Complementary Neural Mechanisms for Tracking Items in Human Working Memory

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Recognition of a specific visual target among equally familiar distracters requires neural mechanisms for tracking items in working memory. Event-related functional magnetic resonance imaging revealed evidence for two such mechanisms: (i) Enhanced neural responses, primarily in the frontal cortex, were associated with the target and were maintained across repetitions of the target. (ii) Reduced responses, primarily in the extrastriate visual cortex, were associated with stimulus repetition, regardless of whether the stimulus was a target or a distracter. These complementary neural mechanisms track the status of familiar items in working memory, allowing for the efficient recognition of a currently relevant object and rejection of irrelevant distracters.

Many everyday tasks require recognition of a specific object among equally familiar alternatives. Examples include looking for a well-known book among many on a bookshelf or searching for a family member's face at a reunion. Neuroimaging studies in humans have shown that neural responses are altered as stimuli become more familiar or new associations are learned (*1*). However, alterations of neural responses that reflect familiarity or learned associations are of no use in distinguishing a specific object that is currently the focus of attention from equally familiar distracters that should be ignored. We used event-related functional magnetic resonance imaging (fMRI) to investigate what neural mechanisms mediate the recognition of a visual target stimulus among equally familiar distracter stimuli (*2*).

We designed a face working memory task in which the recognition of a target face could not be based on the familiarity of that face or on how recently that face was seen previously (*3*). Additionally, every face was used as a target to be recognized in some trials and as a distracter to be ignored in other trials. Under these conditions, we found that enhanced neural responses, primarily in the prefrontal cortex, signaled the identification and maintenance in working memory of a currently attended target item. At the same time, reduced neural responses, primarily in the ventral temporal and parietal cortices, were associated with repetition of stimuli, whether they were targets or distracters.

Each trial of the memory task consisted of a target face to be remembered, presented for 4 s, followed by 13 faces presented in rapid succession at a rate of 2 s per face (Fig. 1). The target and one of the distracters were repeated up to five times in a given trial, separated by 4 to 20 s. fMRI scans were obtained from six right-handed normal participants while they performed the task. Multiple regression analysis was used to identify and differentiate the cortical regions associated with responses to targets, distracters, and repeated distracters (*4*).

We first examined the cortical responses to correctly recognized target faces. Relative to nonrepeated distracters, detection of target faces was associated with increased activity in bilateral inferior/mid-frontal (mean volume = 2.6 cm^3, $N = 4$), left insular (volume = 0.74 cm^3, $N = 5$), bilateral superior temporal (volume = 0.45 cm^3, $N = 6$), and bilateral ventral temporal/fusiform cortices (volume = 0.79 cm^3, $N = 3$) (*5*). This increase in neural response may signal recognition of the target among equally familiar faces. In addition, strongly enhanced responses were also observed in left primary motor (volume = 4.1 cm^3, $N = 6$) and supplementary motor areas (volume = 2.2 cm^3, $N = 6$) (see Fig. 2A), presumably reflecting the motor response to target faces (*6*).

We next examined cortical responses to distracters. Magnetic resonance (MR) responses to repeated distracters were compared with the same baseline that we used for the analysis of the target enhancement effect, namely responses to nonrepeated distracter faces. This analysis revealed reduced neural activity bilaterally in the inferior temporal cortex/fusiform gyrus (volume = 2.3 cm^3, $N = 5$), occipital cortex (volume = 1.0 cm^3,

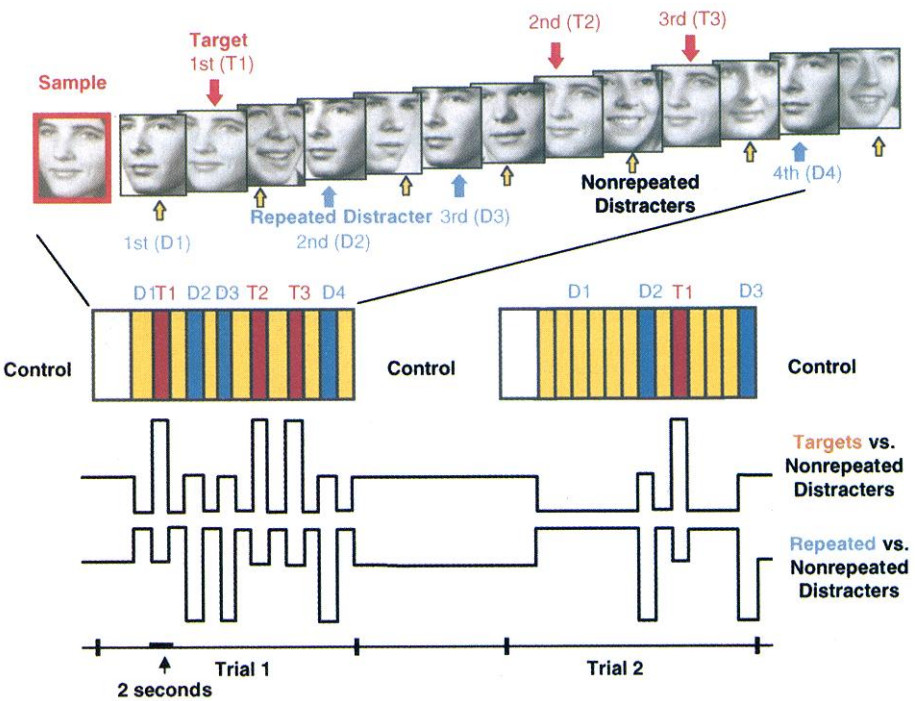

Fig. 1. The working memory task and the fMRI time series. For each memory trial, participants were first presented with a sample face to remember; then they viewed faces presented rapidly in succession. Their task was to press a button with their right hand when they saw a face that matched the sample face (target). Targets (red), as well as some distracter faces (blue), were presented in an unpredictable sequence from one to five times on a given trial and were intermixed with distracter faces (yellow). Working memory trials were separated by 18 s, during which participants passively viewed a series of nine nonmeaningful control stimuli. The MR signals were analyzed with multiple regression (represented by square-wave functions) to reveal regional activation patterns associated with repetition of target faces and repeated distracter faces.

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$N = 4$), superior occipital/intraparietal cortex (volume = 3.5 cm^3, $N = 5$), precuneus (volume = 0.094 cm^3, $N = 3$), and a posterior/mid-frontal region (volume = 0.23 cm^3, $N = 3$) (*7*) (Fig. 2B). In contrast to the enhanced activity associated with detecting target faces, correct rejection of repeated distracter faces was associated primarily with reduced neural responses (*8*).

We determined the regional cortical distribution of the enhanced responses to targets and reduced responses to repeated distracters by counting the associated significant voxels across all six participants and across both hemispheres. As Fig. 2C indicates, enhanced responses to targets outweighed reduced responses to repeated distracter faces in prefrontal and insular areas. In contrast, reduced responses to repeated distracter faces predominated in the posterior visual cortices.

Taken together, these results point to two working memory mechanisms that contribute to the recognition of an object among highly familiar stimuli: One signals the object to be attended; the other indicates that a stimulus has been seen a moment ago. Each mechanism is reflected in distinct neural responses with different regional cortical distributions.

To better understand the characteristics of enhanced and reduced responses to targets and repeated distracters, respectively, we further examined the mean MR responses to repeated items within each trial of the task. We examined within-trial MR responses to repeated targets and distracters in the ventral temporal areas that are face-selective and were associated with reduced responses to repeated distracters (Fig. 2B, 3 in blue). The response to a distracter decreased steadily from its first presentation to its fourth and fifth appearance within a trial ($P < 0.001$) (Fig. 3A). Despite an overall enhanced response to targets in this region, the response to targets also steadily declined with repetition within a trial ($P < 0.001$). These results suggest that reduced neural responses in posterior cortical areas reflect a neural mechanism that signals the repetition of a stimulus (*9*), even if the stimulus is a target. Because the enhanced response to targets in these posterior extrastriate areas is eliminated by the fourth or fifth presentation because of repetition reduction, response enhancement in these areas cannot be a reliable neural signal for identifying targets.

To determine whether response enhancement in anterior areas may be a more reliable signal for identifying targets, we conducted a similar analysis of within-trial changes in response in those areas (Fig. 2A, 4 in red). In contrast to the reduction in response found in ventral temporal areas, the response to targets remained constant with repetition in frontal/insular areas ($P > 0.05$) (Fig. 3B). Activity associated with repeated distracters in these areas also remained at a constant but low level throughout the trial. Thus, the enhanced neural responses in frontal/insular areas may signal the active maintenance of the target object in working memory.

As a further test of the dissociation of the two memory mechanisms, we compared the MR responses to the sample face presented at the beginning of each trial and to the first target within a trial (Fig. 4A). The response to the first target exceeded that to the sample in the same frontal/insular areas previously shown to exhibit enhanced responses to targets. In contrast, a slight decrease in activation was observed for the ventral temporal

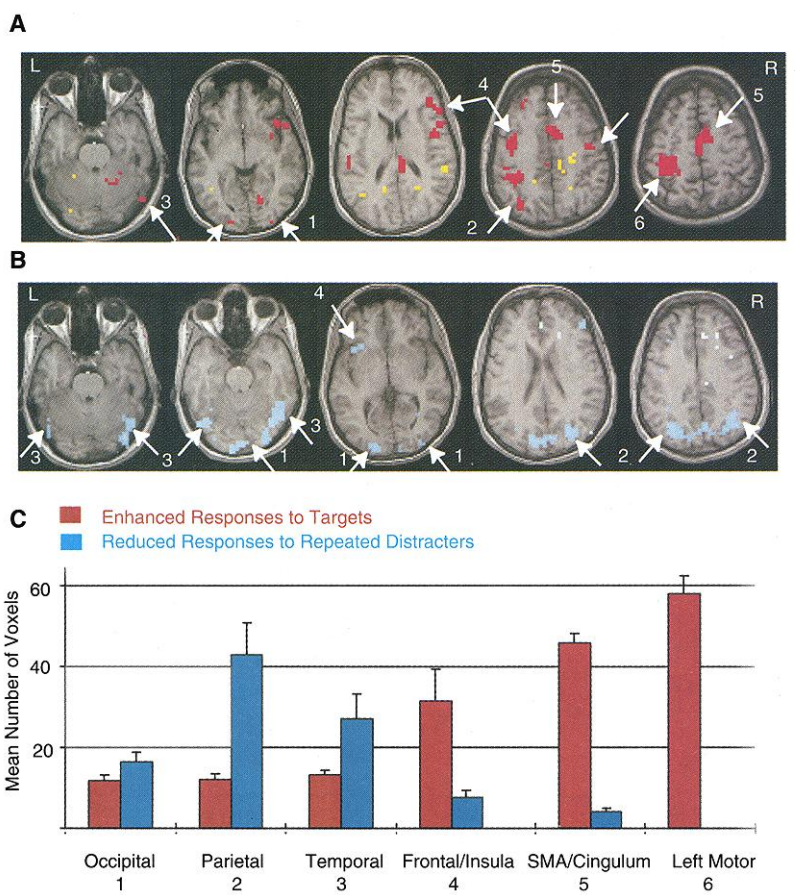

Fig. 2. (**A**) Activation patterns showing enhanced neural responses to targets (red) relative to nonrepeated distracters in one participant. (**B**) Activation patterns for the same participant showing reduced neural responses to repeated distracters (blue) as compared with nonrepeated distracters. Cortical regions: 1, occipital cortices; 2, parietal cortices; 3, temporal cortices; 4, frontal/insular areas; 5, supplemental motor areas or cingulum; and 6, left motor region. (**C**) Regional distribution of the mean number of voxels associated with target enhancement and repetition reduction in each cortical region. 5 and 6 indicate motor response–related activation areas.

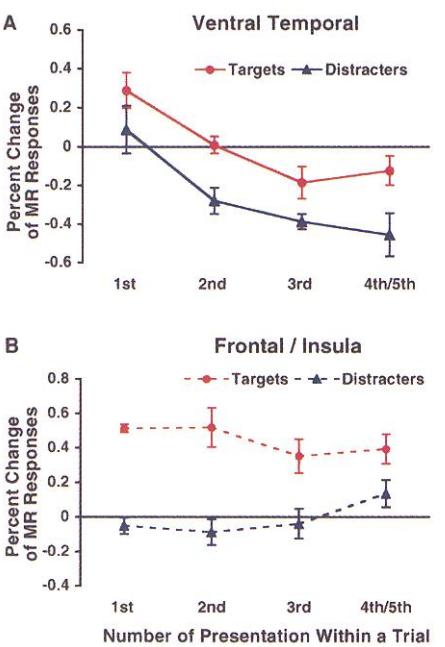

Fig. 3. Mean percentage of increase, relative to nonrepeated distracters, of within-trial MR responses to repeated targets and distracters in ventral temporal (**A**) and frontal/insular (**B**) cortices. (A) In the ventral temporal region, repetition reduction was observed for responses to both targets and distracters within a trial. (B) In contrast, the target enhancement observed in the frontal/insular areas was maintained for repeated presentation. Error bars indicate standard errors after removing the main effect of participant differences in mean response.

areas. The interaction between stimulus type (sample/target) and cortical region (frontal/ventral temporal) was significant ($P < 0.05$). This interaction corroborates the results of our previous analyses that suggest that the enhanced response in frontal/insular areas signals the target status of a stimulus whereas the response to a stimulus in posterior extrastriate areas diminishes with repetition whether that stimulus is a target or not.

It is possible that the repetition reduction we observed reflects a long-term process of increasing familiarity of the stimuli over the course of the experiment. If that is the case, neural responses to the same repeated face should continue to decline in later trials. Alternatively, the neural response to a particular face could "reset" to its initial level for each new trial. We tested these alternative hypotheses by comparing MR responses to a face when used as a repeated distracter for the first time (first trials) with the responses to the same face when used for the second or third time (later trials). In ventral temporal areas, MR signals to repeated distracters decreased within a trial but reset to the initial level for later trials (Fig. 4B). There was no significant difference between the MR responses to the first presentation of the distracter to be repeated in the first and later trials ($P > 0.6$) (*10*). This restoration of response between trials strongly suggests that our observation of a reduction in response with repetition of a familiar item represents a phenomenon distinct from the response reduction associated with long-term familiarization of initially novel stimuli (*11*). Rather, within-trial repetition reduction in the extrastriate cortex may reflect a process that temporarily tags a familiar stimulus so that it can be processed more efficiently when encountered again within the context of the currently active working memory search.

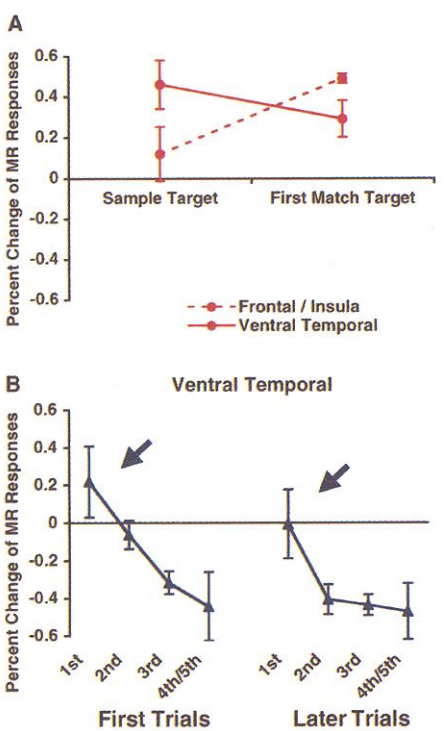

Fig. 4. (**A**) Comparison of mean MR responses to sample and match-to-sample target faces, relative to nonrepeated distracters. (**B**) MR responses to repeated distracters between trials. Mean percentage of increase of MR responses to repeated distracters between trials in ventral temporal area. The results show that repetition reduction, found within both the first and later trials, "reset" between trials during the working memory task (arrows). Error bars indicate standard errors after removing the main effect of participant differences in mean response.

If repetition reduction reflects more efficient processing, reaction times (RTs) to repeated distracters should be faster than for nonrepeated distracters. This prediction was confirmed in a separate behavioral study in which participants responded overtly to both targets and distracters (*12*).

Our results are consistent with studies of single-unit recordings from inferior temporal and prefrontal cortices in monkeys performing delayed match-to-sample tasks with repeated stimuli (*13*). Enhanced neural responses were found when the stimulus was behaviorally relevant (a target). Neurons with enhanced neural responses to targets predominated over repetition suppression neurons in monkey prefrontal cortex, whereas inferior temporal neurons showed the opposite trend. The neural responses to repeated stimuli also "reset" between trials (*14*).

Our results support a role for the active maintenance component of working memory in the selection of targets among distracters. Effective selective attention requires that the neural response to a target stimulus is enhanced and maintained during the period of time the target remains behaviorally relevant (*15*). The sustained enhancement of frontal responses across target repetitions reported here might reflect such top-down control of attention (*16*).

In conclusion, these results demonstrate that equally familiar objects can evoke enhanced or reduced neural responses depending on their working memory status. Enhanced responses were associated with the target, the stimulus that was maintained in working memory. Only the enhanced response in the frontal cortex was sustained across repetitions of the target, suggesting that response enhancement there may signal the target status of a stimulus. Enhanced responses in posterior areas showed increasing reduction of response enhancement with repetition, so that the response magnitude to later presentations did not differ from the responses to nonrepeated distracters. Reductions of neural responses associated with stimulus repetition were found primarily in extrastriate cortices and were found regardless of whether the stimulus was a target or a distracter. This repetition reduction may reflect a process that enables more efficient processing of stimuli when they are encountered repeatedly during an active working memory search. Thus, these complementary neural mechanisms track the status of familiar items in working memory, allowing for the efficient recognition of a currently relevant object and rejection of irrelevant distracters.

References and Notes

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2. With event-related fMRi, it is possible to distinguish neural responses to different stimuli presented in an unpredictable sequence, spaced as short as 2 s apart; e.g., A. Dale and R. Buckner, *Hum. Brain Mapp.* **5**, 329 (1997); V. Clark, J. Maisog, J. V. Haxby, *J. Neurophysiol.* **79**, 3257 (1998).
3. Visual stimuli consisted of 33 photographs of monochromatic faces and 83 photographs of nonmeaningful visual stimuli (scrambled versions of the faces). Before scanning, each participant practiced the working memory task until no errors were made on six successive trials. On average, participants took 12 to 18 trials to reach this criterion, during which each face was seen five to eight times. Participants were also highly accurate in performing the task during MR scanning (98.4% correct identification).
4. J. V. Haxby *et al.*, in *Mapping and Modeling the Human Brain*, P. Fox, J. Lancaster, K. Friston, Eds. (Wiley, New York, in press); K. J. Friston *et al.*, *Hum. Brain Mapp.* **2**, 189 (1995). The delay for the hemodynamic response was evaluated for every activated cortical voxel (0.07 cm^3) with Fourier analysis to estimate the phase for alterations between the working memory and control tasks. Regions showing significant signal enhancement or reduction were defined as voxels with $Z > 3.09$ ($P < 0.001$, one-tailed) for the overall experimental effect and $|Z| > 1.96$ ($P < 0.05$, two-tailed) for either the contrast between responses to targets and nonrepeated distracters or the contrast between repeated distracters and nonrepeated distracters. Each participant completed 72 memory trials inside the scanner. A GE 1.5 T magnet was used to obtain T2*-weighted gradient echo echo-planar images with blood oxygen level–dependent signals. Whole brain volumes, each consisting of 22 5-mm-thick axial slices, were acquired for each participant (repetition time = 3 s, echo time = 40 ms, flip angle = 90°).
5. J. Talairach and P. Tournoux, *Co-planar Stereotaxic Atlas of the Human Brain* (Thieme, New York, 1988). The face-sensitive fusiform regions identified here are in the same areas noted in previous studies of face perception and working memory (within 1 to 9 mm) [e.g., S. M. Courtney *et al.*, *Nature* **386**, 608 (1997); J. V. Haxby *et al.*, *Hum. Brain Mapp.* **3**, 68 (1995); J. V. Haxby *et al.*, *Neuron* **22**, 189 (1999)]. Average Talairach coordinates for enhanced responses to targets were localized to the inferior frontal (left: −34, 9, 10; right: 43, 36, 9; BA 44), left insular (−27, −9, 9), superior temporal (left: −36, −19, 10; right: 56, −13, 6; BA 22/42), ventral temporal cortices/fusiform gyrus (left: −30, −48, −18; right: 30, −60, −20; BA 20/37), and left primary motor (−27, −33, 51; BA 4) and supplementary motor areas (1, −11, 47; BA 6).
6. Identification of motor-related activation associated with right-hand button responses to the target faces validated our rapid, event-related fMRI method. All six participants showed robust activation in the left primary motor cortex and the supplementary motor area (SMA). These findings provide an important internal control for our rapid, event-related fMRI design.
7. Average Talairach coordinates for reduced responses to repeated distracter faces were as follows: ventral temporal cortex/fusiform gyrus (left: −31, −54, −19; right: 30, −64, −18; BA 37), occipital (left: −2, −100, −15; BA 17; right: 27, −78, 9/6, −97, 9; BA 18), superior occipital/intraparietal sulcus cortex (left: −24, −66, 39; right: 35, −65, 39; BA 19), the precuneus (2, −66, 35; BA 7), and posterior frontal (−33, 2, 28; BA 44).

8. Increased MR responses to repeated distracters were found in only two participants. In both participants, these increased responses were in the intraparietal sulcus.
9. Analysis of the MR responses to nonrepeated distracters at each of the 13 stimulus positions within a trial did not show the same trend as the targets or distracters. Thus, we discount the possibility that the repetition reduction effect reflects a "position effect" or linear trend within a trial. Similar results were found for the intraparietal area responses.
10. MR responses to repeated distracters reset in subsequent trials to initial levels in both left and right ventral temporal areas (Fig. 4B presents the mean over two hemispheres) as well as in the left and right intraparietal areas. In all cases, the response to the first presentation in later trials was not significantly different from the response to the first presentation in first trials ($P > 0.1$).
11. R. Buckner *et al.*, *J. Neurosci.* **15**, 12 (1995); J. Demb *et al.*, *J. Neurosci.* **15**, 5870 (1995); D. Schacter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 321 (1996); L. Squire *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 1837 (1992); C. Büchel, J. T. Coull, K. J. Friston, *Science* **283**, 1538 (1999).
12. Six participants performed the same working memory task as in the fMRI study, except that they responded to both targets and distracters. The median RT for repeated distracters (429 ms) was significantly shorter than that for nonrepeated distracters (491 ms): $F(1,5) = 6.7$, $P < 0.05$. RTs for repeated distracters were computed separately for each of five repetitions. A significant main effect of repetition, $F(4,20) = 7.2$, $P < 0.001$, indicated that RT declined with repeated presentation (from 447 to 396 ms). Thus, repetition of familiar objects during the working memory task was associated with improved performance in detecting distracters.
13. E. K. Miller and R. Desimone, *Science* **263**, 520 (1994); E. K. Miller, C. Erickson, R. Desimone, *J. Neurosci.* **16**, 5154 (1996).
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16. R. Desimone and J. Duncan, *Annu. Rev. Neurosci.* **18**, 193 (1995).
17. We thank R. Desimone for insightful comments on an earlier version of the manuscript, J. Maisog for implementing data analysis software, J. Schouten and E. Hoffman for participant recruitment and training, L. Kikuchi and C. Chavez for conducting the behavioral study, J. Szczepanik for help with data analysis, S. Courtney and L. Petit for valuable discussions, and the NIH in vivo Nuclear Magnetic Resonance Center for assistance with MR imaging. Y.J. and R.P. were supported by NIH grant AG07569.

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Inhibitors of Strand Transfer That Prevent Integration and Inhibit HIV-1 Replication in Cells

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Integrase is essential for human immunodeficiency virus–type 1 (HIV-1) replication; however, potent inhibition of the isolated enzyme in biochemical assays has not readily translated into antiviral activity in a manner consistent with inhibition of integration. In this report, we describe diketo acid inhibitors of HIV-1 integrase that manifest antiviral activity as a consequence of their effect on integration. The antiviral activity of these compounds is due exclusively to inhibition of one of the two catalytic functions of integrase, strand transfer.

The development of chemotherapeutic agents for the treatment of HIV-1 infection has focused primarily on two viral enzymes: reverse transcriptase and protease. Although regimens including agents directed at each of these biochemical targets are effective in reducing viral load and morbidity and mortality, the long-lived nature of the infection and the genetic plasticity of the virus have made it apparent that new antiretroviral agents are required to deal with the appearance and spread of resistance (*1*). HIV-1 integrase catalyzes the insertion of the viral DNA into the genome of the host cell. Integration is essential for viral replication and is thus an attractive target for novel chemotherapy (*2, 3*). Many inhibitors of HIV-1 integrase have been identified; however, their in vitro activity has not translated into antiviral activity in cells (*4*).

Integration is a multistep process that occurs in discrete biochemical stages: (i) assembly of a stable complex with specific DNA sequences at the end of the HIV-1 long terminal repeat (LTR) regions, (ii) endonucleolytic processing of the viral DNA to remove the terminal dinucleotide from each 3′ end, and (iii) strand transfer in which the viral DNA 3′ ends are covalently linked to the cellular (target) DNA (Fig. 1) (*4*). Each of the catalytic reactions (3′ processing and strand transfer) requires integrase to be appropriately assembled on a specific viral DNA (or donor) substrate (*5*). In general, compounds identified in assays with purified, recombinant integrase interfere with assembly in vitro (*6, 7*). Because assembly is a prerequisite for catalysis, such compounds may appear to inhibit 3′ processing and strand transfer, but they have no effect on either reaction when assayed subsequent to assembly on HIV-1–specific oligonucleotides (*6*). These compounds are also ineffective in assays wherein viral preintegration complexes isolated from HIV-1–infected cells are used (*8*).

To identify inhibitors of catalysis, we biased the strand transfer reaction by means of preassembling recombinant integrase on immobilized oligonucleotides as a surrogate for preintegration complexes (*6*) (Fig. 1). In a random screen of more than 250,000 samples, a variety of inhibitors was identified; however, the most potent and specific compounds each contained a distinct diketo acid moiety, and thus these inhibitors segregate into a single structural class (Fig. 1). The diketo acid functionality is an intrinsic feature of these inhibitors but is not sufficient for activity, as structural analogs exhibit a range of inhibitory potency. For most analogs, the activity observed in strand transfer assays with recombinant integrase correlated with their relative activity in assays using HIV-1 preintegration complexes (*9*). Analogs that were more potent in these biochemical assays also inhibited HIV-1 replication in cell culture.

L-731,988 and L-708,906 were two of the most active diketo acids in strand transfer assays with recombinant integrase. With 50% inhibitory concentrations (IC_{50}'s) of 80 and 150 nM, respectively, L-731,988 and L-708,906 are also the most potent inhibitors of preintegration complexes described to date. In a single-cycle assay for acute infection (*10*), L-731,988 and L-708,906 inhibited HIV-1 replication with IC_{50}'s of 1 to 2 μM; higher concentrations prevented the spread of HIV-1 in cell culture for several weeks (Fig. 2). L-731,988 and L-708,906 were comparably active against both macrophage- and T cell line–tropic strains of HIV-1, clinical isolates, and variants resistant to reverse transcriptase and protease inhibitors (*11*). Consistent with the effect of an early stage inhibitor, the compounds did not affect virus production from persistently infected cells (up to 50 μM) (*11*).

To validate integrase as the molecular target responsible for the antiviral effect, we selected HIV-1 variants resistant to L-708,906 and L-731,988. At concentrations of inhibitor sufficient to block replication of the wild-type virus (20 μM), the resistant variants replicated nearly as well as the wild-type (or resistant) virus in the absence of inhibitor (Fig. 2). Sequencing of the cDNA derived from four resistant populations consistently identified specific mutations

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