Two Modulatory Effects of Attention That Mediate Object Categorization in Human Cortex

Geraint Rees,* Richard Frackowiak, Chris Frith

Attentional modulation of cortical activity was examined by varying the rate of visual stimuli in object categorization tasks according to single and conjoined features. Activation of dorsolateral frontal cortex was independent of the stimulus presentation rate and elicited by the participant's attention to conjoined compared with single features. Several cortical regions showed attentionally modulated activity. In inferior temporal cortex, modulation was due to an additional bias signal underlying normal rate-correlated activity. In two other regions (premotor cortex and cerebellum), attention modified the correlation of activity and the stimulus presentation rate. Attentional effects in the human cortex are expressed by at least two physiologically distinct mechanisms acting on spatially distributed areas.

Selection is the essence of attention, whether of a train of thought, a particular location, or a specific object (*1*). A general account of selectivity must deal with both spatial and nonspatial selection. The biased competition model (*2, 3*) suggests that selectivity arises from competition among relevant and nonrelevant stimuli according to both bottom-up and top-down factors. Bottom-up factors are those concerning stimulus saliency, whereas top-down factors are a means of biasing bottom-up competition in favor of signals currently relevant to behavior. The neuroanatomical basis for such a model is not clearly established. Objects in the visual field compete for processing in several cortical areas (*3, 4*). Some signals pass from primary visual cortex into inferior temporal cortex and are important for object recognition, whereas other signals pass to posterior parietal cortex and are important for spatial perception (*5*). A great deal of work has implicated the parietal lobe in the context of a larger functional network, which includes dorsolateral frontal cortex and prestriate cortex, in spatial selection through shifts of attention (*6, 7*). Object recognition involves selectivity among factors other than spatial location, and we would expect to find a basis for it in inferior temporal structures. Several lines of evidence suggest the involvement of inferior temporal cortex (*8*), and it has been suggested that modulatory biases to inferior temporal cortex arise in dorsolateral frontal cortex (*9, 10*). We sought evidence for functional interactions between dorsolateral frontal and inferior temporal cortex during selective attention to objects.

The presentation of a visual stimulus evokes transient neural activity. The neural implementation of selective attention (top-down influences on sensory processing) implies a modulation of that transient activity. This modulatory influence could take two distinct forms. First, the transient activity associated with processing the visual stimulus may be enhanced (or suppressed) directly ("phasic" modulation). It has been suggested that this sort of modulation is active in prestriate cortex during selective attention to visual motion (*11*). However, modulation could occur in a second way, by an increase in the "tonic" neural activity in a cortical area even in the absence of stimulus-related neural signals ("tonic" modulation). This type of modulation takes the form of a "bias" signal that is task-dependent, but stimulus-independent, and is suggested by the biased competition model. Functional imaging techniques can be used to detect the difference between these two types of modulation if the rate of presentation of the stimulus is varied across scans. Several studies with positron emission tomography (PET) have shown that the blood flow in the relevant brain areas is strongly correlated with the rate of stimulus presentation or response production (*12*). This finding leads us to suggest that the slope relating neural activity to presentation rate is an index of the amount of transient activity associated with the presentation of a single stimulus. Phasic and tonic modulation by attention can now be distinguished by their different effects on the relation between activity and presentation rate (Fig. 1, A and B). Phasic modulation directly affects the processing of each stimulus, analogous to an amplifier gain control or the contrast control on a television set. However tonic modulation repre-

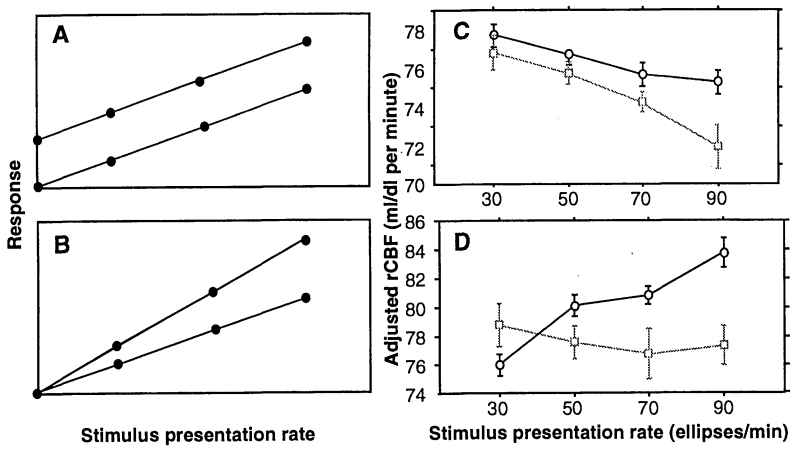

Fig. 1. On the left is a theoretical illustration of how integrated stimulus or response-related phasic activity produces a correlation between regional cerebral blood flow (rCBF) and stimulus presentation rate. A modulatory influence on the processing of each stimulus or response can have a tonic effect that results in no change of the slope describing rate dependency, but a shift due to the task at hand that can be quantified by a change in the intercept (**A**). This implies that there may be activity even in the absence of stimulus-related signals and represents an offset or bias signal. Alternatively, a modulatory influence can have an effect on the phasic activity, increasing the slope of the line (**B**); that is to say, the rate dependency is determined by the task at hand. This illustration shows a positive relation between response and stimulus presentation rate, but concepts apply equally to situations where there is a negative relation, or where the modulatory influence is negative. On the right is actual rCBF data from our experiment. The graphs relate rCBF to stimulus presentation rate in left inferior temporal cortex (**C**) and left premotor cortex (**D**). Squares represent average adjusted rCBF values over all participants for the conjunction task; circles represent similar values from the feature tasks. Error bars represent one standard error. The plots illustrate a modulation of activity by the conjunction task relative to the feature tasks that is tonic in inferior temporal cortex (A and C) and phasic in premotor cortex (B and D). Analysis of variance on the adjusted rCBF values displayed here confirms that there is a significant main effect of condition ($P < 0.01$) and rate ($P < 0.001$) with interaction (condition × rate) (P = not significant) in inferior temporal cortex (C). In premotor cortex (D) there is a significant main effect of condition ($P < 0.01$) and rate ($P < 0.05$) with interaction (condition × rate) ($P < 0.001$).

The Wellcome Department of Cognitive Neurology, Institute of Neurology, Queen Square, London WC1N 3BG, UK.

*To whom correspondence should be addressed.

sents a change in activity in a rate-responsive area that does not alter the correlation between activity and presentation rate. Tonic modulation might be thought of as a modification of stimulus processing by the addition of an offset or bias signal. This mode of action is directly analogous to the brightness control on a television set (*13*). We applied this technique, varying the rate of presentation of items in a nonspatial visual attention task, to characterize the anatomy and neural dynamics of selective visual attention.

Participants categorized serially presented single visual targets by individual features (color or orientation) or the conjunction of color and orientation. Each of these three tasks was performed at four different rates of stimulus presentation (*14*) during a series of 12 PET regional cerebral blood flow (rCBF) scans with $H_2{}^{15}O$ as the tracer (*15*). Reaction times were recorded during the scanning (*16*). We compared appropriately weighted scans to identify cortical areas with three distinct types of response characteristic. First, we identified areas that were activated by the conjunction task relative to the feature tasks but that showed no rate-related effects. Second, we identified those areas in which activity covaried with presentation rate to delineate a distributed network responsible for stimulus identification and response production irrespective of task. Finally, we identified those areas in which there was both stimulus-related activity and modulation of activity in the conjunction task. These areas represent the sites of attentional modulation, and we specifically characterized the modulatory influence of attention in these areas as either showing tonic or phasic effects (*17*).

We identified a distributed network of areas in which activity covaried with the stimulus presentation rate (Table 1) that included striate and extrastriate cortex, motor and premotor cortex, and cerebellum. Large relative deactivations with increasing presentation rate were found in medial prefrontal structures and inferotemporal cortex [probably Brodmann area 20 (BA 20)] bilaterally. The only brain area that showed greater activity in the conjunction task relative to the feature tasks was located in the right dorsolateral frontal cortex (Fig. 2) (*18*). The Talairach coordinates and surface anatomy derived from an averaged magnetic resonance image (MRI) of the six participants suggested that the activation was located in BA 8. Significant modulatory effects due to attention to conjunctions were seen in five areas (Table 2). In three areas, left premotor cortex, left cerebellum, and left precuneus, the modulation took the form of a change in slope of the relation between rCBF and stimulus presentation rate, that is, a phasic effect of attention (Fig. 1B). In two areas, left inferior temporal cortex and right cerebellum, the modulation took the form of a change in the intercept, that is, a tonic effect of attention (Fig. 1A). In inferior temporal cortex this tonic modulation took the form of a decrease in the intercept in the context of a negative relation between rCBF and stimulus presentation rate (Fig. 1, A and C).

Lateralized activity in BA 8 is uniquely seen with performance of the conjunction task (Fig. 2). Inspection of the statistical parametric map failed to identify any stimulus rate–related activity, even at low statistical thresholds. Because this activity is stimulus- and response-independent, we suggest that this area is the source of top-down attentional influences on components of the distributed network performing the task. BA 8 is well placed to be a source of modulatory influence. It receives inputs from dorsal and ventral prestriate cortices (*19*). Neuronal responses in this area are task-related in go–no go and match-to-sample tasks (*20*). Moreover, the excision of frontal cortex around the posterior arcuate sulcus (BA 8) in the macaque selectively impairs performance on a cross-modal conjunction task compared with the corresponding feature responses (*21*). The activation we observed was lateralized to right BA 8, and we have no clear explanation of this result (*22*). Right frontal cortex is selectively implicated in tasks requiring sustained attention (*23*), but there is no reason to suggest that the conjunction task requires sustained attention whereas the feature tasks do not. One previous functional imaging study examined the difference between conjunction and feature categorization (6), but in the context of a spatial task, with analysis restricted to parietal cortex. Our task contained no spatial component, and no parietal activation was apparent.

There are five areas that show a modulatory influence due to attention in addition to rate effects (Table 2). The presence of activity related to both the stimulus presentation rate and to the task in these areas suggests that they are the site at which top-down attentional effects act to influence behavior. The findings in inferior temporal cortex are of particular interest to our experimental hypothesis of frontotemporal interaction as the basis of object selectivity.

Increases in the stimulus presentation rate results in progressive decrements in activity in inferior temporal cortex. Decreases in activity of inferior temporal cortex and cingulate cortex have also been seen in a study where the activity associated with identification of familiar stimuli was compared with that associated with passive observation (*24*). Transient suppression of

Table 1. Areas showing activity varying with stimulus presentation rate. All data shown reaches the $P < 0.05$ level of significance corrected for multiple comparisons, except in inferior temporal cortex, for which $P < 0.001$, uncorrected because of prior hypotheses about these regions. L., left; R., right

Area	Coordinates			*Z* score
Increases				
L. inferior occipital gyrus	−42	−88	−10	4.60
R. fusiform	48	−60	−18	4.68
L. cerebellum	−32	−66	−22	4.87
R. cerebellum	2	−54	−8	4.75
L. precentral gyrus	−56	−2	50	4.14
L. premotor cortex	−12	−10	64	4.36
Decreases				
Anterior cingulate	−2	36	−6	5.13
R. inferior frontal gyrus	22	48	16	4.46
R. inferior temporal gyrus	62	−20	−24	4.08
L. inferior temporal gyrus	−62	−16	−18	4.20

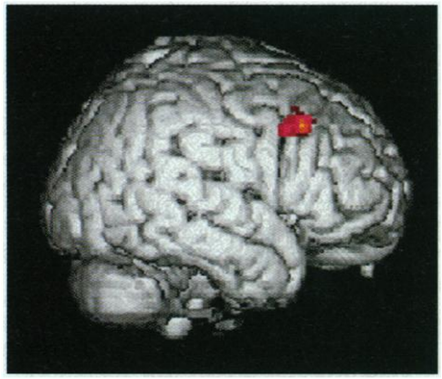

Fig. 2. The area activated by the conjunction task relative to the feature tasks shows no effect of stimulus presentation rate and is located in right BA 8 [Talairach coordinates are (42 22 40), and the *Z* score is 4.22].

Table 2. Areas that show both stimulus-dependent and task-dependent activity. All data shown reaches the $P < 0.05$ level of significance corrected for multiple comparisons.

Area	Coordinates			*Z* score	Type of modulation
L. inferior temporal gyrus	−62	−16	−18	4.77	Tonic
R. precuneus	12	−58	44	4.96	Phasic
L. premotor cortex	−16	−12	62	5.69	Phasic
L. cerebellum	−32	−62	−20	5.47	Phasic
R. cerebellum	28	−54	−26	4.95	Tonic

activity in visually responsive single neurons is seen in monkey inferior temporal cortex during match-to-sample tasks (*25*). We therefore interpret the changes we observed as representing neural activity associated with the identification of familiar stimuli. The modulation of rate-related activity by attention to conjunctions in inferior temporal cortex results in a further tonic decrement of activity (a constant decrement across all presentation rates). Our results therefore demonstrate two independent neural mechanisms operating in parallel in inferior temporal cortex (*26*). One appears to be involved in object identification and shows stimulus rate–related activity. The second mechanism appears to be related to the task and is independent of the stimulus presentation rate, perhaps biasing inferior temporal neurons during the conjunction task (*27*). We speculate that the modulation we observed in left inferior temporal cortex is therefore a neural correlate of the "attentional template" suggested by the biased competition model; it is a bias signal altering the processing of object-related signals in inferior temporal cortex. The bias signal may arise in right BA 8; a functional interaction between prefrontal and inferior temporal cortex is consistent with both the neuroanatomical connectivity of the frontal cortex and the present data (*28*, *29*). Cooling of prefrontal cortex, including BA 8, in monkeys results in both increases and decreases of stimulus-evoked and spontaneous neural activity in inferior temporal cortex (*10*).

In contrast to the tonic modulatory effect of attention observed in inferior temporal cortex, phasic modulation by attention of rate-related activity occurs in left premotor cortex (Fig. 1D) and left cerebellum. The correlation coefficient between the presentation rate and local neural activity changed significantly in the conjunction task. This demonstrates that attention can act in two physiologically distinct ways to influence neural activity in the human cortex. Phasic modulation implies that signals specifically associated with stimulus identification and response production are directly enhanced or suppressed. This contrasts with the tonic modulation seen in inferior temporal cortex, which represents activity that is in addition to, and independent of, stimulus rate–related activity. One prediction from our characterization of tonic attentional modulatory influences is that attention-related activity may be present within a cortical area even in the absence of stimulus presentation.

The modulation of multiple areas in the temporal cortex, premotor cortex, and cerebellum is broadly compatible with theoretical approaches to attention that consider the predominant role of attention as shaping behavior through influencing motor output (*30*). Nonspatial selective attention modulates cortical activity in two physiologically distinct ways. Our results support a model of visual attention in which prefrontal cortex modulates activity in a distributed network of cortical and cerebellar structures that are relevant both to the processing of sensory signals and to appropriate motor output.

REFERENCES AND NOTES

1. W. James, *The Principles of Psychology* (Harvard Univ. Press, Cambridge, MA, 1976); M. I. Posner *et al.*, *Psychobiology* **15**, 107 (1987); J. Duncan, *J. Exp. Psychol. Gen.* **113**, 501 (1984); S. P. Vecera and M. J. Farah, *ibid.* **123**, 146 (1994); M. I. Posner and S. Dehaene, *Trends Neurosci.* **17**, 75 (1994).
2. D. E. Broadbent, *Perception and Communication* (Pergamon, London, 1958); U. Neisser, *Cognitive Psychology* (Appleton-Century-Crofts, New York, 1967); M. R. Harter and C. J. Aine, in *Varieties of Attention*, R. Parasuraman and D. R. Davies, Eds. (Academic Press, Orlando, FL, 1984), pp. 293–321.
3. R. Desimone and J. Duncan, *Annu. Rev. Neurosci.* **18**, 193 (1995).
4. J. Duncan, *Perception* **22**, 1261 (1993).
5. L. G. Ungerleider and M. Mishkin, in *Analysis of Visual Behaviour*, D. J. Ingle, M. A. Goodale, R. J. W. Mansfield, Eds. (MIT Press, Cambridge, MA, 1982), pp. 549–586.
6. M. Corbetta, G. L. Shulman, F. M. Miezin, S. E. Petersen, *Science* **270**, 802 (1995).
7. M. Eglin, L. C. Robertson, R. T. Knight., *Cereb. Cortex.* **1**, 263 (1991); M. Marsel Mesulam, *Ann. Neurol.* **28**, 597 (1990).
8. K. Tanaka, *Science* **262**, 685 (1993); J. V. Haxby *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 1621 (1991).
9. F. A. W. Wilson, S. P. O. Scalaidhe, P. S. Goldman-Rakic, *Science* **260**, 1955 (1993).
10. J. M. Fuster, R. H. Bauer, J. P. Jervey, *Brain Res.* **330**, 299 (1985).
11. M. Corbetta *et al.*, *J. Neurosci.* **11**, 2383 (1991).
12. P. T. Fox and M. E. Raichle, *Ann. Neurol.* **17**, 303 (1985); C. Price *et al.*, *Neurosci. Lett.* **146**, 179 (1992); C. Price, C. Moore, R. S. J. Frackowiak, *Neuroimage* **3**, 40 (1996).
13. This analogy is purely illustrative. The neural instantiation of phasic modulation might, for example, represent modulation of the spiking frequency, number of spiking neurons, or any number of other effects. Functional imaging reveals the activity in populations of neurons, so the theoretical analysis developed here should not be thought of as making predictions about the spiking behavior of individual neurons.
14. All participants were right-handed males aged 31 to 76 (mean 40.5 years) who gave their informed consent for participation in this study. Isoluminant ellipses subtending ~7° of visual angle were presented at regular intervals (30, 50, 70, and 90 ellipses per minute) for 500 ms at fixation on a computer monitor ~57 cm from the participant. The ellipses could differ in color (red, green, or blue) and orientation (horizontal or vertical). A participant indicated a categorization choice of target or nontarget by pressing a button with either the right or left index finger. A response was required for every ellipse presented. The proportion of targets was kept constant across conditions at 50%; in all other respects the stimuli and presentation were identical in all conditions. The assignment of fingers was made before the scanning session and was the same for all scans for any one participant but counterbalanced across participants for the experiment. The order of task presentation was counterbalanced across participants.
15. PET scans were performed with an ECAT EXACT HR+ scanning system (CTI Siemens, Knoxville, TN) in the three-dimensional (3D) mode with septa retracted. A venous cannula to administer the tracer was inserted in the left antecubital fossa vein. About 350 megabecquerels of $H_2{}^{15}O$ in 3 ml of normal saline was loaded into intravenous tubing and flushed into individuals over 20 s at a rate of 10 ml/min by an automatic pump. After a delay of ~35 s, a rise in counts could be detected in the head that peaked 30 to 40 s later (depending on individual circulation time). The interval between successive $H_2{}^{15}O$ administrations was 8 min. The data were acquired in one 90-s frame, beginning 5 s before the rising phase of the head curve. Correction for attenuation was made with a transmission scan collected at the beginning of each study. Images were reconstructed by filtered back projection (Hanning filter, cut off frequency of 0.5 cycles per pixel) into 63 image planes (separation 2.4 mm) and into a 128 by 128 pixel image matrix (size 2.1 mm). For data analysis we used the general linear model and theory of Gaussian fields, as implemented in SPM96 (Wellcome Department of Cognitive Neurology, London, UK). Images were realigned with the first as a reference, and then stereotactically transformed into the space of Talairach and Tournoux [J. Talairach and P. Tournoux, *Co-Planar Stereotaxic Atlas of the Human Brain* (Thieme, New York, 1988)]. The images were smoothed with a Gaussian filter of 12-mm full-width half maximum. Statistical parametric maps were derived with prespecified contrasts between images as discussed in the text [K. J. Friston *et al.*, *Hum. Brain Mapp.* **2**, 165 (1994); K. J. Friston *et al.*, *ibid.* **3**, 189 (1995)].
16. The reaction time (RT) for correct responses for the color, orientation, and conjunction tasks was 352 ms (standard deviation 86 ms), 350 ms (standard deviation 95 ms), and 368 ms (standard deviation 104 ms), respectively. This significant difference in RT between conjunction and feature tasks is not in accord with Treisman's feature integration theory, if one regards our task as a conjunction search without distractors. However, similar elevated RTs in a conjunction search without distractors have been seen in the only functional imaging study previously reported of conjunction search (*6*). The RT declined with increasing rate of presentation, being 378 ms, 358 ms, 346 ms, and 357 ms for rates of 30, 50, 70, and 90 ellipses per minute, respectively. This may reflect participants finding it easier to stay "on task" at higher rates, keeping relevant behavioral goals more active. Error rates were highest (15.5%) with the color condition; for the orientation condition there were 5.05% errors, and for the conjunction condition 6.98% errors averaged over all participants. There is thus no suggestion from the error data that increased difficulty of the conjunction task might account for our results.
17. Tonic effects were indexed as an increase in baseline activity without an alteration of the correlation between activity and presentation rate. Phasic effects were indexed as a change in the correlation of activity and presentation rate. See Fig. 1 and Table 2.
18. The Z score was 4.22, Talairach coordinates (42 22 40), and $P < 0.001$ uncorrected for multiple comparisons because of our prior hypothesis.
19. B. Seltzer and D. N. Pandya, *J. Comp. Neurol.* **281**, 97 (1989); C. Cavada and P. S. Goldman-Rakic, *ibid.* **287**, 422 (1989); H. Barbas, *ibid.* **276**, 313 (1988); M. Petrides and D. N. Pandya, *ibid.* **273**, 52 (1988); H. Barbas and M.-M. Mesulam, *ibid.* **200**, 407 (1981).
20. For example, M. Sakagami and H. Niki, *Exp. Brain Res.* **97**, 423 (1994); E. B. Pragay *et al.*, *Exp. Neurol.* **96**, 481 (1987).
21. M. Petrides and S. D. Iversen, *Neuropsychologia* **16**, 527 (1978); A. Sahgal *et al.*, *Nature* **257**, 672 (1975).
22. BA 8 in monkey contains the frontal eye fields, and an alternate explanation for our results is that eye movements were responsible for the differences in neural activity between conjunction and feature tasks. However all stimuli were presented in an identical fashion over all tasks at fixation, and there is no reason to suspect differential eye movements in the conjunction condition. Furthermore, in humans the reported coordinates for the frontal eye fields are not located within BA 8 [T. Paus, *Neuropsychologia* **34**, 475 (1996); G. A. O'Driscoll *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 925 (1995); P. T. Fox *et al.*, *Neuro-*

physiology **54**, 349 (1985).
23. J. V. Pardo *et al.*, *Nature* **349**, 61 (1991).
24. R. Vandenberghe, P. Dupont, G. Bormans, L. Mortlemans, G. Orban, *Neuroimage* **2**, 306 (1995).
25. E. K. Miller and R. Desimone, *Science* **263**, 520 (1994); E. K. Miller, L. Li, R. Desimone, *J. Neurosci.* **13**, 1460 (1993); *Science* **254**, 1377 (1991). "Adaptive mnemonic filtering" refers to the reduction in the response from a cell to a stimulus, which has been observed independently of task. However an extrapolation from single-cell data to the evoked hemodynamic response from activity in a population of neurons should be made with caution. Other effects are also seen in inferior temporal cells; for example, an enhancement of response to a stimulus when it matches a searched for target. Our data suggest that the aggregate outcome of activity in several subsets of neurons with different response characteristics is an overall decrease in inferior temporal activity.
26. There are intriguing parallels with single-cell electrophysiology work, where both tonic and phasic signals are seen in inferior temporal cortex cells when monkeys attend to stimuli [(*25*); B. J. Richmond and T. Sato, *Soc. Neurosci. Abstr.* **8**, 812 (1982); *J. Neurophysiol.* **58**, 1292 (1987)]. However, there are important differences with our data; in terms of modulation by attention our data suggest only a tonic modulation of inferior temporal cortical activity. There is no phasic modulation by attention (Fig. 1). Rather, we see a correlation between the rate of presentation of stimuli and inferior temporal cortex activity in addition to the attention-related modulation of this activity.
27. An alternate explanation for the modulatory effect seen in inferior temporal cortex might be that it reflects differences in the stimuli presented. Activity decreases in inferotemporal cortex units in monkey have been observed associated with target identification, and also with repetition of visual items [for example, (*29*)]. The probability of a target was kept constant at 50% in our study, and targets were physically identical in the conjunction condition but shared one feature in the feature tasks; therefore, there were more repetitions of physically identical targets per unit time in the conjunction task. However, if unequal repetition probabilities were to have an effect on our results, they should have led to increased activity with increasing rate of presentation. Such an effect would lead to a change in the slope relating rCBF to presentation rate in conjunction and feature tasks. We found no such evidence; the modulatory effect is tonic and so is task-dependent and stimulus-independent.
28. M. J. Webster, J. Bachevalier, L. G. Ungerleider, *Cereb. Cortex* **4**, 470 (1994); D. N. Pandya *et al.*, *Brain Res.* **31**, 35 (1971); D. N. Pandya and H. G. J. M. Kuypers, *ibid.* **13**, 13 (1969).
29. D. A. Chavis and D. N. Pandya, *Brain Res.* **117**, 369 (1976); S. Jacobson and J. Q. Trojanowski, *ibid.* **132**, 209 (1977); E. G. Jones and T. P. S. Powell, *Brain* **93**, 793 (1970).
30. For example, A. Allport, in *Perspectives on Perception and Action*, H. Heuer and A. F. Sanders, Eds. (Erlbaum, Hillsdale, NJ, 1987).
31. We are grateful to the Wellcome Trust for their support, and to all our colleagues in the Functional Imaging Laboratory for their help in the conduct of this study. We also would like to thank D. Passingham, M. Husain, and our anonymous reviewers for their helpful comments and discussion.

5 August 1996; accepted 5 December 1996

Dopaminergic Neurons Protected from Degeneration by GDNF Gene Therapy

Derek L. Choi-Lundberg, Qing Lin, Yung-Nien Chang, Yawen L. Chiang, Carl M. Hay, Hasan Mohajeri, Beverly L. Davidson, Martha C. Bohn*

Glial cell line–derived neurotrophic factor (GDNF) supports growth and survival of dopaminergic (DA) neurons. A replication-defective adenoviral (Ad) vector encoding human GDNF injected near the rat substantia nigra was found to protect DA neurons from the progressive degeneration induced by the neurotoxin 6-hydroxydopamine (6-OHDA) injected into the striatum. Ad GDNF gene therapy reduced loss of DA neurons approximately threefold 6 weeks after 6-OHDA lesion, as compared with no treatment or injection of Ad lacZ or Ad mGDNF (encoding a biologically inactive deletion mutant GDNF). These results suggest that Ad vector–mediated GDNF gene therapy may slow the DA neuronal cell loss in humans with Parkinson's disease.

Parkinson's disease is characterized by the progressive loss of DA neurons in the substantia nigra (SN) that project to the striatum. Current therapies do not prevent the continuing degeneration of DA neurons. GDNF, a neurotrophic factor for DA neurons (*1*), protects DA neurons in several rodent and primate models of Parkinson's disease when administered to the adult nigrostriatal system (*2*). Although these studies elucidated GDNF as a therapeutic molecule for limiting the neuronal damage caused by Parkinson's disease, single, repeated, or continuous infusions of recombinant GDNF protein in microgram quantities directly into brain parenchyma or cerebrospinal fluid were used. Continuous targeted delivery of neurotrophic factors to specific neurons in the central nervous system (CNS) in amounts that are therapeutic, but not deleterious to other cells, is a challenge that remains to be met. In vivo gene therapy has the potential to meet this challenge by delivering neurotrophic factors continuously to a focal brain area. In this study we delivered GDNF via an adenoviral (Ad) vector in a progressive degeneration rat model of Parkinson's disease.

Ad vectors were constructed for human GDNF, for a mutant form of GDNF (mGDNF) with 12 amino acids deleted, and for nuclear-localizing lacZ encoding β-galactosidase (β-Gal) (*3*). The GDNF vectors were tested in vitro for bioactivity. PC12 cells (*4*) were infected with 300 to 1000 plaque-forming units (PFU) of Ad GDNF or Ad mGDNF per cell or were mock-infected. Five days later, 24-hour conditioned medium (CM) was analyzed by enzyme-linked immunosorbent assay (ELISA) (*5*); 0.5 to 3.2 ng of human GDNF was secreted per 10^4 infected cells per day, as compared to 0 to 0.2 ng from Ad mGDNF– or mock-infected cells. DA bioactivity conferred by the vectors was assessed with embryonic day 14 (E14) ventral mesencephalon cultures, as described previously (*6*). Cultures were either maintained on 50% CM from PC12 cells infected with Ad vectors or were directly infected with 10 PFU per cell for 2 hours. Seven days later, cultures were stained for tyrosine hydroxylase immunoreactivity (TH-IR) to identify DA neurons. Ad GDNF led to a 65 to 84% increase in TH-IR neuron number, whereas Ad mGDNF did not improve survival (Fig. 1). These results confirmed that bioactive

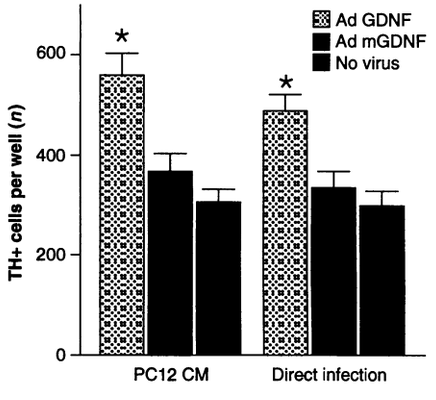

Fig. 1. Bioactive GDNF is produced after infection with Ad GDNF. The number of TH-IR (DA) neurons was counted in E14 ventral mesencephalon cultures maintained on 50% PC12 CM or directly infected with Ad GDNF or Ad mGDNF or mock-infected (mean ± SEM, $n = 6$). Ad GDNF increased survival versus Ad mGDNF and no virus in both groups [analysis of variance (ANOVA), $F = 10.49$, $P < 0.001$; asterisk indicates Tukey's post hoc pairwise comparisons at a family error rate of 0.02].

D. L. Choi-Lundberg, Q. Lin, H. Mohajeri, M. C. Bohn, Department of Neurobiology and Anatomy, University of Rochester, Box 603, 601 Elmwood Avenue, Rochester, NY 14642, USA.
Y.-N. Chang, Y. L. Chiang, C. M. Hay, Genetic Therapy, 19 First Field Road, Gaithersburg, MD 20878, USA.
B. L. Davidson, Department of Internal Medicine, University of Iowa College of Medicine, 200 Hawkins Drive, Iowa City, IA 52242, USA.

*To whom correspondence should be addressed. E-mail: mbohn@troi.cc.rochester.edu