

binds Sos through its SH3 domains and tyrosine-phosphorylated IRS-1 and Shc through its SH2 domain, and after insulin stimulation the GRB2-Sos complex associates with IRS-1 and Shc (Fig. 5) (8, 27).

It is not clear how binding of GRB2 to IRS-1 or Shc potentiates the ability of GRB2 to activate Sos. The interaction of GRB2 with IRS-1 or Shc or with both molecules simultaneously may serve to reposition Sos adjacent to Ras, which is located in the plasma membrane. In support of this mechanism, IRS-1 has been shown to relocate to the plasma membrane in insulin-stimulated cells (28). Alternatively, the binding of GRB2 to Shc or IRS-1 may cause a conformational change in GRB2, leading to activation of Sos. Another possibility is that the binding of GRB2 to IRS-1 or Shc promotes the phosphorylation of Sos, thus stimulating guanine nucleotide-releasing activity. The studies reported here demonstrate that, as for EGF and other growth factors, insulin stimulation of Ras signaling pathways is mediated by GRB2. However, in contrast to the EGF receptor that interacts directly with the GRB2-Sos complex, stimulation of the insulin receptor leads to phosphorylation of the two docking proteins IRS-1 and Shc. The ensuing interaction of the GRB2-Sos complex with IRS-1 and Shc in insulin-stimulated cells provides an additional level of control by the insulin receptor of this pivotal signaling pathway.

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- Abbreviations for the amino acid residues are: E, Glu; F, Phe; K, Lys; L, Leu; N, Asn; R, Arg; S, Ser; and V, Val. Mutations referred to herein are also indicated with this single-letter code. Thus, Arg⁶⁶ → Lys is R86K and Ser¹⁷ → Asn is S17N.
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- GRB2 and GRB2(R86K) were subcloned into the eukaryotic expression vector PMJ30 (29) and co-transfected with a neomycin resistance plasmid into an L6 myoblast cell line by calcium phosphate precipitation (30). Stable cell lines overexpressing GRB2 and the GRB2 mutant were isolated. Mutation in the SH2 domain of GRB2 was done as described (3). Cell lines overexpressing GRB2 were selected for further study. To analyze for phosphotyrosine, we incubated cells for 24 hours in medium containing 0.5% fetal bovine serum. Cells were then either unstimulated or stimulated with insulin as indicated and lysed in lysis buffer [50 mM Tris (pH 7.4), 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 0.2 mM sodium orthovanadate, 0.2 mM phenylmethylsulfonyl fluoride, and 0.5% NP-40]. Cell lysates were separated by SDS-PAGE (10% gel). Bound antibodies were visualized with ¹²⁵I-labeled protein A (ICN, Irvine, CA). The antibodies to phosphotyrosine were rabbit polyclonal antibodies (15). Antibodies to ERK-1 and ERK-2 were from Zymed (South San Francisco, CA), clone 2033.
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Dissociation of Object and Spatial Processing Domains in Primate Prefrontal Cortex

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Areas and pathways subserving object and spatial vision are segregated in the visual system. Experiments show that the primate prefrontal cortex is similarly segregated into object and spatial domains. Neurons that code information related to stimulus identity are dissociable, both by function and region, from those that code information related to stimulus location. These findings indicate that the prefrontal cortex contains separate processing mechanisms for remembering "what" and "where" an object is.

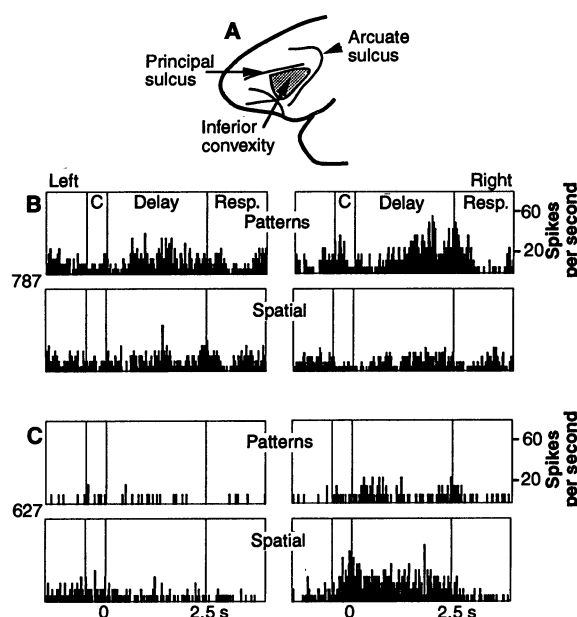
In the primate visual system, segregated pathways convey information pertaining to an object's identity, such as color and shape, and to its location in space (1). Although segregation and regional specialization are clearly established within the

sensory processing areas of the cortex, they may also exist in the prefrontal cortex, which mediates cognitive and executive functions (2). Physiological recordings in the principal sulcus and arcuate regions of monkeys trained to perform delayed-re-

sponse tasks demonstrate that neurons in these areas hold representations of spatial stimuli "on line" for brief periods when such stimuli are no longer present and must be recalled (3). This activity is considered a neuronal correlate of working memory, a process for updating information on a trial-by-trial basis. These prefrontal areas are connected with the posterior parietal cortex, from which they presumably access spatial information (4). Numerous other findings support the idea that the working memory function of the dorsolateral prefrontal region is specialized for spatial information (2). Experiments presented here were designed to reveal if other areas of the prefrontal cortex provide comparable working memory mechanisms that encode nonspatial information about the color and form of an object, as opposed to its location.

The cortex of the inferior prefrontal convexity ventrolateral to the principal sulcus (Fig. 1A) is a candidate for processing color and form information because lesions of this area produce deficits on tasks requiring memory for objects (5). Further, the receptive fields of neurons in this area represent the fovea (6), the region of the retina specialized for the analysis of the detail in patterns and color—stimulus attributes important for the recognition of objects. We recorded neuronal activity from the inferior convexity in two monkeys trained to perform oculomotor delayed-response tasks in which spatial or pattern memoranda had to be remembered on independent, randomly interleaved trials (7). On spatial delayed-response (SDR) trials, stimuli were presented to the left or right of fixation while the monkeys gazed at a fixation point on a video monitor. After a subsequent 2.5-s delay, the fixation point disappeared, which instructed the monkey to direct its gaze to the remembered location of the stimulus. On pattern delayed-response (PDR) trials, a stimulus pattern was presented in the center of the screen; one pattern required a left eye and the other a right eye movement after the delay. Thus, both spatial and pattern trials required identical responses but differed in the type of memory that guided those responses.

Neurons in the inferior convexity were especially responsive ($P < 0.05$) on PDR trials. For example, neuron 787 (Fig. 1B) (upper panel) showed a greater activation in the delay period after offset of one of the two pattern cues (8). This type of neuron processed information about the pattern because it was unresponsive during the delay period when an identical eye movement was guided by the memory of a spatial cue



on SDR trials (Fig. 1B, lower panels). Of 31 inferior convexity neurons with delay-period activity, 24 (77%) neurons responded selectively to pattern memoranda; 6 (19%) neurons responded in the delay preceding right or left responses on both spatial and pattern trials, thus encoding the response direction. However, only one (3%) inferior convexity neuron encoded locational information, although such neurons were more common in the dorsolateral prefrontal cortex (3). For example, neuron 627 (Fig. 1C) responded during the delay period when a spatial cue on the right was the memorandum but was unresponsive in the delay when a pattern stimulus required the identical rightward response on PDR trials (9). These results demonstrate two important points about frontal lobe mechanisms: (i) that information about object identity and location may be processed separately from each other and (ii) that selective

neuronal activity in the delay period does not necessarily reflect a motor signal related to the direction of the impending saccadic movement.

In the course of these experiments, we discovered that neurons in the inferior convexity were highly responsive to stimuli presented in foveal vision, and we explored this property in experiments that assessed neuronal responsiveness to visual stimuli in a picture fixation task (10). We found that 137 inferior convexity neurons tested on the picture task showed significant stimulus specificity. They responded differentially ($P < 0.05$), strongly to some stimuli and less so or not at all to the other stimuli within a set (11) (Fig. 2A). Monochromatic color fields tended to be the least effective stimuli, indicating that texture and contour within the image were important for the neuronal activity and that brightness was not. Moreover, the responses of certain neurons con-

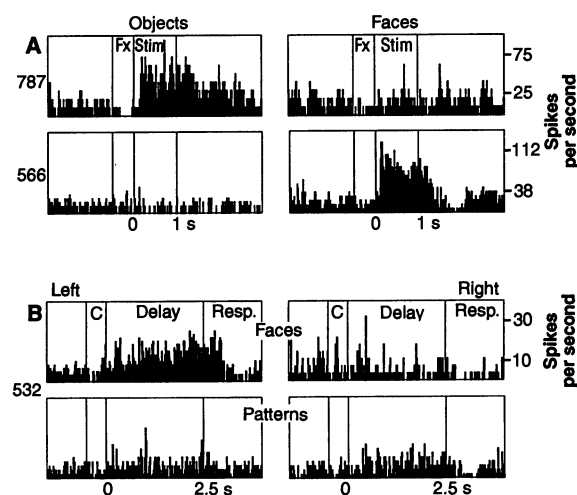


Figure 2A shows stimulus and face-selective responses in the picture fixation task. Neuron 787 (upper panels) is responsive to a pattern stimulus and unresponsive to a face. Conversely, neuron 566 (lower panels) responds selectively to faces and is unresponsive to patterns; Fx, fixation; Stim, stimulus. Figure 2B shows responses of inferior convexity neuron 532 with delay-period activity after a specific monkey face in the memory task. Upper panels show differential responses to two faces (requiring left or right responses); lower panels show the lack of response to two patterns requiring left or right responses.

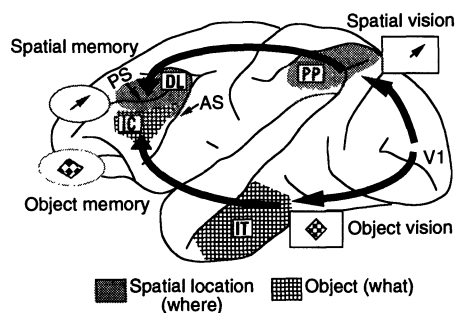


Fig. 3. Schematic diagram illustrating the primate visual systems implicated in foveal and peripheral vision and their connections with the prefrontal cortex; PS, principal sulcus; AS, arcuate sulcus. The posterior parietal (PP) cortex is concerned with spatial perception, and the inferior temporal (IT) cortex with object recognition. These regions are connected with the dorsolateral (DL) and inferior convexity (IC) prefrontal cortices (2, 17), where memoranda pertaining to spatial location and object identity are encoded in working memory.

sistently outlasted a preferred stimulus by 0.5 to 3.0 s after its disappearance. This sustained activity was generally specific to certain stimuli; for example, neuron 787 (11) (Fig. 2A) responded differentially to 22 stimuli, but only one elicited sustained activity. As these prolonged responses are unlike activity that has been reported in earlier parts of the visual system, which tends to be either transient or locked to the onset and offset of stimuli (12), the post-stimulus activity of inferior convexity neurons may reflect transient memory of a stimulus in a task that did not formally require memory but also did not preclude it.

We tested the extent of the receptive fields of stimulus-selective neurons in the inferior convexity by presenting stimuli in nine locations, at the fovea and at 13° eccentric to the fixation point (7). Typically, these neurons responded strongly to their optimal stimulus when it was presented foveally, with weaker responses to peripheral stimuli. These findings provide further evidence of a dissociation between spatial and nonspatial processing mechanisms, in keeping with the results of studies showing that the receptive fields of inferior convexity neurons include the fovea (6).

Evidence for the object-oriented nature of inferior convexity neurons was provided by the finding that 11 of 137 stimulus-selective neurons responded specifically to faces of monkeys and humans. Neuron 566 (Fig. 2A) (13) responded to monkey faces but not to objects presented on picture fixation trials. We also manipulated the attributes of certain faces by changing their size, orientation, and color to gray-scale values; we found that, although the magnitude of the responses changed with these manipulations, the stimulus specificity re-

mained largely invariant. To test the possibility that memory for faces elicits delay activity in inferior convexity neurons, we trained one monkey to use pictures of faces as memoranda in the PDR task. Figure 2B illustrates the responses of a neuron that was specifically activated in the delay period after the offset of one face but not the other (14). The same neuron was not activated on PDR (or SDR) trials even when the same eye movements were required. This finding demonstrates that inferior convexity neurons can encode configurational features of visual stimuli and their involvement in nonspatial working memory.

The response properties of inferior prefrontal convexity neurons to visual stimuli closely resemble those of the inferior temporal (IT) cortex (15, 16). Their receptive fields are relatively large, they respond maximally to complex stimuli presented at the fovea, and they are less responsive to stimuli presented in peripheral space and to simple stimuli. Their selectivity is relatively invariant over transformations of size, inversion, and color versus black and white. Neurons in both structures respond selectively to different visual stimuli including faces and are active in working memory tasks that require identification of objects. These properties suggest that stimulus-selective activity, when it can be related to an entity such as a face, reflects the identity of objects rather than their local features. However, one difference between the inferior prefrontal cortex and the IT cortex is that the responses of certain IT neurons decline with changes in the familiarity of the stimuli (16). We did not detect such changes in inferior prefrontal neurons with our paradigms. The IT cortex projects to the inferior convexity (17), and these connections presumably provide signals about the attributes of foveal visual stimuli on which prefrontal circuits operate, as evidenced by metabolic studies (18).

Our findings in the inferior convexity of the prefrontal cortex contrast with recordings in adjacent areas: the dorsolateral cortex above and within the principal sulcus and the anterior bank of the arcuate sulcus. With the exception of a few stimulus-selective neurons in orbital prefrontal cortex, we rarely encountered pattern-selective neurons outside of the inferior convexity (19), although we did encounter neurons with spatial and directional tuning. These latter findings are consistent with recordings in the regions of the principal sulcus and anterior arcuate cortex where neurons are responsive to visuospatial stimuli in SDR tasks similar to that used in the present study (3, 6). These considerations strongly suggest that there are areal specializations in the processing of spatial and nonspatial visual information in the pre-

frontal cortex, supporting the domain-specific working memory hypothesis of prefrontal organization (2). Our findings suggest that the inferior convexity may be involved in mnemonic processing of objects and faces, whereas the principal sulcus and arcuate area of the dorsolateral convexity is dedicated to spatial processing (Fig. 3).

Clinical studies reveal that patients with damage to the inferior frontal cortex are impaired in recognizing recently seen faces and words and in the classification of visual patterns (20). Furthermore, blood flow studies in humans have shown that these regions of the prefrontal cortex are functionally active when subjects perform tasks that require the processing of pattern information in short-term memory or in the categorization of features as in the Wisconsin Card Sort Test (21). Although it is not yet clear whether the inferior prefrontal areas in these studies of human cognition correspond to the inferior convexity area described here, their relative position in the human brain appears homologous with the area identified in the present study of non-human primates.

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7. In the standard task, monkeys were presented with two spatial (left or right) cues, each subtending 0.5° at 13° eccentricity, and two pattern (subtending 3°) cues presented on the fovea. Two variants of the standard task used color (blue, yellow; subtending 3°) and face (subtending 10°) cues. All stimuli were presented for 0.5 s and required saccades 13° to the left or right of fixation. A version of the standard task with eight spatial cues (with 45° of angular separation between them) was used to map responses to peripheral spatial stimuli. Correct responses were rewarded with apple juice. We monitored eye movements by using the scleral search coil technique [D. A. Robinson, *IEEE Trans. Biomed. Eng.*

- 10, 137 (1963)]. Analysis of variance was used to compare neuronal responses for each trial type within the cue and delay conditions. The source of significant variance was then determined by the Tukey HSD test. All delay-related and stimulus-specific neuronal responses were based on significant comparisons. Care of the monkeys was in accordance with the Yale University Animal Care Committee.
8. On PDR trials, neuron 787 responded during the delay period (26 spikes per second) after a pattern that required a rightward saccade and was significantly less responsive (12.5 spikes per second) after the other pattern, and after spatial cues (11 and 10 spikes per second) on SDR trials. Spontaneous activity was 8.4 spikes per second.
 9. The delay period activity of neuron 627 on SDR trials was 13.5 spikes per second after spatial cues requiring saccades 13° to the right, significantly greater than the delay activity (2.9 spikes per second) for leftward-going saccades, and the delay activity (4.2 and 0.9 spikes per second) on PDR trials. Spontaneous activity was 1.9 spikes per second.
 10. The picture fixation task required the monkey to fixate while stimuli (subtending 10°) were presented in foveal vision for 1 s, and juice was delivered 0.5 s after stimulus offset; directional saccades were not required. This task utilized a library of 280 stimuli, grouped into 40 sets of seven images (five objects, one color field, and one face). The stimuli were digitized photographs of laboratory

objects; each color field was of uniform hue, generated by mixing red-green-blue values with graphics software.

11. Neuron 787: a total of 30 objects was presented (294 trials), of which 22 elicited a response (range, 37.6 to 14.3 spikes per second, mean, 24 spikes per second; spontaneous activity, 8.4 spikes per second); the other 8 objects failed to elicit any change in firing rate. The neuron (Figs. 1B and 2A) was unresponsive on SDR trials (80 trials) when the monkey had to remember and respond to the locations of 8 spatial cues; the average response to these 8 cues was 8.1 spikes per second.
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13. The responses of neuron 566 to faces ranged from 99 to 30.6 (mean, 64) spikes per second for 12 face stimuli presented, significantly greater than the responses to 14 objects ranging from 23 to 8.2 (mean, 16.4) spikes per second; data were obtained on 240 trials of the picture task. The neuron was unresponsive (mean, 11.8 spikes per second) in the eight-location SDR task (80 trials). Spontaneous activity was 16.2 spikes per second.
14. Neuron 532 fired during the delay period over which the monkey was required to remember one particular face (11.8 spikes per second), a response significantly greater than the responses to another face (3.9 spikes per second) and to the two patterns (6.1 and 5.9 spikes per

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TECHNICAL COMMENTS

Unusual Mutational Mechanisms and Evolution

R. E. Lenski and J. E. Mittler argue that studies purporting to demonstrate directed mutation lack certain controls and do not account adequately for population dynamics (1). In addition, none of the novel, even unorthodox, mechanisms invoked to explain the directed mutation hypothesis is supported by secure evidence, and some of the ideas have proved untenable (1–5). In concluding that no evidence has been presented to deny the classical tenet that mutation and selection are independent, Lenski and Mittler point out that mutation rates may vary both between and within genomes, and they raise the possibility that variable mutation rates may be an evolved response that specifically promotes increased genetic variation under stress. We lean favorably toward this suggestion and wish to expand the proposition with reference to documented genetic mechanisms.

Central to the directed mutation debate is a means of explaining the increased frequency of altered phenotypes, which those who support the hypothesis have said arises “specifically when (and even because) it is advantageous” (1). We suggest that an increase in the frequency of altered phenotypes could occur as the result of an increase in the frequency of gene expression mediated by two classical mechanisms, namely, alterations in DNA sequence and alterations in DNA topology. Mechanisms facilitating alterations in the fre-

quency of gene expression include reiterative oligonucleotide sequence motifs, which introduce frameshifts and affect translation (6); homopolymeric tracts, which, because of the likelihood of insertions or deletions occurring within regions of reiterated bases, affect transcription (7); and differential inhibition of site-specific methylation, which induces extrinsic alterations in DNA conformation (8). These are but a few examples, possibly the tip of the iceberg, of documented mechanisms with potential for mediating high-frequency changes in DNA sequence, or DNA confirmation. We wish to emphasize that, in addition to being compatible with neo-Darwinian theory, these mechanisms contribute an intrinsically stochastic component to the regulation of gene expression, the potential outcome of which is polymorphism, that is, population heterogeneity. Far from being directed, such phenotypic variations arise from mechanisms that are blind.

In an in-press article we have placed considerable emphasis on the potential importance of polymorphism within bacterial populations generated through genetically based stochastic mechanisms (9). We also outlined evidence suggesting a role for stress in regulation of the frequency of altered gene expression.

Mechanisms capable of generating random variation might provide a satisfying solution to the problem of responding to

unpredictable environmental change. It is likely that evolution has favored a balance between biological memory (as stored within a sequence of nucleotides) and probabilistic mechanisms (“contingency behavior”) that enables an optimal response to situations that cannot be anticipated.

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The article by Lenski and Mittler (1) prompted us to reevaluate our data about the conjugative transposable element Tn916 (2). Our data suggest that control of