nell, *J. Cell Biol.* **95**, 876 (1982); G. Tarone *et al.*, *ibid.* **94**, 179 (1982).
10. C. H. Damsky, K. A. Knudsen, C. A. Buck, *J. Cell. Biochem.* **18**, 1 (1982).
11. N. Oppenheimer-Marks and F. Grinnell, *Exp. Cell Res.* **152**, 467 (1984); J. D. Aplin *et al.*, *ibid.* **134**, 488 (1981).
12. P. A. Harper and R. L. Juliano, *J. Cell Biol.* **87**, 755 (1980).
13. ———, *Nature (London)* **290**, 136 (1981a); *J. Cell Biol.* **91**, 647 (1981); P. A. Harper, P. Brown, R. L. Juliano, *J. Cell Sci.* **63**, 287 (1983).
14. M. A. Schwarz and R. L. Juliano, *Exp. Cell Res.* **153**, 550 (1984).
15. E. G. Hayman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 4003 (1983).
16. E. Engvall and E. Ruoslahti, *Collagen Relat. Res.* **3**, 359 (1983); V. P. Terranova, D. H. Rohrbach, G. R. Martin, *Cell* **22**, 719 (1980).
17. S. F. St. Groth and D. Scheidegger, *J. Immunol. Methods* **35**, 1 (1980).
18. B. B. Mishell and S. M. Shiigi, *Selected Methods in Cellular Immunology* (Freeman, San Francisco, 1980).
19. T. Stachelin *et al.*, *J. Biol. Chem.* **256**, 9750 (1981).
20. R. L. Juliano, *Curr. Top. Membr. Transp.* **11**, 107 (1978).
21. L. A. Lampson, in *Monoclonal Antibodies*, R. Kennett, T. J. McKearn, K. Bechtol, Eds. (Plenum, New York, 1980).
22. M. Bretscher, *Science* **224**, 681 (1984).
23. R. Ehrismann, *J. Biol. Chem.* **257**, 7381 (1982).
24. M. A. Markwell and C. F. Fox, *Biochemistry* **17**, 4807 (1978).
25. B. Oesch and W. Birchmeier, *Cell* **31**, 671 (1982).
26. H. P. Vollmers and W. Birchmeier, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3729 (1983).
27. N. T. Neff *et al.*, *J. Cell Biol.* **95**, 654 (1982); J. M. Greve and D. I. Gottlieb, *J. Cell. Biochem.* **18**, 221 (1982).
28. A. F. Horwitz, C. Decker, R. Greggs, *J. Cell Biol.* **98**, 123a (1983).
29. E. Hasegawa, W. T. Chen, K. M. Yamada, *ibid.* **99**, 165a (1984).
30. K. A. Knudsen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6071 (1981); C. Damsky, D. Wylie, C. Buck, *J. Cell Biol.* **80**, 401 (1979); F. Giancotti, *et al.*, *Exp. Cell Res.* **156**, 182 (1985).
31. M. Schramm and Z. Selinger, *Science* **225**, 1350 (1984).
32. R. L. Juliano and V. Ling, *Biochim. Biophys. Acta* **455**, 152 (1976).
33. Supported by grant PCM8117019 from NSF and NIH GM26165. We thank E. Ruoslahti for providing samples of purified laminin and vitronectin, E. Cheung, M. Schwarz, and P. Kelleher for valuable advice and assistance, and S. Hobbs for assistance.

13 December 1984; accepted 29 March 1985

Morphine-Induced Delay of Normal Cell Death in the Avian Ciliary Ganglion

Abstract. *Repeated administration of morphine in increasing doses delayed normal cell death in the ciliary ganglion of the chick embryo; the effect was completely blocked by naloxone. Survival of spinal motoneurons was not affected. Morphine also inhibited potassium-stimulated synthesis of acetylcholine in ganglion cells cocultured with muscle, suggesting that morphine can influence neurotransmission. Morphine's effect on cell death may be due to an inhibition of transmission at the neuromuscular junction, but opiates may also directly affect cell death. Although it is not known whether the endogenous opiates in the ciliary ganglion influence neuronal survival during embryogenesis, exogenous opiates can affect normal cell death in the autonomic nervous system.*

Neuronal death during development occurs naturally throughout the nervous system (*1*) and can be influenced by muscular activity (*2*), the target, and the trophic support (*3, 4*). In the avian ciliary ganglion, cells die during the period of synapse formation with the target (*5*). Ciliary ganglion cells project to the muscles of the iris, ciliary body, and choroid coat. Normal cell death in this system results in a reduction from 6260 ± 339 cells (mean ± standard error) on embryonic day 8 to 3704 ± 241 cells on embryonic day 14, and can be altered by neuromuscular blockade, by administration of guanosine 3′,5′-monophosphate (cyclic GMP), and by varying the amount of target tissue in the developing chick embryo (*3, 6*).

Erichsen *et al.* (*7*) observed enkephalin-like immunoreactivity in presynaptic terminals in the ciliary ganglion before the onset of cell death. Cultures of ciliary ganglion cells, which lack preganglionic terminals, also show enkephalin-like immunoreactivity (*8*). These findings suggest that opiate peptides may exist both pre- and postganglionically in vivo. Since opiates modulate neurotransmission in many systems (*9*), we examined the possibility that opiates play some role in regulating normal cell death in the ciliary ganglion.

We administered morphine or naloxone or both daily to the vascularized chorioallantoic membrane through a window in the egg's shell on embryonic days 7 to 14. All experimental embryos survived; motility (*10*) was slightly less than in controls (9 versus 14 kicks per minute, respectively). The stages of the experimental and control embryos were determined with the criteria of Hamburger and Hamilton (*11*), including beak length, appearance of feather germs, third toe length, and eyelid morphology. The experimental animals were indistinguishable from the controls at all embryonic ages examined, suggesting that morphine does not have a general effect on growth and development. Some eggs were injected continuously until hatching; these chicks did not differ from the controls in their ability to hatch or their weight at hatching.

The embryos were killed on embryonic day 14 and the ciliary ganglia were fixed in Bouin's and embedded in paraffin. Serial sections (8 μm) were stained with hematoxylin and eosin orange and ganglion cells containing at least one nucleolus were counted at ×400 (Table 1). Daily treatment with 20 μg (*12*) of morphine sulfate (Mallinckrodt) resulted in only a moderate increase in cell survival; daily treatment with a larger dose (200 μg) had no significant effects. In contrast, daily injection of a progressively larger dose of morphine (20 to 200 μg) rescued most of the cells that would have died (total cell number being 5948 ± 345 on day 14), and this effect was reversed by naloxone. Daily treatment with 20 μg of naloxone alone did not significantly affect cell survival when administered during the period of normal cell death.

Table 1. Effect of morphine treatment on cell number (mean ± standard error) in the ciliary ganglion. All raw cell counts were corrected for overcounting (*24*). Values in parentheses give the number of experiments.

Treatment	Embryonic day			1 day after hatching
	8	14	16	
Control	6260 ± 339(9)	3704 ± 241(5)		3315 ± 116(8)
Morphine (20 μg/day)		4996 ± 584(3)*		
Morphine (200 μg/day)		4354 ± 275(3)		
Morphine† (20 to 200 μg/day)		5948 ± 345(3)‡		
Morphine† and naloxone (20 to 200 μg/day)		4199 ± 125(3)		
Morphine§ (20 to 200 μg/day)			4302 ± 509(3)	
Naloxone (20 μg/day)		4098 ± 307(3)		

*Significantly different from corresponding control value [$P < 0.05$, one-tailed Student's t-test with the Bonferroni inequality (*25*)]. †Eggs were injected daily with an increasing dose of morphine as follows: 20, 20, 40, 40, 100, 100, 200 and 200 μg/day. ‡$P < 0.025$. §Eggs were injected daily with the same increasing dose of morphine, followed by 2 days without injection.

It is possible that continuous administration of the high dose of morphine failed to rescue cells significantly because it produced tolerance in the embryo, as has been reported for morphine treatment in myenteric plexus neurons (*13*). Accordingly, the embryo would not be expected to develop tolerance as easily with the lower dose of morphine (*14*). The possibility that the high dose had a cytotoxic effect was investigated in cultured ciliary ganglion neurons. Repeated exposure of these neurons to 100 μM morphine had no effect on their survival.

When morphine treatment was discontinued on day 14, cell death increased toward control levels: after 2 days only 4302 ± 509 cells remained of the 5948 ± 345 cells that were alive on day 14. Therefore morphine in increasing doses appears to delay cell death only until the treatment is discontinued. This effect is similar to the ability of neuromuscular blocking agents to delay cell death in the spinal cord (*15*).

To determine whether morphine can affect transmission between ciliary ganglion neurons and muscle, we used previously characterized cocultures of ganglionic neurons and striated muscle (*16*) in a routine assay for acetylcholine (ACh) synthesis that is very sensitive to alterations in presynaptic function (Fig. 1). Embryonic ciliary ganglion neurons (day 9) were cocultured with striated muscle for 7 days. Incorporation of tritiated choline into ACh during a 30-minute incubation is indicative of neuronal ACh synthesis. This synthesis can be stimulated by a 10-minute preincubation in high potassium, which causes ACh release, decreasing internal stores (*16*). As shown in Fig. 1, addition of micromolar levels of morphine to the preincubation solution blocked the release-induced acceleration of ACh synthesis, and this block was reversed by naloxone. Presynaptic effects of morphine on neurotransmission have been observed (*9*), and recently enkephalin was shown electrophysiologically to decrease transmitter release in cultured avian ciliary ganglion cells (*17*). Thus it is possible that morphine also decreases transmission at the neuromuscular junction by inhibiting presynaptic release of ACh.

Since pharmacological blockade of the neuromuscular junction also rescues ciliary ganglion cells from normal death (*6*), it is tempting to conclude that exogenous opiates act by the same principle. However, neuromuscular blockade has never been shown to rescue the entire neuronal population in the ciliary ganglion, whereas morphine does. If morphine is acting at the ganglionic synapses [and there is good evidence of an inhibitory effect in the rabbit ciliary ganglion (*18*)] as well as at the neuromuscular junction, then a functional inhibition of these synapses might be thought to contribute to the morphine-induced prevention of cell death. However, treatment of the embryos with chlorisondamine, an inhibitor of transmission at ganglionic synapses, increases cell death in the ciliary ganglion (*19*). Thus it is apparent that simple blockade of transmission cannot fully explain morphine's effect on cell death.

We were interested in determining whether these effects are restricted to the ciliary ganglion or whether morphine affects other motoneuron populations, such as those in the chick embryo spinal cord, where cell death has been well documented (*15*). During normal development motoneurons in the lumbosacral lateral motor column (LMC) decrease from about 22,000 on embryonic day 6 to about 13,000 on day 10 (*2, 15*). Morphine was administered in increasing doses as described above, except that the treatment began on embryonic day 6 and continued through day 10 in order to coincide with the period of motoneuron death in the spinal cord. This treatment resulted in the survival of 12,751 ± 728 lumbosacral LMC cells at day 10, similar to the number of control motoneurons at the same embryonic age. Therefore morphine's influence on cell survival does not seem to affect all motoneurons, and may be restricted to cells with endogenous opiates.

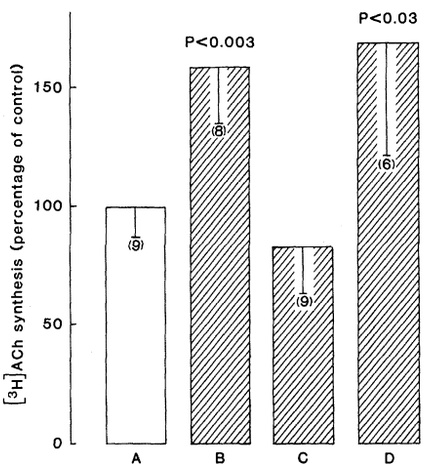

Fig. 1. Effect of morphine on K^+-stimulated [^{3}H]ACh synthesis. Neurons were derived from 9-day chick embryo ciliary ganglia and cocultured for 7 days with 11-day chick embryo pectoral muscle (*16*). Acetylcholine synthesis in neuronal cells was measured by incorporation of [^{3}H]choline (8.4 Ci/mmol) into ACh during a 30-minute incubation at 37°C in normal Tyrode's solution. Values (means ± standard errors) are expressed as percentages of the control synthesis rate per cell (A). Cells represented in (B), (C), and (D) were exposed to Tyrode's containing 55 mM K^+ for 10 minutes before the synthesis assay. In (C) the preincubation solution also contained 5 μM morphine sulfate and in (D) the preincubation solution contained 5 μM morphine and 5 μM naloxone HCl.

Weill and Greene (*20*) reported that addition of cyclic GMP to the egg can delay motoneuron death in the chick embryo spinal cord (*20*). This effect also occurs in the ciliary ganglion (*6*). It is possible that exogenous morphine may regulate neuronal survival by an activity-dependent mechanism or a neurotrophic mechanism mediated by an intracellular second messenger such as cyclic GMP. Opiates elevate cyclic GMP levels in striatal slices and neuroblastoma (*21, 22*).

The insignificant effect on neuronal survival of daily naloxone during the period of synapse formation argues against the possibility that an endogenous peptide regulates cell death. However, the hypothesis is still valid since neither the identity of the pre- or postganglionic opiate peptide nor the pharmacological nature and ontogeny of opiate receptor appearance in the ganglion or at the neuromuscular junction have been determined.

Whether or not endogenous opiates play a role in regulating neuronal cell death, it is clear that morphine can dramatically alter development of the chick autonomic nervous system. This may have teratogenic implications for humans, since infants born to heroin-addicted women can show signs of central and autonomic nervous system dysfunction (*23*).

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References and Notes

1. V. Hamburger, *J. Comp. Neurol.* **160**, 535 (1975); W. M. Cowan, *The Nervous System* (Academic Press, New York, 1973), pp. 19–41; T. J. Cunningham, *Int. Rev. Cytol.* **74**, 1 (1982).
2. R. H. Pittman and R. W. Oppenheim, *Nature (London)* **271**, 364 (1978).
3. C. H. Narayanan and Y. Narayanan, *J. Embryol. Exp. Morphol.* **44**, 53 (1978); W. R. Boydson and G. S. Sohal, *Brain Res.* **178**, 403 (1979).
4. V. Hamburger and J. W. Yip, *J. Neurosci.* **4**, 767 (1984).
5. G. Pilar, L. Landmesser, L. Burstein, *J. Neurophysiol.* **43**, 233 (1980).
6. S. D. Meriney, G. Pilar, R. Nunez, *Soc. Neurosci. Abstr.* **10**, 640 (1984).
7. J. T. Erichsen, H. J. Karten, W. D. Eldred, N. C. Brecha, *J. Neurosci.* **2**, 994 (1982).
8. G. Crean, M. Ogawa, G. Pilar, *Soc. Neurosci. Abstr.* **10**, 429 (1984).
9. A. H. Mulder, G. Wardeh, F. Hogenboom, A. L. Frankhuyzen, *Nature (London)* **308**, 278 (1984); J. L. Bixby and N. C. Spitzer, *ibid.* **301**, 431 (1983); D. M. Michaelson, G. McDowall, Y. Sarne, *Brain Res.* **305**, 173 (1984); S. Konishi, A. Tsunoo, M. Otsuka, *Nature (London)* **294**, 80 (1981).
10. Motility was determined by counting the number of rapid, discrete flexion and extension

motions of the embryo's hind limb during periods when the shell window was removed for injections.
11. V. Hamburger and H. Hamilton, *J. Morphol.* **88**, 49 (1951).
12. In the absence of information on drug absorption, diffusion, or metabolism, a dose of 20 μg per egg was assumed to reach an effective concentration of 1 μ*M*, given simple dilution of the drug into an egg with a volume of 25 ml. The highest dose administered, 200 μg per egg, was thus assumed to reach a concentration of 10 μ*M*.
13. P. J. Karras and R. A. North, *J. Pharmacol. Exp. Ther.* **217**, 70 (1981).
14. L. S. Goodman and A. Gilman, *The Pharmacological Basis of Therapeutics* (Macmillan, London, 1970), p. 23.
15. R. Pittman and R. Oppenheim, *J. Comp. Neurol.* **187**, 425 (1979).
16. J. B. Tuttle, K. Vaca, G. Pilar, *Dev. Biol.* **97**, 255 (1983).
17. J. F. Margiotta and D. K. Berg, *Soc. Neurosci. Abstr.* **10**, 805 (1984).
18. Y. Katayama and S. Nishi, *J. Physiol. (London)* **351**, 111 (1984).
19. L. Wright, *Dev. Brain Res.* **1**, 283 (1981).
20. C. Weill and D. P. Greene, *Nature (London)* **308**, 452 (1984).
21. K. Minneman and L. Iversen, *ibid.* **262**, 313 (1976).
22. R. Gullis, J. Traber, B. Hamprecht, *ibid.* **256**, 57 (1975).
23. G. Wilson *et al.*, *Pediatrics* **63**, 135 (1979).
24. M. Abercrombie, *Anat. Rec.* **94**, 239 (1946).
25. G. W. Snedecor and W. G. Cochran, *Statistical Methods* (Iowa State Univ. Press, Ames, 1980), p. 116.
26. We thank L. Landmesser for critically reviewing the manuscript, B. Wautlet for technical assistance, and S. Putnam for assistance in the preparation of the manuscript. Supported by NSF grant BNS 8410581, NIH grants NS 19640 and NS 10338, and the University of Connecticut Research Foundation.

30 November 1984; accepted 29 March 1985

Knowledge Without Awareness: An Autonomic Index of Facial Recognition by Prosopagnosics

Abstract. *Prosopagnosia, the inability to recognize visually the faces of familiar persons who continue to be normally recognized through other sensory channels, is caused by bilateral cerebral lesions involving the visual system. Two patients with prosopagnosia generated frequent and large electrodermal skin conductance responses to faces of persons they had previously known but were now unable to recognize. They did not generate such responses to unfamiliar faces. The results suggest that an early step of the physiological process of recognition is still taking place in these patients, without their awareness but with an autonomic index.*

Patients with prosopagnosia are unable to recognize visually the faces of persons they previously knew or ought to have learned without difficulty. They fail to experience any familiarity with those faces, and, even after they recognize the faces through other cues, such as voices, their physiognomies remain meaningless. Prosopagnosia is due to a complete failure to evoke memories pertinent to specific faces or to a defective evocation that fails to reach awareness. The condition is caused by bilateral damage to mesial occipitotemporal cortices or their connections.

Investigators of prosopagnosia have generally relied on the verbal report of the patient's experience as the sole index of recognition, an approach that does not address potential covert processes of which there may be no subjective awareness. In this study we used the electrodermal skin conductance response (SCR) as a dependent measure and found that two prosopagnosic patients generated significantly larger SCR's and responded more frequently to familiar faces than to unfamiliar ones (*1*). These results indicate that, despite their inability to experience familiarity with the visual stimulus and to provide verbal evidence of recognition, prosopagnosics still carry out some steps of the recognition process for which there is an autonomic index.

The subjects were two female patients with stable prosopagnosia caused by bilateral occipitotemporal damage, as determined from computerized tomography (CT) and nuclear magnetic resonance (NMR) imaging (*2*). We conducted several experiments. In each the patient was shown 50 black-and-white photographs of faces, depicting a full frontal pose on a white background (*3*). Forty-two of the faces were of persons entirely unfamiliar to the patient ("nontarget" faces) and eight were of persons with whom the patient was well acquainted ("target" faces). Both subjects were shown two sets of target faces selected from a period preceding the prosopagnosia (these target faces were randomly interspersed among the nontargets). In one of the sets, "family" faces, the target faces included those of the patient herself, family members, and close friends; in the other set, "famous" faces, the targets were famous politicians and actors. Subject 2 was exposed to a third set of target stimuli, "anterograde" faces, in which the targets were persons with whom the patient had had extensive contact since the onset of her illness but not before (physicians, psychologists, and so forth).

The subjects were given two presentations of each of the two sets of stimuli (or three sets, in the case of subject 2). During the first presentation skin conductance was recorded with Ag-AgCl electrodes from the thenar and hypothenar eminences of the nonpreferred hand on a Beckman type RM Dynograph recorder. Slides were presented for 2 seconds at intervals of 20 to 25 seconds. During the first viewing, no response was required of the subject; during the second, she was asked to verbally rate the familiarity of each face (*4*). Skin conductance was not recorded during the second presentation.

The results are presented in Table 1. As expected on the basis of her pervasive syndrome, subject 1 showed a complete failure to recognize any of the targets in the family and famous faces sets. Yet not only did she produce more frequent and consistent SCR's to the target stimuli, she also generated larger SCR's to the target faces than to the nontargets. The amplitude data were compared by the Mann-Whitney *U* test, a nonparametric test that avoids statistical assumptions not fulfilled by the data sets generated in this study. The average SCR amplitude for the target faces was significantly larger than that observed for the nontargets for both family faces ($U = 241$, $z = 4.01$, $P < 0.001$) and famous faces ($U = 265.5$, $z = 1.80$, $P < 0.05$) (*5*).

Subject 2 also evidenced more frequent and significantly larger SCR's to the target stimuli in the family faces ($U = 362$, $z = 4.63$, $P < 0.001$) and famous faces ($U = 204$, $z = 3.19$, $P < 0.001$) sets (Table 1), but, consistent with her lack of retrograde prosopagnosia, she also recognized accurately the familiar faces in these two sets. In the anterograde faces set, however, in which she was not able to recognize the target faces, she again produced more consistent and significantly larger SCR's to the target faces ($U = 283$, $z = 3.95$, $P < 0.001$). Thus this subject also showed a highly accurate autonomic index of recognition of familiar faces, despite a complete inability to experience familiarity with these faces and to recognize them formally.

The dissociation between the absence of an experience of recognition and the positive electrodermal identification may mean that in these subjects an early step of the physiological process of recognition is still taking place, but that the results of its operation are not made available to consciousness. Dissociations between overt recognition and unconscious discrimination of stimuli have been reported (*6*). Healthy subjects can show accurate autonomic discrimination of certain target stimuli, even when they are presented in a degraded or camou-