cial layers of the superior colliculus and lamina II of the spinal cord and extremely light immunoreactivity was present in the cerebellar molecular layer. Staining of these regions may represent localization of a different antigen with the same antigenic determinant as we found in the limbic system, or may indicate a true functional relation between these and the other classically defined limbic structures. For example, the stain in lamina II is present on cells innervated by afferents concerned with sensations that have a strong emotional component, such as pain and sex.

The density of 2G9 staining varied among areas (Table 1). At the light microscopic level, the immunofluorescence appeared to reside on the surface of neurons and their dendritic processes (Fig. 1C). Glial cells were not immunopositive. Preliminary electron microscopic immunocytochemical data on prefrontal cortex and amygdala confirm the presence of stain on the soma and dendrites of the neurons; thus far, we have not seen immunoreactivity on axons in these regions.

We have identified, therefore, a molecule whose distribution in the vertebrate brain is correlated with the conventional anatomic organization of the limbic system. In addition to its possible functional importance, the antigen may serve as a marker during the assembly of limbic pathways. Only in invertebrates have monoclonal antibodies previously been generated that detect neurons composing a functional system (*12*). Thus, the limbic system antigen is part of an increasingly complex cell-surface molecular organization in the vertebrate CNS. In previous studies three groups of surface molecules have been described: (i) cell class–specific molecules such as those on neuronal or glial cells (*3*); (ii) cell type–specific molecules, which may be present on one type of neuron in many different brain regions (*1*); and (iii) molecules that are nonspecific with respect to cell type, but whose distribution in the CNS may be related to a specific developmental event (*4, 5*). We now propose a fourth class, molecules distributed in a system-specific manner on neurons that are interconnected.

PAT LEVITT

Department of Anatomy,
Medical College of Pennsylvania,
Philadelphia 19129

References and Notes

1. P. Broca, *Rev. Anthropol.* **1**, 385 (1878).
2. J. W. Papez, *Arch. Neurol. Psychiatry* **38**, 725 (1937).
3. C. Lagenaur, M. Schachner, D. Solter, B. Knowles, *Neurosci. Lett.* **31**, 1811 (1982); M. Schachner, in *Neuroimmunology*, J. Brockes, Ed. (Plenum, New York, 1982); R. Mirsky, L. M. B. Wendon, P. Black, C. Stolking, D. Bray, *Brain Res.* **148**, 251 (1978); R. D. McKay and S. J. Hockfield, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6747 (1982); M. Hirn, M. S. Ghandour, H. Deagostin-Bazin, C. Goridis, *Brain Res.* **265**, 87 (1983); C. J. Barnstable, *Nature (London)* **286**, 231 (1980); J. Cohen and S. Y. Selvendran, *ibid.* **291**, 421 (1981); R. Hawkes, E. Niday, A. Matus, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2410 (1982); L. A. Sternberger, L. W. Harwell, N. W. Sternberger, *ibid.*, p. 1326; R. McKay, M. C. Raff, L. F. Reichardt, Eds., *Monoclonal Antibodies to Neural Antigens* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1981).
4. G. D. Trissler, M. D. Schneider, M. Nirenberg, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2145 (1981).
5. J.-P. Thiery, J. L. Duband, U. Rutishauser, G. M. Edelman, *ibid.* **79**, 6737 (1982).
6. G. Galfre, S. C. Howe, C. Milstein, G. W. Butcher, J. C. Howard, *Nature (London)* **266**, 550 (1977); T. J. McKearn, in *Monoclonal Antibodies: Hybridoma, A New Dimension in Biological Analysis*, R. H. Kenneth, T. J. McKearn, K. B. Bechtol, Eds. (Plenum, New York, 1981).
7. Antibody subclass was determined by Ouchterlony double-immunodiffusion with antibodies to mouse immunoglobulin (Miles Laboratories) and by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.
8. We used different fixation protocols to eliminate the possibility of selective regional reduction of antigenicity due to the fixative. Staining patterns were identical with any of the following: 4 percent paraformaldehyde in 0.1*M* sodium phosphate (*p*H 7.2); paraformaldehyde-lysine-periodate (PLP) fixative [I. W. McLean and P. K. Nakane, *J. Histochem. Cytochem.* **22**, 1077 (1974)]; 2 percent paraformaldehyde and 0.1 percent glutaraldehyde in 0.1*M* sodium phosphate buffer (*p*H 7.2); and Bouin's fixative.
9. Incubation with 0.3 percent Triton X-100 eliminated 2G9 staining in all CNS regions.
10. P. D. MacLean, *Electroencephalogr. Clin. Neurophysiol.* **4**, 407 (1952); W. J. H. Nauta and V. B. Domesick, in *The Neural Basis of Behavior*, A. L. Beckman, Ed. (Spectrum, New York, 1982); J. E. Krettek and J. L. Price, *J. Comp. Neurol.* **178**, 225 (1978); A. E. Kelley, V. B. Domesick, W. J. H. Nauta, *Neuroscience* **7**, 615 (1982); R. M. Beckstead, *J. Comp. Neurol.* **184**, 43 (1979); R. T. Robertson, S. S. Kaitz, M. J. Robards, *Neurosci. Lett.* **17**, 161 (1980); J. M. Wyss, L. W. Swanson, W. M. Cowan, *Neuroscience* **4**, 463 (1979); J. N. Riley and R. Y. Moore, *Brain Res. Bull.* **6**, 437 (1981); J. S. Schwaber, B. S. Kapp, G. A. Higgins, P. R. Rapp, *J. Neurosci.* **2**, 1424 (1982); L. W. Swanson and W. M. Cowan, *J. Comp. Neurol.* **172**, 49 (1977); M. Palkovitz and L. Zaborszky, in *Handbook of the Hypothalamus*, vol. 1, *Anatomy of the Hypothalamus*, P. J. Morgane and J. Panksepp, Eds. (Dekker, New York, 1979).
11. Cytoarchitectonic areas of rat cerebral cortex were defined as described by W. J. S. Krieg, *J. Comp. Neurol.* **84**, 221 (1946).
12. B. Zipser and R. McKay, *Nature (London)* **289**, 549 (1981).
13. I thank V. Cooper and A. S. McNeal for excellent technical assistance, S. Sidney for secretarial assistance, and T. Cunningham, M. Goldberger, and M. Murray for critical review of the manuscript. Supported by NIH grant NS 19606, March of Dimes Birth Defects Foundation Basil O'Conner starter grant 5-348, and a National Down Syndrome Scholar Award. P.L. is a Sloan Foundation fellow.

2 August 1983; accepted 10 November 1983

Activation of Pontine Cholinergic Sites Implicated in Unconsciousness Following Cerebral Concussion in the Cat

Abstract. *Low levels of cerebral concussion in the cat produce reversible behavioral suppression presumably associated with unconsciousness. This injury is also associated with increased rates of glucose utilization in regions within the dorsomedial pontine tegmentum. Microinjection of carbachol into these regions produced behavioral suppression resembling that following concussion. These data, together with previously published observations on cholinergic responses to brain injury, suggest that concussive unconsciousness may be attributable in part to activation of cholinergic pontine sites.*

Cerebral concussion is usually defined as a reversible syndrome occurring without detectable pathology and principally characterized by immediate loss of consciousness. The neurophysiological basis of the concussive syndrome is not known. Most investigators have related the consequences of cerebral concussion

Table 1. Local cerebral glucose utilization (LGU) in midbrain regions of uninjured control cats ($N = 4$) and cats subjected to experimental cerebral concussion ($N = 4$). Values of LGU are means (± standard errors) of readings from four to eight sections studied in each cat. All differences between control and injured animals were statistically significant (*t*-tests for independent samples, $\alpha = 0.05$). Regions correspond to those from P1.0 to P2.0 (Fig. 1B).

Area	Glucose utilization (μmole per 100 g per minute)		Change (%)
	Control	Concussion	
Gray matter			
Inferior colliculi	73.9 ± 2.1	56.8 ± 2.0	−23
Ventral central gray	48.6 ± 3.4	33.9 ± 1.7	−30
Central superior nucleus	48.9 ± 4.0	36.7 ± 1.2	−25
Locus coeruleus	38.2 ± 2.6	27.6 ± 1.2	−28
Tegmental reticular nucleus	36.9 ± 2.9	32.2 ± 1.2	−13
Dorsomedial pontine tegmentum	47.6 ± 2.1	57.3 ± 1.7	+20
Nucleus of lateral lemniscus	54.8 ± 3.7	36.9 ± 1.4	−32
White matter			
Pyramidal tract	28.0 ± 2.0	22.3 ± 1.6	−20
Brachium conjunctivum	31.6 ± 3.2	23.5 ± 1.2	−25
Medial longitudinal bundle	32.7 ± 2.3	25.0 ± 1.9	−24
All regions	44.2 ± 3.7	35.2 ± 3.2	−21

to neuronal injury and reduced brain activity (*1*). We now present evidence that at least some components of the behavioral suppression associated with the reversible loss of consciousness following low levels of concussive brain injury may be attributable to the activation of cholinergic pontine sites.

Concussion was produced by a device that produces graded brain injury associated with brief distortion of neural tissue (*2*). The effects of similar magnitudes of injury on cerebral glucose metabolism and behavior were studied in two separate groups of cats. Pontomesencephalic lesions are a significant prognostic feature of closed head injury in humans (*3*). Systematic transections of the brain stem have indicated that functional changes in pontomesencephalic sites are necessary to produce components of spinal reflex suppression following concussion (*4*). Thus, analyses of changes in glucose metabolism focused on this region. Behavioral effects of microinjection of drugs into pontine sites were examined in a third group of cats.

The method of Sokoloff *et al.* (*5*) was used to examine changes in rates of local cerebral glucose utilization (LGU) after concussive injury. [^{14}C]Deoxyglucose (DG) was injected intravenously (75 μCi per kilogram of body weight) 1 hour after injury (N = 4) or completion of surgery (N = 4). Timed plasma samples of DG and glucose were taken to calculate LGU. Forty-five minutes after injections, animals were killed by a barbiturate overdose, and their brains were prepared for autoradiographic analyses by standard DG techniques. Horseradish peroxidase was injected intravenously (50 to 75 mg/kg) 5 minutes before injury to assess the status of the blood-brain barrier and the presence of contusions or hemorrhage (*6*). Alternate serial sections were collected for horseradish peroxidase visualization and hematoxylin and eosin staining. Heart rate, mean arterial blood pressure, arterial blood gases, intracranial pressure, and cortical electroencephalogram (EEG) were continuously monitored. In behavioral experiments, four cats were injured and four were surgically prepared but not injured. Cats were removed from the injury-inducing device, surgical wounds were sutured, and behavioral assessments of 5 hours' duration were begun within 60 minutes after injury or completion of surgery. Behavior was assessed at 12-hour intervals for the next 6 days. Behaviors studied included reflex function as well as assessments of muscle tone, locomotion, and oculomotor responses (*7*).

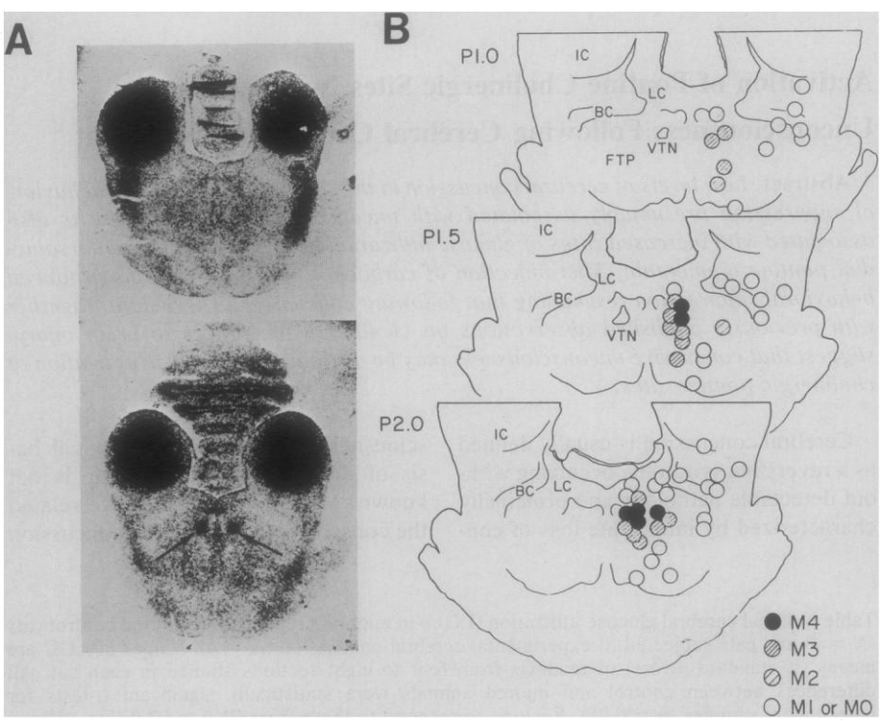

Fig. 1. (A) Photomicrographs of DG autoradiographs from midbrains (P2.0) of an uninjured control cat (top) and a cat subjected to experimental cerebral concussion (bottom). Uptake of DG increased in a region caudal to the ventral tegmental nuclei (*VTN*) in the injured cat (arrows). (B) Schematic representation of the behavioral consequences of microinjection of carbachol into midbrain loci of awake, unanesthetized cats. Behavioral M scores indicate the maximum response elicited by noxious mechanical stimulation: 0, normal responsiveness; 1, ability to orient to and localize stimulation by supporting body weight but noticeable weakness; 2, ability to orient to and localize stimulation with head or limbs but unable to support weight; 3, ability to move including reflexive responses but not to orient to and localize stimulation; and 4, no response to stimulation. Microinjections into regions caudal to and including VTN produced temporary behavioral suppression resembling that seen after concussive injury; during suppression, animals were unresponsive even to intense stimulation. Suppression resulting from microinjections into the regions including and caudal to VTN, corresponding to postconcussion hypermetabolic foci (modal score ≧ M3) was significantly greater than that seen after injections into surrounding zones including the paralemniscal tegmental field (*FTP*) (modal score ≦ M2) [$\chi^2(1) = 6.7$, $P < 0.05$]. Abbreviations: *IC*, inferior colliculi; *LC*, locus coeruleus; and *BC*, brachium conjunctivum.

Behavioral, anatomical, and pharmacological features of bilateral microinjections of drugs into the brain stem were studied in 20 cats. Two guide tubes (26 gauge) were permanently implanted bilaterally into regions encompassing the dorsomedial pontine tegmentum. Cholinergic neurons were diffusely scattered throughout this area (*9*), which includes zones that showed increased LGU after concussive injury. After recovery from surgery, carbachol (0.4 μg per 0.2 μl) was microinjected into 58 brain sites of awake, unanesthetized cats through 33-gauge injection cannulas. Atropine sul-

Concussive brain injury decreased LGU in every area studied (Table 1 and Fig. 1A) except the dorsomedial pontine tegmentum ventromedial to the locus coeruleus. This hypermetabolic zone, which was not associated with histopathology, extended bilaterally from the vicinity of the ventral tegmental nuclei of Gudden and coursed caudally for 0.5 to 1.5 mm. Systemic cardiovascular variables, blood gases, intracranial pressure, and EEG were normal and stable for the 45 minutes after injection of DG. Brain injury temporarily (< 5.0 minutes) abolished corneal and blink reflexes. As reported by others (*8*), concussion temporarily suppressed EEG. Alpha activity was suppressed more than other frequency ranges. Slow-wave activity did not increase after concussion. For as long as 12 hours after injury, resting muscle tone decreased, righting and placing reflexes were disturbed, and thresholds to flexion reflexes increased. Cats were unable to locomote, produce organized escape responses, or track or blink to visual stimuli. Palpebrae were relaxed, nictitating membranes extended, and pupils miotic. No cats showed signs of decorticate or decerebrate posturing. By 48 hours after injury, cats were behaviorally normal. Control animals showed no acute changes in muscle tone or oculomotor or reflex function, and they were behaviorally normal within 12 hours after surgery.

fate (1.2 μg per 0.6 μl, $N = 9$) or mecamylamine hydrochloride (0.4 to 1.2 μg per 0.6 μl, $N = 9$) was also microinjected at the same sites into which carbachol had been injected 8 to 16 minutes earlier. Atropine (0.5 to 1.0 mg/kg, intravenous, $N = 4$) was also administered systemically after carbachol injection. Tetracaine hydrochloride (0.5 μg per 0.5 μl, $N = 4$) or normal saline (0.2 to 2.0 μl, $N = 4$) were microinjected at the same sites at which carbachol produced maximal behavioral effects. Six hours of behavioral assessments and EEG monitoring, as described above, began after completion of injections.

Carbachol injected into areas corresponding to hypermetabolic foci seen in DG studies produced relatively greater behavioral suppression than that produced at other sites (Fig. 1B). This suppression resembled that seen after concussion. Within 8 minutes after injections in these regions, there was a complete loss of muscle tone and abolition of flexion, righting, and placing reflexes. Corneal and blink reflexes were relatively unaffected. Palpebrae were relaxed, nictitating membranes extended, and pupils miotic. Cats were unresponsive to intense stimuli. EEG's were desynchronized. Cats appeared responsive within 30 to 90 minutes after receiving injections. The behavioral effects of carbachol were antagonized by atropine either microinjected or administered systemically. Tetracaine or saline had no behavioral effects.

These data indicate that low levels of concussive injury in the cat are associated with increased LGU in the dorsomedial pontine tegmentum. It is likely that this increased LGU represents a functional activation of those regions (*10*). The behavioral effects of carbachol microinjection also probably resulted from local activation of neurons since no behavioral suppression resulted when tetracaine was microinjected at doses that produce reversible inactivation of other neural systems (*11*). Spontaneous activity of neurons in the dorsomedial pontine tegmentum also increases during cholinergically produced decreases in muscle tone (*12*).

This research demonstrates that sites showing postconcussion increases in LGU were the most effective in producing cholinergically mediated behavioral suppression resembling that following concussion. Other investigators have reported that carbachol microinjected into pontomesencephalic sites can produce a transient behavioral state showing some characteristics of coma (*13*). However, these studies did not relate the behavioral consequences of microinjections to traumatic unconsciousness, nor did they precisely localize the most sensitive injection sites for producing a coma-like state. Cholinergic stimulation of other pontine tegmental regions can also suppress behavior, although not so profoundly. This observation has been discussed in relation to neural processes mediating desynchronized sleep and catalepsy (*14*). Additional anatomical features of these data are incomplete since connections of the hypermetabolic brain regions have not been described (*15*).

Other researchers have reported that concentrations of acetylcholine in the cerebrospinal fluid of experimentally injured animals increase (*16, 17*) and that systemic atropine administration can antagonize the behavioral suppression seen in concussed cats (*17*). Our observations further suggest that components of the feline behavioral response to minor concussive injury are mediated by increased functional activity of pontine cholinergic neurons. However, other processes such as neuronal damage could contribute to certain neurological consequences of head injury, especially to prolonged coma or permanent neurological disturbances following higher magnitudes of brain trauma. Our studies provide no data on neural processes accompanying concussion in humans, although concentrations of acetylcholine in cerebrospinal fluid have been reported to be negatively correlated with the clinical states of patients with head injuries (*18*), and atropine has been reported to abolish or reduce coma after human head injury (*19*).

R. L. HAYES, C. M. PECHURA
Y. KATAYAMA, J. T. POVLISHOCK
M. L. GIEBEL, D. P. BECKER
Division of Neurosurgery, Richard Roland Reynolds Neurosurgical Research Laboratories, Medical College of Virginia, Virginia Commonwealth University, Richmond 23298

References and Notes

1. F. Plum and J. Posner *The Diagnosis of Stupor and Coma* (Davis, Philadelphia, ed. 3, 1980), pp. 11–301; A. Ommaya and T. Gennarelli, *Brain* **97**, 633 (1974).
2. H. Sullivan *et al.*, *J. Neurosurg.* **45**, 520 (1976). Cats (2.5 to 3.0 kg) were anesthetized with sodium methohexital, paralyzed with a continuous infusion of succinylcholine, and artificially ventilated with 70 percent N_2O and 30 percent O_2, and prepared for induction of mechanical brain injury. Nitrous oxide anesthesia does not significantly alter cerebral energy metabolism [R. A. Ratcheson, L. Bilezikjian, J. A. Ferrendelli, *J. Neurochem.* **28**, 223 (1977)]. Some cats were surgically prepared, maintained on N_2O anesthesia, and not injured; others were injured and anesthesia was discontinued 10 minutes later. The technique used to produce the injury also produces pressure transients similar to those recorded in human cadaver skulls during sudden impact and reproduces in animals many clinical features of concussive injury, including temporary behavioral suppression and disturbances in the regulation of cerebral blood flow and intracranial pressure.
3. W. Rosenblum, R. Greenberg, J. Seelig, D. Becker, *Neurosurgery* **9**, 613 (1980).
4. Y. Katayama, D. Becker, R. Hayes, in preparation.
5. L. Sokoloff, M. Reivitch, C. Kennedy, M. DesRosiers, C. Patlah, K. Pettigreu, O. Sakurada, M. Shinohara, *J. Neurochem.* **8**, 897 (1977). The lumped constant value for the cat was 0.411. Analyses of autoradiographs and calculation of LGU were done with a computerized image-processing system [C. Goochee, W. Rasband, L. Sokoloff, *Ann. Neurol.* **7**, 359 (1980)].
6. J. Povlishock, D. Becker, J. Miller, L. Jenkins, W. Dietrick, *Acta Neuropathol.* **47**, 1 (1979).
7. Muscle tone was assessed as normal, hypotonic, or atonic. Locomotion was scored with a modified version of the Tarlov scale [I. Tarlov, *Spinal Cord Compression: Mechanisms of Paralysis and Treatment* (Thomas, Springfield, Ill., 1957)]. Pupillary size, palpebral fissure, nictitating membrane extension, corneal and flexion reflexes, and eye tracking and blink to visual stimuli were examined.
8. J. Stockard, R. Bickford, M. Aung, in *Handbook of Clinical Neurology*, P. Vinken and G. Bruyn, Eds. (American Elsevier, New York, 1975), vol. 23, pp. 317–367.
9. H. Kimura, P. McGeer, J. Peng, E. McGeer, *J. Comp. Neurol.* **200**, 151 (1981).
10. Several observations indicate that the assumptions of the DG method were met in these experiments. The technique has been usefully applied to other studies of brain pathologies in which there is no evidence of tissue damage [for example, M. Shinohara *et al.*, *Science* **203**, 188 (1979); L. Sokoloff in (*5*), C. Goochee *et al.* in (*5*)]. Plasma glucose concentrations, although elevated both before and after the injury (means ± standard errors, 168.6 ± 17.9 mg/dl and 260.1 ± 18.7 mg/dl, respectively), were stable and within acceptable limits for the method [L. Sokoloff, in *Ciba Foundation Symposium 56, Cerebral Vascular Smooth Muscle and Its Control* (Elsevier North-Holland, Amsterdam, 1978), pp. 171–197]. Even higher magnitudes of concussive injury in the cat are associated with normal values of regional cerebral blood flow 60 minutes after insult [D. DeWitt *et al.*, *J. Cereb. Blood Flow Metab.* **1** (Suppl. 1), S579 (1981)].
11. H. Proudfit, *Brain Res.* **201**, 459 (1980).
12. K. Hoshino and O. Pompeiano, *Arch. Ital. Biol.* **114**, 244 (1976).
13. H. Peon, *Prog. Brain. Res.* **18**, 96 (1965); B. Baxter, *Exp. Neurol.* **23**, 220 (1969); R. George, W. Haslett, D. Jenden, *Int. J. Neuropharmacol.* **3**, 541 (1964).
14. P. VanDongen, *Exp. Neurol.* **67**, 52 (1980); ———, C. Broekkamp, A. Cools, *Pharmacol. Biochem. Behav.* **8**, 527 (1977); M. Mitler and W. Dement, *Brain Res.* **68**, 335 (1974); T. Amatruda *et al.*, *ibid.* **98**, 501 (1975).
15. T. Briggs and W. Kaelber, *Brain Res.* **29**, 17 (1971). We are addressing this issue [S. Carlton *et al.*, *Soc. Neurosci Abstr.* **8**, 167 (1982)].
16. D. Ruge, *J. Neurosurg.* **11**, 77 (1948).
17. M. Bornstein, *J. Neurophysiol.* **9**, 349 (1946).
18. D. Tower and D. McEachern, *J. Clin. Invest.* **27**, 558 (1948).
19. A. Ward, Jr., *J. Neurosurg.* **7**, 398 (1950); G. Chatrian, L. E. White, Jr., D. Daly, *Electroencephalogr. Clin. Neurophysiol.* **15**, 272 (1963).
20. Supported by NIH grant NS 12587.

18 August 1983; accepted 31 August 1983