Molecular Systems. Briefly, RNA was extracted from 200 μl of serum and the equivalent of 50 μl was subjected to RT-PCR amplification with *rTth* DNA polymerase in the presence of an internal RNA standard. The relative amounts of the internal standard and HIV-1–specific PCR products were quantitated after 28 cycles of PCR with a microtiter-format enzyme-linked immunosorbent–like assay. The lower limit of detection of this assay is ~10 RNA copies (200 copies per milliliter), with a linear dynamic range of at least 4 log units and a coefficient of variation of <25% (*18*).
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19. Nested RT-PCR was performed with 0.25 μg of oligonucleotide primers A-35 (5′-TTGGTTGCACTTTAAATTTTCCCATTAGTCCTATT-3′) and NE1-35 (5′-CCCACTAACTTCTGTATGTCATTGACAGTCCAGCT-3′) in the first round of PCR, and primers Comb2 (5′-CTGTACCAGTAAAATTAAAGCCAGG-3′) and biotinylated primer Comb3 (5′-ATAGGCTGTACTGTCCATTTATCAGG-3′) in the second round, in a Perkin Elmer 9600 thermal cycler. RNA was extracted from serum as described (*17*) and 25-μl portions were reverse transcribed in a total volume of 50 μl containing 200 U of Superscript II RT (Life-Technologies), 13% (w/v) glycerol, 10 mM dithiothreitol, 75 mM KCl, 50 mM tris (pH 8.3), 3 mM $MgCl_2$, 200 μM deoxynucleoside triphosphates (dNTPs), and primer NE1-35. This RT reaction was separated from a lower PCR buffer containing 50 mM tris (pH 8.3), 25 mM KCl, 1.5 mM $MgCl_2$, 10 μg of bovine serum albumin, 200 μM dNTPs, primer A-35, and 5 U of *Taq* DNA polymerase by a layer of wax (Ampliwax). After incubation for 45 min at 45°C, the temperature was increased to 95°C for 20 s. Five cycles of denaturation at 95°C (20 s), annealing at 55°C (10 s), and extension at 72°C (60 s) were performed, followed by 30 additional PCR cycles of denaturation at 90°C (10 s), annealing at 55°C (10 s), and extension at 72°C (60 s for the first cycle, increasing by 5 s with each additional cycle). Portions (1 to 4 μl) of the PCR products were carried over to a second-round PCR, performed in a buffer containing 50 mM tris (pH 8.3), 25 mM KCl, 2.5 mM $MgCl_2$, 10% (w/v) glycerol, 10 μg of bovine serum albumin, 200 μM dNTPs, and 2.5 U of *Taq* DNA polymerase for 35 cycles of denaturation at 95°C (10 s), annealing at 60°C (10 s), and extension at 72°C (30 s). DNA sequencing was performed on an ABI 373 automated sequencer with standard T7 DNA polymerase sequencing procedures (*26*).
20. Recombinant viruses were constructed with a previously described system (*21*); however, the HIV proviral RT-deleted clone pHIVΔRTBstEII was replaced by a partially RT-deleted clone (pHIVΔBst1107I, with a 578–base pair deletion spanning codons 40 to 231 in RT). In addition, PCR products used in the recombination experiments were from the oligonucleotide primer pair Comb2 and Comb3 (*19*).
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Excision of Deoxyribose Phosphate Residues by DNA Polymerase β During DNA Repair

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Eukaryotic DNA polymerase β (pol β) can catalyze DNA synthesis during base excision DNA repair. It is shown here that pol β also catalyzes release of 5′-terminal deoxyribose phosphate (dRP) residues from incised apurinic-apyrimidinic sites, which are common intermediate products in base excision repair. The catalytic domain for this activity resides within an amino-terminal 8-kilodalton fragment of pol β, which comprises a distinct structural domain of the enzyme. Magnesium is required for the release of dRP from double-stranded DNA but not from a single-stranded oligonucleotide. Analysis of the released products indicates that the excision reaction occurs by β-elimination rather than hydrolysis.

Pol β, one of the four known nuclear DNA polymerases, consists of a single polypeptide (39 to 45 kD in vertebrates) that is highly conserved among higher eukaryotes. Pol β has been implicated in DNA repair on the basis of three observations: It is expressed at relatively constant concentrations throughout the cell cycle (*1*), its concentrations increase after treatment of cells with certain DNA-damaging agents (*2*), and it can fill small gaps and nicks in DNA (*3*). Studies with in vitro repair systems indicate that pol β catalyzes DNA synthesis during base excision repair (*4*–*6*).

Base excision repair is a major pathway for correction of modified bases. According to one proposed model (*7*), modified bases are repaired by five sequential reactions: (i) removal of a modified base by a specific DNA *N*-glycosylase to leave an apurinic-apyrimidinic (AP) site, a common intermediate product; (ii) incision of the AP site at its 5′ side by a class II AP endonuclease; (iii) excision of the 5′-terminal dRP to leave a single-nucleotide gap; (iv) DNA synthesis by DNA polymerase to fill the gap; and (v) sealing by DNA ligase. In the case of higher eukaryotes, characterization of in vitro repair reactions including pol β supports this model (*5*), although there is an alternative pathway for AP site repair that uses proliferating cell nuclear antigen and pol δ for the excision and DNA synthesis reactions (*6*). In the pol β–dependent base excision repair, pol β had been thought to act only to fill a single-nucleotide gap. Although some distinct activities such as deoxyribonuclease V and adenosine triphosphatase are known to interact with pol β (*8*), no intrinsic activities other than DNA polymerization have been attributed to pol β.

In a reconstituted system with proteins derived from *Xenopus laevis* ovaries, AP sites are efficiently repaired by AP endonuclease, pol β, and a partially purified fraction, BE-1B (*6*, *9*). For further analysis of pol β–dependent AP site repair, we used rat pol β that had been purified after overexpression in bacteria and bacteriophage T4–encoded DNA ligase as substitutes for *X. laevis* pol β and the BE-1B fraction, respectively. AP sites were successfully repaired in reactions containing these two proteins and *X. laevis* AP endonuclease (Fig. 1, A and B), which indicates that at least one of the three proteins is responsible for excision of the 5′-terminal dRP.

In prokaryotes, two proteins—Fpg and RecJ—have been reported to possess dRP excision activity. The Fpg protein has a DNA *N*-glycosylase activity specific for

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formamidopyrimidines and 8-oxoguanines and an AP lyase activity, as well as an activity for excision of the 5′-terminal dRP (*10*). This excision activity of Fpg is suppressed by Mg^{2+} (*11*). The RecJ protein was originally isolated as a 5′→ 3′ exonuclease specific for single-stranded (ss) DNA (*12*) and was subsequently found to be identical to DNA deoxyribophosphodiesterase (dRPase), which releases the 5′-terminal dRP in a Mg^{2+}-dependent manner (*11*). It has also been reported that RecJ is involved in base excision repair in vitro (*13*). In eukaryotes, an activity similar to bacterial dRPase was identified in human cell lines and calf thymus (*14*) but was not purified.

We tested X. *laevis* AP endonuclease, rat pol β, and T4 DNA ligase for the excision activity. Analysis of the intermediate products indicated that the recombinant rat pol β, but not the other proteins, released the 5′-terminal dRP from the incised AP site (Fig. 1C). The excision activity associated with pol β required prior incision at AP sites by AP endonuclease. The rat pol β did not cleave intact AP sites (*9*). We also detected the same excision activity in pol β purified from X. *laevis* ovaries (*9*). These results suggest that the association of the excision activity with pol β is conserved among higher eukaryotes. In subsequent experiments, we purified recombinant rat pol β and its derivatives from a *recJ*$^-$ bacterial strain to avoid contamination with one of the major bacterial dRP excision enzymes (*15*).

Structural analyses of rat pol β (*16*) indicate that this enzyme consists of four domains: an 8-kD domain (amino acids 1 to 87), a "finger" domain (amino acids 88 to 151), a "palm" domain (amino acids 152 to 262), and a "thumb" domain (amino acids 263 to 355). The catalytic center for the nucleotidyl transfer reaction is in the palm domain, whereas the DNA polymerase catalytic activity is localized to a 31-kD polypeptide composed of fingers, palm, and thumb domains. To identify the catalytic domain for the excision activity of the rat pol β, we prepared three polypeptides that were devoid of various domains of the enzyme (Fig. 2A). Whereas the 31-kD fragment lost the excision activity, both the 16-kD and 8-kD fragments retained activity to a degree comparable to that of the intact enzyme. The 8-kD domain of the rat pol β is reported to bind to ssDNA but only weakly to double-stranded (ds) DNA (*17*). The excision activity of the intact enzyme and of the 8-kD fragment was dependent on Mg^{2+} and was suppressed by EDTA (Fig. 2C). This suppression by EDTA was alleviated by addition of an excess amount of Mg^{2+}, Ca^{2+}, or Mn^{2+} but not Zn^{2+} (*9*).

These data strongly suggest that pol β itself carries out the release of the 5′-terminal dRP. To test the possibility that another protein or proteins, possibly in association with pol β, were responsible for the excision activity, we extracted the intact rat pol β, 16-kD, and 8-kD polypeptides from an SDS-containing polyacrylamide gel after electrophoresis and assayed the proteins after renaturation for excision activity (Fig. 3). Activity was not detected in the eluted sample of the 39-kD intact enzyme. The DNA polymerase activity was also lost during the procedures (*9*), which suggests that the 39-kD polypeptide may not be eluted efficiently from the 15% polyacrylamide gel or may not renature properly. In contrast, the excision activity was recovered from the gel slices

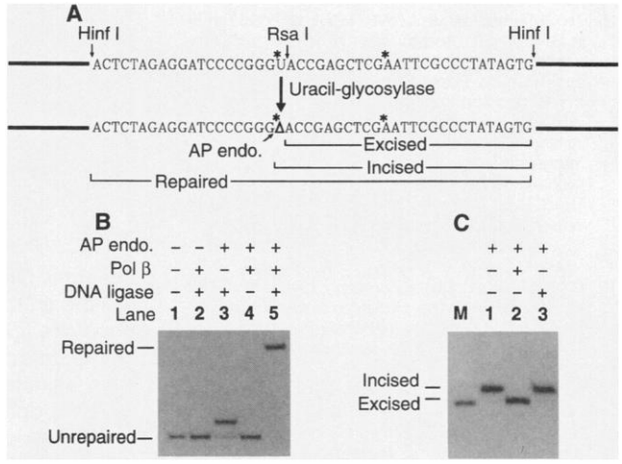

Fig. 1. AP site repair and excision of 5′-terminal dRP by the pol β–dependent pathway. (**A**) Structure of substrate DNA for repair and excision assay. Only the strand that contains an AP site is depicted, although the closed-circular dsDNA was used for most assays. The AP site and AP endonuclease are designated by a triangle and AP endo., respectively. The DNA was prelabeled with ^{32}P at either of the positions indicated by an asterisk. The DNA labeled on the 3′ side of the AP site was prepared as in (*6*) and was used for the repair and excision assays, whereas the DNA labeled at the 5′ side of the AP site was used for experiments to analyze the released products (*24*). (**B**) AP site repair with AP endonuclease purified from *X. laevis* ovaries, recombinant rat pol β expressed in *Escherichia coli*, and bacteriophage T4 DNA ligase (Promega, Madison, Wisconsin). Reaction conditions were as in (*6*). After digestion with Hinf I and AP endonuclease, the DNA samples were analyzed by electrophoresis through an 8 M urea–containing 20% polyacrylamide gel (67 V/cm for 2 hours). The slower mobility of the unrepaired DNA in lane 3 is due to the addition of adenosine monophosphate to the 5′ terminus of the incised DNA by DNA ligase (ligation intermediates). (**C**) Excision of the 5′-terminal dRP by pol β. The AP site containing DNA was incised with AP endonuclease, incubated with the protein or proteins indicated, and then exposed to the reducing agent $NaBH_4$ (*6, 25*). After digestion with Hinf I, the DNA was analyzed by electrophoresis through an 8 M urea–containing 20% polyacrylamide gel (67 V/cm for 7 hours). The substrate DNA, which was not treated with uracil-DNA-glycosylase, was digested with Rsa I and Hinf I and loaded in lane M as a molecular weight standard.

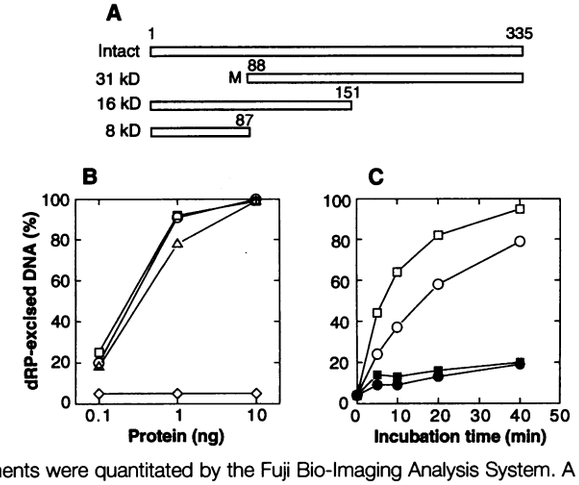

Fig. 2. Identification of the domain in pol β that catalyzes excision of 5′-terminal dRP. (**A**) Schematic summary of partial polypeptides of the rat pol β. All the polypeptides were expressed from truncated cDNAs introduced into bacteria (*15*). Numbers indicate amino acid position. (**B**) Excision of 5′-terminal dRP by polypeptide fragments of pol β. DNA containing the preincised AP site was incubated with the indicated amounts of intact pol β (circles), of the 31-kD fragment (diamonds), of the 16-kD fragment (triangles), or of the 8-kD fragment (squares) for 30 min in the presence of 5 mM $MgCl_2$. After analysis by gel electrophoresis as in Fig. 1C, the incised and dRP-excised DNA fragments were quantitated by the Fuji Bio-Imaging Analysis System. A corresponding protein fraction from control bacteria that carried the expression vector only did not show excision activity more than 0.2% of the intact rat pol β fraction. (**C**) Requirement of Mg^{2+} for excision. DNA containing the AP site was preincised with AP endonuclease in the presence of 1 mM $MgCl_2$ and then incubated for the indicated time with 1 ng of the intact pol β (circles) or 2 ng of the 8-kD polypeptide (squares) in the presence of additional 4 mM $MgCl_2$ (open symbols) or 5 mM EDTA (solid symbols). The incised and dRP-excised DNA fragments were quantitated as in (B).

containing either the 16-kD or 8-kD polypeptide, which supports the notion that it is pol β, rather than a contaminating protein, that catalyzes the release of dRP.

Pol β–catalyzed release of 5′-terminal dRP could proceed by hydrolysis, as is the case with RecJ and the human excision activity (*11*, *14*, *18*), or by β-elimination pathway, as is the case with the Fpg protein (*10*). Hydrolysis would generate a normal form of dRP, whereas β-elimination would generate an unsaturated derivative of the dRP. These two products can be separated by anion-exchange chromatography in the presence of sodium thioglycolate, which modifies only the unsaturated dRP. To determine the excision mechanism used by pol β, we analyzed the reaction products by anion exchange chromatography as in (*10*). In reactions containing dsDNA prelabeled with ^{32}P at the 5′ side of the AP site, the rat pol β released unsaturated dRP in a Mg^{2+}-dependent manner (Fig. 4, A and B), which is indicative of a β-elimination mechanism. When an ss oligonucleotide that had a [^{32}P]AP site at its 5′ terminus was used as a substrate, the liberation of dRP was not suppressed by EDTA (Fig. 4C). This result indicates that the β-elimination catalyzed by pol β does not require divalent cations. The requirement of Mg^{2+} for the release of dRP from dsDNA suggests that the 8-kD domain of pol β may bind efficiently to dsDNA only in the presence of Mg^{2+}. The 8-kD domain has been shown to bind to the phosphorylated 5′ position in gapped DNA substrate when the gap was as large as five nucleotides, whereas it did not bind to a single-nucleotide gapped region (*19*). It is possible that Mg^{2+}, which was not included in these experiments, may modify the binding character of the 8-kD domain to small gaps or nicked regions.

Several studies have examined the substrate specificity of the 5′-dRP excision activity. Both bacterial and human dRPase activities liberate dRP from a natural AP site but not from a reduced form of the AP site (*5*). The Fpg protein cannot release dRP from the reduced AP site, which is not susceptible to β-elimination. We tested three substrates—a natural AP site, the reduced form of the AP site, and a synthetic analog, 3-hydroxy-2-hydroxymethyltetrahydrofuran (*20*)—in the excision assay. The rat pol β released dRP only from the natural AP sites and not from the other β-elimination–resistant AP sites (*9*). This is consistent with the excision mechanism of pol β and with the substrate specifici ty of the pol β–dependent AP site repair, which removes natural AP sites but not tetrahydrofuran sites (*6*).

β-Elimination at AP sites is also catalyzed by basic proteins such as histones and polyamines (*21*). We found that more than 1 μg of histone H1, 1 mM spermine, or 10 mM spermidine is required to provide the same degree of dRP excision activity as 1 ng of pol β (*9*), which suggests that pol β catalyzes this reaction more efficiently than do histones and polyamines.

Our finding that pol β can catalyze excision of 5′-terminal dRP from incised AP sites is consistent with the postulated role of pol β in base excision repair. Although pol β may not be the only enzyme in eukaryotic cells to release dRP, the physical association of this activity with DNA polymerase activity may enhance the efficiency of repair. The coordinated action of excision and DNA synthesis by a single enzyme may also minimize the possibility that the reaction intermediates become substrates for aberrant reactions catalyzed by other nuclear proteins.

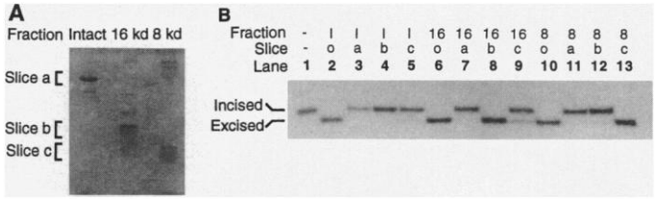

Fig. 3. Recovery of the dRP activity after electrophoresis through an SDS-polyacrylamide gel. (**A**) Copper-stained polyacrylamide gel. The intact rat pol β (39 kD) and the 16-kD and 8-kD fragments were subjected to electrophoresis in an SDS-containing 15% polyacrylamide gel and negatively stained with copper as in (*26*). Gel slices a, b, and c, which contained the 39-kD, 16-kD, and 8-kD polypeptides, respectively, were excised from all three lanes. The proteins in the gel slices were extracted and renatured as in (*27*). (**B**) Excision of dRP by the eluted proteins. The sample eluted from each gel slice [denoted by the fraction name and the gel slice name in (A)] was assayed for the excision activity as in Fig. 1C. Lanes 2, 6, and 10 show samples that had not been subjected to gel electrophoresis, as positive controls.

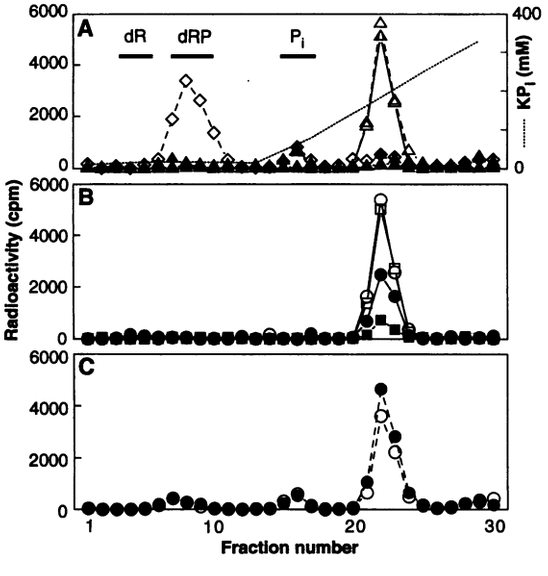

Fig. 4. Analysis of the excision reaction products by anion exchange column chromatography. (**A**) Products released by treatment of substrate DNA with alkali or snake venom phosphodiesterase I (Pharmacia). The DNA labeled with ^{32}P at the 5′ side of the AP site on the dsDNA (solid lines) or the ss oligonucleotide (broken lines) (*28*) was incubated without (solid triangles) or with 0.2 N NaOH (open triangles) or with 1 U of snake venom phosphodiesterase I plus 5 mM $MgCl_2$ (open diamonds; for the ss substrate only) as controls. After incubation at 25°C for 30 min, the reaction mixtures were diluted to 1 ml with 20 mM KH_2PO_4 and loaded on a 1-ml HiTrap Q column (Pharmacia). After being washed with 4 ml of 20 mM KH_2PO_4, the released ^{32}P-labeled products were eluted from the column with a 10-ml linear gradient of 20 to 400 mM KH_2PO_4. The radioactivity of 0.5-ml fractions was measured by Cerenkov counting. Deoxyribose and saturated dRP (Sigma) were used as markers and monitored by the absorbance at 600 nm of the diphenylamine reaction (*29*). The elution position of inorganic phosphate (P_i) is also indicated. Both the dsDNA and the ss oligonucleotide remained bound to the column under these elution conditions. (**B**) Products released by pol β from the incised AP site on the circular dsDNA. (**C**) Products released by pol β from the ss oligonucleotide. The same DNAs were incubated in the presence of 50 mM sodium thioglycolate with 100 ng of rat pol β plus 5 mM $MgCl_2$ (open circles), 100 ng of rat pol β plus 5 mM EDTA (solid circles), 10 ng of rat pol β plus 5 mM $MgCl_2$ (open squares; for the ds substrate only), or 10 ng of rat pol β plus 5 mM EDTA (solid squares; for the ds substrate only).

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Motor Cortical Activity in a Context-Recall Task

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A monkey was trained to respond on the basis of the serial position of a test stimulus in a sequence. First, three stimuli were presented successively on a circle. Then one of them (except the last) changed color (test stimulus) and served as the go signal: The monkey was required to produce a motor response in the direction of the stimulus that followed the test stimulus. When the test stimulus was the second in the sequence, there was a change in motor cortical activity from a pattern reflecting the direction of this stimulus to the pattern associated with the direction of the motor response. This change was abrupt, occurred 100 to 150 milliseconds after the go signal, and was evident both in the activity of single cells and in the time-varying neuronal population vector. These findings identify the neural correlates of a switching process that is different from a mental rotation described previously.

The elucidation of the neural mechanisms underlying cognitive processing is a basic goal of behavioral neuroscience (*1*). The recording of the activity of single cells in the brains of behaving animals has provided a powerful tool by which these mechanisms can be studied. In a previous study (*2*, *3*), we identified the neural correlates of a mental rotation process as the orderly rotation of the neuronal population vector (*4*) from a stimulus to a movement direction, through successive directions within a specified angle. This rotation exemplified the spatial rule operating in the mental rotation task, which required the production of a movement at an angle from a stimulus direction. In the present study, we sought instead to determine the neural correlates of a cognitive process, the rule of which was based not on a spatial constraint but on the serial position of stimuli in a sequence: Given an arbitrary sequence of stimuli on a circle, one of which was identified as the test stimulus, the motor response had to be

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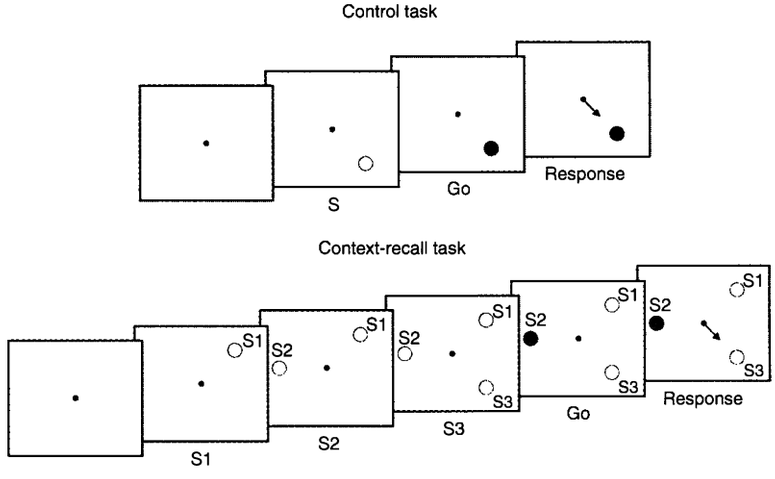

Fig. 1. Schematic diagram of two trials of the tasks used. In the control task (**top**), the yellow stimulus S changed to blue after 400 ms, which gave the go signal. The correct motor response was in the direction of this stimulus. In the context-recall task (**bottom**), three yellow stimuli (S1, S2, and S3) were presented sequentially at 400-ms intervals and stayed on the screen; these stimuli defined the sequence for this trial. In this trial, S2 changed to blue, which now dictated a motor response toward S3.