2DG infusion (*11, 13*). This result might be expected if cells in the spots receive inputs from neurons with high spontaneous activity (for example, from the LGN) or if these regions had a higher endogenous metabolic activity than surrounding regions. The V2 strips seem to behave similarly in this kind of 2DG experiment. Both macaque and squirrel monkeys that viewed a blank screen or total darkness during 2DG infusion showed a slightly higher uptake of 2DG in the strips of high cytochrome oxidase activity. The idea that regions of high cytochrome oxidase activity in V1 and V2 also have higher metabolic activity is supported by electron microscopic evidence (*28*).

We also find that both the V1 spots and the V2 strips respond similarly (in terms of 2DG uptake) to various visual stimuli. In the macaque, certain visual stimuli produce robust, stimulus-specific 2DG uptake confined to the cytochrome oxidase spots in V1; the same visual stimuli also produce 2DG uptake in V2, confined to the cytochrome oxidase strips (*16*). The visual patterns producing such 2DG results include stimuli that are unoriented, diffuse, and of low spatial frequency (Fig. 3A). The converse is also true: those visual stimuli which produce patterns of high 2DG uptake in regions between the V1 spots also produce patterns of high 2DG uptake extending between the V2 strips. This latter pattern of 2DG uptake is produced by oriented stimuli of high spatial frequency (*16, 17*). Thus, strong circumstantial evidence suggests that the V1 spots and the V2 strips (as well as adjacent areas between the spots and strips) are functionally related to each other.

Oriented visual stimuli of high spatial frequency invariably produce isolated columns of high 2DG uptake in the regions between the V2 strips. Since these isolated V2 columns are not produced by nonoriented or multioriented stimuli of low spatial frequency, they may be analogous to the orientation and spatial frequency columns in V1 (*9, 17*).

Studies of single units have shown that cells in the input layers of V1 are largely monocular, but that the cells in other layers (including those projecting to V2) are largely binocular and disparity-specific (*19, 29*). The binocular cells in V2 may also be grouped into disparity columns (*19*). Our anatomical evidence is consistent with this model. (i) Although 2DG and cytochrome oxidase evidence exists for a segregation of the monocular inputs into ocular dominance columns in macaque V1 (*8, 10*), we find no such anatomical evidence for ocular dominance columns in V2. (ii) The V2 strips (like the V1 ocular dominance columns) are seen only in the binocular representation of the visual field. These results suggest that the transformation of visual information in V1 and V2 includes a systematic conversion of monocular inputs into a binocular disparity code, presumably as a prerequisite for the neural computation of stereoscopic depth. A columnar arrangement of orientation and spatial frequency sensitivity may facilitate this computation (*30*).

Note added in proof: Livingston and Hubel (*31*) have recently confirmed that the thalamus projects onto the V2 strips.

ROGER B. H. TOOTELL
Department of Psychology, University of California, Berkeley 94720
MARTIN S. SILVERMAN
Department of Physiology, University of California, San Francisco 94143
RUSSELL L. DE VALOIS
Department of Psychology, University of California, Berkeley
GERALD H. JACOBS
Department of Psychology, University of California, Santa Barbara 93106

References and Notes

1. J. M. Allman, J. F. Baker, W. T. Newsome, S. T. Petersen, in *Cortical Sensory Organization*, C. N. Woolsey, Ed. (Humana, Clifton, N.J., 1981), vol. 2, p. 502.
2. D. C. Van Essen, W. T. Newsome, J. L. Bixby, *J. Neurosci.* **2**, 265 (1982).
3. In some primates, area V2 (as mapped electrophysiologically) is not coextensive with area 18 (as described by Broadman). In the macaque, the anatomical patterns we describe appear coextensive with V2 rather than with area 18.
4. R. Gattas, C. G. Gross, J. H. Sandell, *J. Comp. Neurol.* **201**, 519 (1981).
5. J. M. Allman and J. H. Kaas, *Brain Res.* **76**, 247 (1974).
6. D. C. Van Essen, J. H. R. Maunsell, J. L. Bixby, *Soc. Neurosci. Abstr.* **5**, 812 (1979).
7. P. H. Schiller and J. G. Malpeli, *Brain Res.* **126**, 366 (1977).
8. C. Kennedy, M. H. D. Rosiers, O. Sakurda, M. Shinohara, M. Reivich, J. W. Jehle, L. Sokoloff, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4230 (1976).
9. D. H. Hubel, T. N. Wiesel, M. P. Stryker, *J. Comp. Neurol.* **177**, 361 (1978).
10. J. C. Horton and D. H. Hubel, *Nature (London)* **292**, 762 (1981).
11. A. Humphrey and A. Hendrickson, *Soc. Neurosci. Abstr.* **6**, 315 (1980).
12. J. C. Horton and D. H. Hubel, *ibid.*, p. 315.
13. A. L. Humphrey and A. E. Hendrickson, *J. Neurosci.* **3**, 345 (1983).
14. A. E. Hendrickson, S. P. Hunt, J.-Y. Wu, *Nature (London)* **292**, 605 (1981).
15. R. B. H. Tootell, M. S. Silverman, R. L. De Valois, *Invest. Ophthalmol. Visual Sci.* **19** (Suppl.), 226A (1980).
16. R. Tootell and M. Silverman, *Soc. Neurosci. Abstr.* **7**, 356 (1981).
17. R. B. H. Tootell, M. S. Silverman, G. Switkes, R. L. De Valois, *ibid.* **8**, 707 (1982).
18. D. H. Hubel and T. N. Wiesel, *J. Comp. Neurol.* **158**, 267 (1974).
19. ———, *Nature (London)* **225**, 41 (1970); P. G. H. Clarke, I. M. L. Donaldson, D. Whitteridge, *J. Physiol. (London)* **256**, 509 (1976).
20. R. L. De Valois and P. L. Pease, in *Methods in Physiological Psychology*, R. F. Thompson, Ed. (Academic Press, New York, 1973), p. 95; R. B. Tootell, M. S. Silverman, R. L. De Valois, *Science* **214**, 813 (1981).
21. M. Wong-Riley, *Brain Res.* **171**, 11 (1979).
22. J. Horton, thesis, Harvard University, in preparation.
23. D. H. Hubel and M. S. Livingston, *Soc. Neurosci. Abstr.* **7**, 357 (1981).
24. D. Fitzpatrick, K. Itoh, I. T. Diamond, *J. Neurosci.*, in press.
25. A. Cowey, *J. Neurophysiol.* **27**, 366 (1964); J. Tigges, W. B. Spatz, M. Tigges, *J. Comp. Neurol.* **158**, 219 (1974).
26. D. H. Hubel and T. N. Wiesel, *Soc. Neurosci. Abstr.* **4**, 632 (1978).
27. C. A. Curcio and J. K. Harting, *Brain Res.* **143**, 155 (1978).
28. E. Carroll and M. Wong-Riley, *Soc. Neurosci. Abstr.* **8**, 811 (1982); M. Wong-Riley and E. Carroll, *ibid.*, p. 706.
29. J. D. Pettigrew, T. Nikara, P. O. Bishop, *Exp. Brain. Res.* **6**, 391 (1968); H. B. Barlow, C. Blakemore, J. D. Pettigrew, *J. Physiol. (London)* **193**, 327 (1967); D. Ferster, *ibid.* **311**, 623 (1981); G. F. Poggio and B. Fischer, *J. Neurophysiol.* **40**, 1392 (1977).
30. D. Marr and T. Poggio, *Proc. R. Soc. London Ser. B* **204**, 301 (1979); J. P. Frisby and J. E. W. Mayhew, *Philos. Trans. R. Soc. London Ser. B* **290**, 95 (1980).
31. M. S. Livingston and D. H. Hubel, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6098 (1982).
32. Supported by PHS grants EY-00014 and EY-02052 and by NSF grant BNS 78-86171. We thank L. Thorell, J. Horton, and G. Switkes for incisive comments on the manuscript and G. Switkes for technical assistance.

2 August 1982; revised 2 December 1982

Lymphoid Cell–Glioma Cell Interaction Enhances Cell Coat Production by Human Gliomas: Novel Suppressor Mechanism

Abstract. *Certain human glioma lines produce mucopolysaccharide coats that impair the generation of cytolytic lymphocytes in response to these lines in vitro. Coat production is substantially enhanced by the interaction of glioma cells with a macromolecular factor released by human peripheral blood mononuclear cells in culture. This interaction thus constitutes an unusual mechanism by which inflammatory cells may nonspecifically suppress the cellular immune response to at least one class of solid tumors in humans.*

Although it is well documented that most glioma patients make humoral immune responses to their tumors (*1, 2*), there is little evidence of significant cellular immune responses (*3–6*). We studied the generation of allogenic cytolytic lymphocytes in response to cultured human glioma cells in vitro in order to identify those properties of glioma cells that enable them to escape cellular immune attack. We found that certain glioma lines produce thick coats of mucopolysaccharide that impair the generation of cytolytic lymphocytes specific for

Table 1. Hyaluronidase-induced increase in the generation of effectors specifically cytotoxic for LM and FG gliomas, which possess mucopolysaccharide coats. The enzyme did not enhance the generation of effectors cytotoxic for NN gliomas, which lack such a coat. Cytolytic lymphocytes were generated in MLTC (*6*). In experiment 1 bovine testicular hyaluronidase (Sigma) was used at a final concentration of 10 U/ml; in experiment 2 *Streptomyces* hyaluronidase (Miles) was added (2 U/ml). Effector-to-target ratios were 24 to 1 for gliomas and 40 to 1 for line K562 in experiment 1, and 5 to 1 for gliomas and 7.5 to 1 for line K562 and PHA-activated lymphocyte blasts in experiment 2. Spontaneous ^{51}Cr release in the two experiments varied from 17 to 27 percent for gliomas and from 8 to 17 percent for K562 cells and PHA-activated blasts. Values are means ± standard errors for triplicate determinations. Specificity of effectors was evaluated by determining the ratio of lytic activity in cultures containing glioma stimulators to the lytic activity in equivalent cultures lacking glioma stimulators and comparing these ratios for different targets. Note the reciprocal specificity controls in experiment 2.

Contents of cultures				Percent specific ^{51}Cr release from				
Responder leukocytes	Stimulator leukocytes	Glioma	Hyaluronidase	LM glioma	FG glioma	NN glioma	K562	PHA-activated stimulator blasts
				Experiment 1				
+				1.2 ± 1.5		7.2 ± 1.8	8.7 ± 0.4	
+			+	1.4 ± 2.2		3.5 ± 1.1	7.9 ± 0.3	
+		LM		−1.6 ± 1.0		1.7 ± 2.1	13.3 ± 1.0	
+		LM	+	9.7 ± 0.6		5.3 ± 1.3	19.0 ± 0.9	
+	+			34.2 ± 2.0		20.9 ± 5.2	19.6 ± 1.5	
+	+		+	35.7 ± 4.9		24.3 ± 1.3	21.9 ± 1.4	
+	+	LM		33.9 ± 1.0		23.9 ± 3.2	23.8 ± 0.8	
+	+	LM	+	88.3 ± 5.4		34.4 ± 3.8	27.5 ± 1.9	
				Experiment 2				
+					0.5 ± 1.6	5.8 ± 1.4	3.3 ± 0.5	4.0 ± 1.3
+			+		−0.3 ± 0.6	7.1 ± 0.7	7.0 ± 0.6	9.0 ± 1.3
+		FG			2.2 ± 0.8	2.4 ± 1.3	4.3 ± 0.2	2.8 ± 0.9
+		FG	+		6.1 ± 1.5	4.8 ± 1.1	7.0 ± 0.7	4.0 ± 2.1
+		NN			0.1 ± 1.4	5.7 ± 1.2	9.1 ± 0.8	6.9 ± 0.6
+		NN	+		2.0 ± 0.5	4.7 ± 0.9	12.2 ± 0.2	5.3 ± 0.9
+	+				7.7 ± 1.2	10.6 ± 1.3	49.8 ± 4.5	27.9 ± 2.5
+	+		+		7.1 ± 1.5	8.4 ± 1.3	52.1 ± 1.2	26.3 ± 0.3
+	+	FG			12.2 ± 1.0	10.0 ± 1.5	16.1 ± 1.1	10.3 ± 1.8
+	+	FG	+		20.3 ± 1.1	10.1 ± 2.8	19.4 ± 1.3	10.6 ± 0.8
+	+	NN			3.2 ± 0.8	22.8 ± 1.3	27.6 ± 2.8	13.7 ± 1.2
+	+	NN	+		3.4 ± 0.9	23.5 ± 1.4	28.6 ± 2.6	14.6 ± 1.1

Table 2. Enhancement of LM glioma cell coat formation by a macromolecular factor released by peripheral blood mononuclear cells in MLC's. MLC supernatants were prepared by incubating 4×10^6 responder leukocytes with 4×10^6 irradiated allogeneic stimulator leukocytes in 2-ml wells for 3 days. Similar results were obtained with supernatants harvested after 2 to 6 days. Supernatants from glioma and fibroblast cultures were obtained from confluent cultures after 6 days of incubation. A portion of the MLC supernatant was concentrated eightfold by ultrafiltration through an Amicon PM-10 membrane. The retentate was diluted to the original volume by the addition of medium without serum. The filtrate was reconstituted with 5 percent human AB serum. LM glioma cells (1000 per well) were incubated with culture supernatants or supernatant fractions in the wells of a Costar 3596 microtest plate for 3 days. Erythrocytes (1.5×10^6 per well) were then added and allowed to settle overnight in order to outline the halos around the glioma cells. The areas of the cells and their surrounding coats were measured with Omnicon FAS II image analysis system (Bausch & Lomb). Cell and halo margins were outlined manually with a light pen in an image modification mode on the video monitor. Culture supernatants and supernatant fractions were distributed randomly among the wells of the microtest plate, and the individual outlining the cell and halo margins did not know the distribution until the experiment was completed. The areas of the cells and their halos (if any) were measured with the image-processing system and a Data General Nova 3 computer. The area of each cell was subtracted from the area of the cell and its halo to yield the area of the halo itself. Each area listed is the mean ± standard error for 40 cells.

Source of culture supernatant	Supernatant fraction	Equivalent dilution of original culture supernatant	Pericellular halos around LM glioma cells (square micrometers per cell)
None			517 ± 43
MLC	Unfractionated	1/4	3701 ± 443
MLC	Unfractionated	1/40	1946 ± 286
MLC	Unfractionated	1/400	572 ± 82
MLC	PM-10 retentate	1/4	3821 ± 384
MLC	PM-10 retentate	1/40	1557 ± 207
MLC	PM-10 retentate	1/400	517 ± 37
MLC	PM-10 filtrate	1/4	488 ± 39
MLC	PM-10 filtrate	1/40	455 ± 49
LM glioma	Unfractionated	1/4	620 ± 70
GR glioma	Unfractionated	1/4	327 ± 29
GR skin fibroblasts	Unfractionated	1/4	692 ± 104

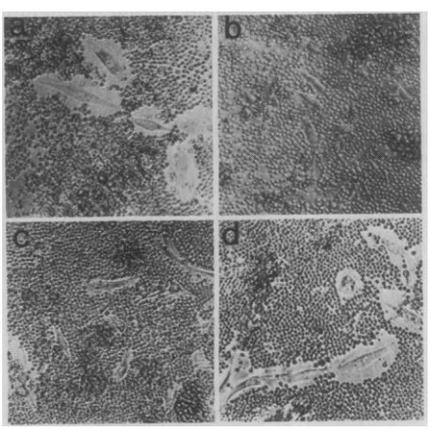

Fig. 1. Phase-contrast photomicrographs of cultures containing LM glioma cells and peripheral blood mononuclear cells (×120). (a) Glioma cells and lymphoid cells were incubated together for 5 days. Lymphoid cells were resuspended and allowed to settle for 14 hours before the photograph was taken. (b) This culture was treated identically to that shown in (a), except that *Streptomyces* hyaluronidase (final concentration, 2 U/ml) was added 14 hours before the photograph was taken. (c) Glioma cells were incubated in the absence of lymphoid cells for 4½ days. Peripheral blood mononuclear cells were then added and the culture was photographed 14 hours later. (d) Glioma cells were incubated for 4½ days in the presence of 20 percent supernatant fluid from an MLC. Blood mononuclear cells were then added and the culture was photographed after 14 hours.

these lines. Production of these coats is stimulated by the interaction of glioma cells with a macromolecular factor released by human peripheral blood mononuclear cells in culture.

Glioma lines were derived from explants of tumor biopsy specimens and characterized by a combination of ultrastructural, biochemical, and biophysical techniques (*7, 8*). The origins and properties of the lines used in these experiments have been reported in detail (*2, 6, 8*). Cytolytic lymphocytes were generated in mixed lymphocyte and tumor cultures (MLTC's) by incubating responder cells with irradiated (10,000 R) allogeneic glioma cells or irradiated (2000 R) stimulator lymphocytes from a third individual or with both (*6*). Lymphoid cells were harvested from MLTC's after 7 to 12 days and their lytic activity was measured in assays of ^{51}Cr release (*6*).

Five of ten glioma lines did not by themselves elicit allogeneic lymphocyte responses, despite the presence of serologically detectable histocompatibility antigens on the surface of the glioma cells (*6*). Although three of the nonstimulatory lines did elicit specific cytolytic lymphocyte responses in the presence of third-party stimulator lymphocytes (*6*), the LM line remained nonstimulatory (Table 1). Microscopic examination of MLTC's containing LM glioma cells revealed that responder lymphocytes were separated from glioma cells by a clear pericellular halo surrounding each glioma cell (Fig. 1a). These halos could be destroyed by hyaluronidase (Fig. 1b). Bovine testicular hyaluronidase, *Streptomyces* hyaluronidase, and chondroitin ABC lyase, at concentrations of 2 U/ml ($\leq$ 10 μg/ml), each destroyed the halos in 45 minutes or less at 37°C. In contrast, treatment for 1.5 hours with trypsin, chymotrypsin, Pronase, plasmin, collagenase, deoxyribonuclease, or ribonuclease (100 μg/ml), or with 20 U of neuraminidase per milliliter, caused no visible change in the halos. Thus the halos appear to be mucopolysaccharide coats. Moreover, their destruction by *Streptomyces* hyaluronidase, an enzyme highly specific in its requirement for hyaluronic acid as a substrate (*9*), suggests that hyaluronic acid is a major constituent of these cell coats.

It seemed reasonable that the cell coats might impair the generation of cytolytic lymphocytes specific for glioma cells by impeding contact between responder lymphocytes and stimulator glioma cells. We previously showed that in MLTC's containing responder and stimulator lymphocytes and glioma cells, two distinct effectors that lyse glioma cells are generated: cytolytic T lymphocytes, which specifically lyse cells of the stimulator glioma line, and non-T effectors, which nonspecifically lyse a variety of targets including glioma cells, fibroblasts, and the erythroleukemia line K562 (*6*). The latter effectors may be identical to "anomalous killers" or "NK-like" cells (*10–12*). The addition of hyaluronidase to MLTC's did not increase the generation of nonspecific effectors, as measured by the lysis of K562 cells, or the generation of specific effectors that lysed phytohemagglutinin (PHA)–activated stimulator lymphocyte blasts. However, the addition of hyaluronidase to MLTC's containing responder and stimulator lymphocytes and LM glioma cells resulted in a strong specific response to the LM glioma (Table 1). Similarly, the addition of hyaluronidase to three-cell MLTC's containing glioma cells of a second coat-producing line, FG, caused a substantial increase in the generation of cytolytic lymphocytes specific for the FG glioma (Table 1). In contrast, hyaluronidase did not affect the specific lymphocyte response to the NN glioma, a line that does not possess a detectable cell coat (Table 1). Thus hyaluronidase enhanced only the generation of cytolytic lymphocytes specific for antigens on glioma lines that possessed cell coats, suggesting that the mechanism of this enhancement was indeed the removal of cell coats by the enzyme.

The hyaluronidase-sensitive cell coats of glioma lines such as LM and FG did not reach maximum thickness until the second or third day of the MLTC. Moreover, this time course was not altered if the glioma cells were incubated for as long as 4 days before the addition of lymphocytes (Fig. 1c). Thus, interaction of the glioma cells with peripheral blood mononuclear cells might be required for the formation of thick cell coats. This was confirmed by the finding that incubation of glioma cells in the absence of blood mononuclear cells but in the presence of supernatants from mixed leukocyte cultures (MLC's) resulted in the formation of large, hyaluronidase-sensitive cell coats by LM glioma cells (Fig. 1d and Table 2). These cell coats could be readily visualized by the addition of erythrocytes, which did not by themselves induce coat formation. Coat area was measured with a Bausch & Lomb image analysis system (Table 2). Supernatants from MLC's, but not from cultures of glioma cells or normal fibroblasts, caused the LM glioma cells to produce substantially larger cell coats. Moreover, the active component in the MLC supernatants was retained on an Amicon PM-10 membrane, which retains molecules greater than 10,000 daltons. Supernatants from cultures of blood mononuclear cells from a single donor were sometimes effective in enhancing coat formation by glioma cells and occasionally displayed activity equal to that of supernatants from MLC's (*13*).

It has been reported that the rate of lysis of human synovial cells (*14*) and mouse fibrosarcoma cells (*15*) by lymphocytes is increased by the removal of mucopolysaccharide cell coats with hyaluronidase. McBride and Bard (*15*) found hyaluronidase-sensitive cell coats on a variety of murine sarcoma and carcinoma lines. They did not examine any cell lines derived from human solid tumors. We have observed hyaluronidase-sensitive cell coats of various thicknesses in seven of ten glioma lines examined to date. The destruction of these coats by hyaluronidase or chondroitin ABC lyase but not by plasmin or other proteases distinguishes them from the fibrin coats observed surrounding some human (*16, 17*) and animal (*18*) tumors. Campbell and Love (*19*) recently found that cultured rabbit fibroblasts produce increased amounts of hyaluronic acid when incubated with supernatants from cultures of antigen-activated rabbit spleen cells. Our finding of increased production of hyaluronic acid–containing cell coats by tumor cells in response to lymphokines or monokines constitutes a novel mechanism by which inflammatory cells nonspecifically suppress the generation of cell-mediated antitumor responses.

STEVEN J. DICK
BEATRICE MACCHI
SAVVAS PAPAZOGLOU
EDWARD H. OLDFIELD
PAUL L. KORNBLITH
BARRY H. SMITH
MAURICE K. GATELY

Surgical Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, Maryland 20205

References and Notes

1. M. Pfreundschuh *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 5122 (1978).
2. H. B. Coakham, P. L. Kornblith, E. A. Quindlen, L. A. Pollock, W. C. Wood, L. C. Hartnett, *J. Natl. Cancer Inst.* **64**, 223 (1980).
3. N. L. Levy, *J. Immunol.* **121**, 903 (1978).
4. R. E. Woosley, M. S. Mahaley, Jr., J. L. Mahaley, G. M. Miller, W. H. Brooks, *J. Neurosurg.* **47**, 871 (1977).
5. K. M. A. Sheikh, M. L. J. Apuzzo, M. H. Weiss, *Cancer Res.* **39**, 1733 (1979).
6. M. K. Gately, M. Glaser, S. J. Dick, R. W. Mettetal, Jr., P. L. Kornblith, *J. Natl. Cancer Inst.* **69**, 1245 (1982).
7. P. L. Kornblith, *Clin. Neurosurg.* **25**, 346 (1978).
8. P. McL. Black, P. L. Kornblith, P. F. Davison, T. M. Liszczak, L. P. Merk, B. H. Smith, P. E. McKeever, E. A. Quindlen, *J. Neurosurg.* **56**, 62 (1982).
9. T. Ohya and Y. Kaneko, *Biochim. Biophys. Acta* **198**, 607 (1970).

10. J. K. Seeley, G. Masucci, A. Poros, E. Klein, S. H. Golub, *J. Immunol.* **123**, 1303 (1979).
11. R. L. H. Bolhuis and H. Schellekens, *Scand. J. Immunol.* **13**, 401 (1981).
12. J. M. Zarling, F. H. Bach, P. C. Kung, *J. Immunol.* **126**, 375 (1981).
13. M. K. Gately, unpublished data.
14. J. R. E. Fraser and B. J. Clarris, *Clin. Exp. Immunol.* **6**, 211 (1970).
15. W. H. McBride and J. B. L. Bard, *J. Exp. Med.* **149**, 507 (1979).
16. R. Hiramoto, J. Bernecky, J. Jurandowski, D. Pressman, *Cancer Res.* **20**, 592 (1960).
17. G. R. Dickersin, E. J. Manseau, A. M. Dvorak, H. F. Dvorak, *Lab. Invest.* **42**, 112 (Abstr.) (1980).
18. H. F. Dvorak, A. M. Dvorak, E. J. Manseau, L. Wiberg, W. H. Churchill, *J. Natl. Cancer Inst.* **62**, 1459 (1979).
19. R. D. Campbell and S. H. Love, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **41**, 557 (Abstr.) (1982).
20. We thank M. Johnson for her excellent technical assistance and M. A. Greenwood for help in taking and processing the photomicrographs.

18 October 1982; revised 30 November 1982

Evidence for Sensory-Selective Set in Young Infants

Abstract. *The existence of low-level filtering of sensory input is a point of debate among cognitive theorists. This present study suggests that filtering by modality exists at levels low enough to modulate the brainstem blink reflex and that it is evident as early as the 16th week of life. During foreground listening or looking conditions, blinks elicited by acoustic or visual probes were larger when probe and foreground modality matched than when they mismatched. "Interesting" foregrounds, by comparison with "dull" ones, intensified the modality-selective effect.*

Reflexes mediated at low levels of the nervous system can be facilitated or inhibited by the neural activity of higher centers (*1*). This fact has recently been exploited in demonstrations that attention directed to selected inputs can modulate the brainstem blink reflex (*2*). The direction of modulation—reflex enhancement when an acoustic reflex-eliciting stimulus was attended and reflex reduction when attention was directed to a tactile or visual stimulus—suggests that selective attending might affect low-level sensory pathways, at least acoustic pathways. These findings were based on studies of human adults; the purpose of this study was to determine whether attentional activity in young infants produces similar reflex modulation. Although attentional or orienting behaviors such as visual fixation and heart rate deceleration can be elicited even in newborn infants (*3*), the functional consequences of the process indexed by these behaviors is not known.

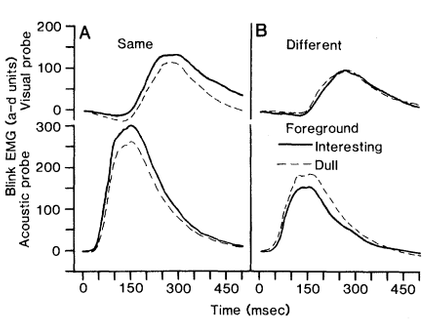

Fig. 1. Averaged evoked EMG activity in analog-to-digital (a-d) units for 500 msec after the onset of visual and acoustic probes for conditions in which modality of probe and concurrent foreground was the same (A) or different (B).

This experiment assessed the magnitude of blink reflexes elicited by visual as well as acoustic probes when probe modality either matched or mismatched the modality of other "foreground" stimulation to which infants were concurrently attending. We also determined whether any effect of modality match was intensified when attention was more strongly engaged, that is, by "interesting" rather than "dull" foregrounds. Heart rate decelerations were measured to verify a difference in attention-engaging quality.

The subjects were 32 medically normal infants, selected at an age (16 weeks ± 9 days) when attentional behaviors have become well established. The infants were divided into two equal groups (*4*). One group was exposed to visual and the other to acoustic foregrounds. The factor of probe modality, also acoustic and visual, and the factor of foreground interest were orthogonally combined to create four conditions varying within groups. Order of conditions was randomized without replacement in four-trial blocks, and each infant could receive as many as eight blocks. Half of the subjects began with block 1 and half with block 5. Because testing had to be discontinued if infants became fussy, the average number of trials completed was 28.3.

Infants were tested in a dimly illuminated and sound-attenuated chamber (Industrial Acoustics). Except for two acoustically shielded flash units (Vivitar), stimulus-generating and recording equipment was located outside the chamber. A PDP12 computer in an adjoining room controlled stimulus presentations, timing, and the digitizing of electromyographic (EMG) activity and heart rate. Present in the chamber were an observer and a parent. The observer rated infant state and visual fixation (*5*) through a peephole in a large projection screen placed 45 cm from the seated parent and infant (*6*). Trials were deleted if the infant was fussy (mean, 2.1) or did not maintain fixation on the center of the screen (mean, 6.5).

To help bring fixations to midline, trials began with 2 seconds of flashing light (200 msec on and off) from a small cold-cathode bulb attached to the screen. Foreground stimulation was then presented for 5 seconds. Visual foregrounds, slides projected on the screen to subtend a visual angle of 40°, were either interesting colored slides—one of 16 smiling faces photographed in natural settings—or dull blank slides—individually matched to the average luminance of a colored slide. As measured by a photometer (Spectra Pritchard), average luminance values ranged from 0.096 to 0.441 mL per slide and from 0.219 to > 0.249 mL per condition (1 L = 3183.099 cd/m^2). Acoustic foregrounds, delivered from a speaker centered behind the screen, were accompanied by one of the blank slides in order to maintain the central fixation. Recorded segments of music-box tunes provided the interesting acoustic foreground, and repetition of a 1000-Hz tone served as the dull foreground. Average sound pressure level re 20 μN/m^2 was 60 dB (scale A).

Four seconds after foreground onset, a blink-eliciting probe was introduced. If visual, it consisted of a < 500-μsec flash from the flash units, reflected from the screen image and having a luminance of approximately 10^3 mL. If acoustic, it was a 50-msec, 109-dB (scale A) burst of broadband noise delivered through

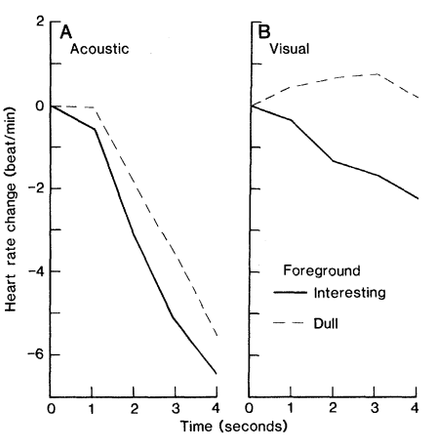

Fig. 2. Heart rate during 4 seconds between foreground and probe onsets, less heart rate during the 1 second preceding foreground onset (mean, 150.9 beats per minute).