If the logarithms of the isoheptane ratios are functions of temperature as suggested above, then some relation between these functions and the rate of thermal cracking should exist. The concentration of light hydrocarbons in petroleum has been suggested as an index to the rate of thermal cracking (*13*). As temperature increases, the rate of thermal cracking should increase exponentially. The Sabine oils show the predicted exponential relation between isoheptane concentration and the postulated temperature functions (Fig. 3).

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15. The distinction in means between the Midland set and the Sabine-like sets very likely reflects a distinction in the types of source rocks that generated the respective sets. This would suggest that the large data set in Fig. 1 is some composite of homologous sets from the various types of source rocks and their mixtures. The similarity in means in the Sabine-like sets probably reflects a common source rock type, possibly the marine shales often found in the Gulf of Mexico. Additional variability in the large database may be attributed to the different forms of compositional alteration.

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Guam Amyotrophic Lateral Sclerosis–Parkinsonism–Dementia Linked to a Plant Excitant Neurotoxin

PETER S. SPENCER, PETER B. NUNN,* JACQUES HUGON,† ALBERT C. LUDOLPH,‡ STEPHEN M. ROSS, DWIJENDRA N. ROY, RICHARD C. ROBERTSON

The decline in the high incidence of amyotrophic lateral sclerosis, parkinsonism, and Alzheimer-type dementia among the Chamorro population of the western Pacific islands of Guam and Rota, coupled with the absence of demonstrable viral and hereditable factors in this disease, suggests the gradual disappearance of an environmental factor selectively associated with this culture. One candidate is seed of the neurotoxic plant *Cycas circinalis* L., a traditional source of food and medicine which has been used less with the Americanization of the Chamorro people after World War II. Macaques were fed the *Cycas* amino acid β-*N*-methylamino-L-alanine, a low-potency convulsant that has excitotoxic activity in mouse brain, which is attenuated by *N*-methyl-D-aspartate receptor antagonists. These animals developed corticomotoneuronal dysfunction, parkinsonian features, and behavioral anomalies, with chromatolytic and degenerative changes of motor neurons in cerebral cortex and spinal cord. In concert with existing epidemiological and animal data, these findings support the hypothesis that cycad exposure plays an important role in the etiology of the Guam disease.

AMYOTROPHIC LATERAL SCLEROSIS (ALS) is a progressive, fatal disorder of adults stemming from degeneration of anterior horn cells in the spinal cord, certain motor nuclei of the brain stem, and neurons in the motor cortex. Elucidation of the etiology of ALS has been sought for over 35 years through intensive longitudinal study of the indigenous (Chamorro) population of the Marianas islands of Guam and Rota, among whom the disease and a parkinsonism-dementia (PD) clinical variant thereof have been remarkably common (*1*). In the 1950s, ALS prevalence ratios and death rates for Chamorro residents of Guam or Rota were 50 to 100 times the estimates for the continental United States and other developed countries (*2*). The decline of ALS after 1955 on Guam (*3*), and the absence of demonstrable inherited (*4*) or viral (*5*) factors in this disease, has led to the search for environmental agents that have been decreasing as the Chamorro population has become Americanized. An early suggestion (*6*) incriminated the highly toxic seed of the false sago palm (*Cycas circinalis* L.), which was used in food and traditional medicine (*7*) until the acculturation of this people after World War II to the contemporary practices of the continental United States led to a decline in cycad use (*8*). Descriptions of a degenerative locomotor disease in animals grazing on cycad species (*9*) fueled interest in the possible etiologic role of this plant in Guam ALS-PD. Laboratory investigation of *C. circinalis* revealed the presence of various glycosides, including cycasin (*10*), and an "unusual" nonprotein amino acid, α-amino-β-methylaminopropionic acid (synonym, β-*N*-methylamino-L-alanine or L-BMAA) (*11*), agents that possessed certain neurotoxic properties but failed to induce an experimental disorder akin to ALS-PD (*12*). Cycad research in relation to Guam ALS-PD was then abandoned (*13*), even though prolonged feeding of "cycasin-free" flour (L-BMAA content unknown) had been noted (*14*) to induce limb muscle atrophy, nonreactive degeneration of anterior horn cells, and degeneration and partial loss of pyramidal neurons of motor cortex in a single rhesus monkey (*Macaca mulatta*). We report here that repeated oral administration of L-BMAA to macaques (*Macaca fascicularis*) produces signs of motor-neuron, extrapyramidal, and behavioral dysfunction, conduction deficits in the central motor pathway, and neuropathological changes of giant Betz cells in motor cortex and of anterior horn cells in spinal cord.

Thirteen 1-year-old male cynomolgus monkeys in six groups received by gavage varying doses of synthetic L-BMAA identical in composition to the natural free amino acid in *C. circinalis* seed (*15*). Treated and control animals were inspected daily, clinically examined weekly, evaluated neurophysiologically before and during the period of treatment (*16*), and subjected to neuro-

Institute of Neurotoxicology, Departments of Neuroscience, Neurology, and Pathology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

*Present addresses: Department of Biochemistry, Kings College London, London, United Kingdom.
†Department of Neurology, University of Limoges, Limoges, France.
‡Department of Neurology, University of Muenster, Muenster, Federal Republic of Germany.

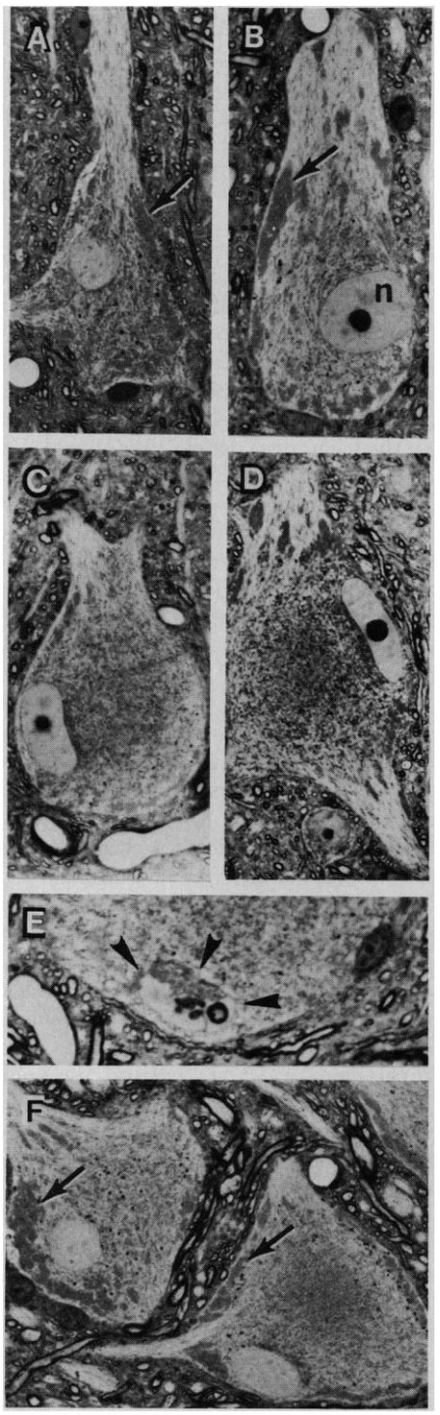

Fig. 1. Motor cortex (A through E) and lumbar spinal cord (F) of *M. fascicularis* (group 4) with prominent signs of L-BMAA–induced motor-system disease. (**A**) Early chromatolysis of pyramid-shaped Betz cell, with partial peripheral displacement of Nissl substance (arrow). (**B**) Later chromatolysis with globular enlargement of perikaryon and apical dendrite (clear spaces correspond to increased neurofilaments) and peripheral displacement of nucleus (n). (**C**) Advanced chromatolysis showing a markedly eccentric nucleus in a globular perikaryon. (**D**) Extreme chromatolysis with increased density of perikaryal cytoplasm (corresponding to numerous mitochondria and multivesicular, vacuolar, and dense bodies). (**E**) Large intranuclear body (arrowheads) in chromatolytic Betz cell perikaryon. (**F**) Severe chromatolysis of a pair of anterior horn cells with partial peripheral displacement of Nissl substance (arrows) (*36*). One-micrometer epoxy sections stained with toluidine blue. Tissue preparation methods as in (*17*). Magnification: ×200 (A through D); ×250 (E and F).

Table 1. Electrophysiological data (mean ± SD) obtained from three animals (group 2) prior to baseline and after 1 month of L-BMAA treatment when clinical signs were apparent. Mean central latencies for the motor pathway regulating hand and (to a lesser extent) foot muscles were abnormally prolonged after administration of L-BMAA; concurrently, conduction velocity of the spinal cord between vertebrae C_7 and L_1 was mildly decreased. Latencies for the whole motor pathway (cortex to abductor pollicis brevis in hand or extensor digitorum brevis in foot) include the peripheral latency (vertebra C_6 to abductor pollicis brevis; L_4 to extensor digitorum brevis) and central latency (whole motor pathway latency minus peripheral latency). Examination of the peripheral and central components of the somatic sensory nervous system was unremarkable, except for a possible decrease in the mean conduction velocity (CV) of the long asecending sensory fibers of the spinal cord between vertebrae L_1 and C_7. The central latency (C_1 to first cortical component) after median nerve stimulation was unchanged. Significance attributed to mean values for treated animals greater than 2 SD from mean baseline values which were comparable with controls (group 7, $n = 5$), data for which are not shown.

Pathway	Baseline (day 0)	L-BMAA for 1 month
Motor pathways		
Cortex/hand (msec)	9.9 ± 0.1	13.8 ± 0.7
Central latency (msec)	4.4 ± 0.1	7.0 ± 0.6
Peripheral latency (msec)	5.5 ± 0.2	6.8 ± 0.2
Cortex/foot (msec)	15.9 ± 0.5	18.2 ± 0.2
Central latency (msec)	9.3 ± 0.5	10.4 ± 1.0
Peripheral latency (msec)	6.6 ± 0.4	7.8 ± 1.0
Sensory pathways		
Median: central latency (msec)	8.1 ± 0.1	8.1 ± 0.3
L_1 to C_7 spinal CV (m/sec)	54.0 ± 3.3	47.7 ± 5.2
Median: wrist to C_7 (m/sec)	77.0 ± 3.0	72.3 ± 4.0
Peroneal: fibula to L_1 (m/sec)	75.7 ± 6.0	73.7 ± 5.2

pathological examination (*17*). Neurological deficits appeared insidiously after 2 to 12 weeks. Signs of motor-neuron dysfunction developed symmetrically or asymmetrically in the extremities. In eight animals, the forelimbs were affected first, with wrist-drop, clumsiness, and difficulty in picking up small objects. Muscle weakness and loss of muscle bulk followed. Six animals displayed unilateral or bilateral extensor hindlimb posturing, with or without leg crossing, a primate sign linked to toxic or traumatic impairment of corticospinal function (*18*). After 1 month of drug administration, all seven animals in groups 2, 3, and 4 exhibited a stooped posture, unkempt coat, tremor in and weakness of upper or all four extremities, and a reduction or loss of characteristic aggressive behavior. Three of these macaques seemed disinterested in their environment and showed changes in their normal diurnal pattern of vigilance (for example, falling asleep during examination). More prolonged treatment (up to 13 weeks) led to periods of immobility with an expressionless face and blank stare, a crouched posture and a bradykinetic, shuffling, bipedal gait performed with the legs flexed and the rump close to the ground. Animals (two of two) treated with an oral antiparkinsonian drug (*19*) showed, within a period of 30 minutes, selective recovery of marked facial muscle movement and spontaneous activity.

Clinical and electrophysiological signs of motor deficit (Table 1) preceded the appearance of concrete structural alterations in the corresponding areas of the central nervous system (CNS). The hierarchy of regional susceptibility appeared to be motor cortex (most affected), spinal cord (less affected), and substantia nigra (mostly unaffected). Light microscope examination of the motor cortex (area 4, arm and leg regions) revealed giant Betz cells (and some smaller pyramidal neurons) undergoing chromatolysis (Fig. 1, A through D). Some Betz cell perikarya and the basal portion of their apical dendrites were swollen with pale-staining material (Fig. 1B) (identified as 10-nm neurofilaments), and a few contained intranuclear inclusion bodies (consisting of altered chromatin) (Fig. 1E) (*20*). Scattered pyramidal neurons were more densely stained, as in chronic cell degeneration (simple atrophy). Large anterior horn cells of the spinal cord were similarly affected to a lesser degree (Fig. 1F), and giant axonal swellings and myelin debris were more frequently encountered in treated animals than controls. After 13 weeks, one animal (group 4) showed isolated neuritic swellings in the pars compacta of the substantia nigra (*20*). Otherwise, the basal ganglia, hippocampus, and cerebellum of all sampled animals were similar to those of controls.

These studies provide clinical, neurophysiological, and neuropathological evidence that L-BMAA induces a primate motor-system disorder with involvement of upper and lower motor neurons, the extrapyramidal system, and possibly other regions regulating various behaviors. Under the dosing conditions used, it is evident that Betz cells in motor cortex are more vulnerable than anterior horn cells in spinal cord, and that the corticomotoneuronal pathway is functionally compromised before the appearance of wrist-drop. The early dominance of the upper-motor-neuron lesion induced by L-BMAA presumably finds clinical expression in the loss of skilled finger movements and in the extensor posturing of the lower limb. Signs of upper-motor-neuron dysfunction are also early and prominent features of Guam ALS or PD (*2*). The motor-neuron deficit in macaques is attended later in the course of L-BMAA administration by L-dopa–sensitive features resembling the extrapyramidal signs reported in animals receiving 1,2,5,6-methylphenyltetrahydropyridine (MPTP) (*21*), an agent that induces nigrostriatal degeneration. The presence of minor pathological changes in the substantia nigra of animals receiving L-BMAA suggests, as in the case of primates receiving low doses of MPTP, that pharmacologically mediated parkinsonism may be expressed without loss of neurons in the pars compacta. Experiments with a more prolonged period of L-BMAA administration will be required to determine if more extensive neuronal damage is the sequel to functional compromise of the extrapyramidal system, as shown for pyramidal neurons of the motor cortex.

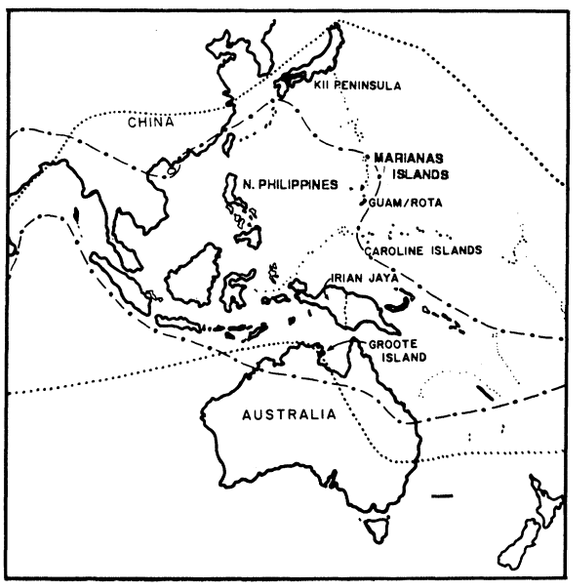

Fig. 2. Distribution of *Cycas* spp. in relation to high-incidence foci of ALS in Guam and Rota, Kii Peninsula (Japan), and Irian Jaya (Indonesia). Area contained within the dotted lines shows earlier distribution [*Encyclopedia Americana* (*30*)]; that within the broken punctuated line is taken from Read and Solt (*30*). Interest in possible foci of ALS has also centered on the North Philippines and Groote Island, Australia (*30*). No cases of ALS-PD have been reported in the Caroline Islands or in the islands east (Marshall and Gilbert Islands) or west (Palau Islands) thereof, although *C. circinalis* was formerly used for food in Palau, Sonsorol (Palau Islands), Yap, Nukuoro (Caroline Islands), Majuro, and Arno (Marshall Islands) [see Fosberg and Sachet (*7*)]. Other *Cycas* species were highly prized for food in Japan in the 18th century (*37*) and, more recently, used in the Ryukyu Islands (South Japan) and Batanes Islands (North Philippines), and by the aborigines of northern Australia [see Whiting (*7*)]. Cycads are common in many parts of New Guinea, and the stem of *C. circinalis* was a source of sago in the Moluccas and Timor (Indonesia). Delayed-onset locomotor dysfunction of cattle, sheep, and horses has been recorded on numerous occasions in northeastern Australia (*9*). Banks (*38*) and Grey (*39*) mention the acute poisonous effects on humans of eating seed of the Australian cycad *Macrozamia* spp., which also contain L-BMAA [see Bell *et al.* (*12*)].

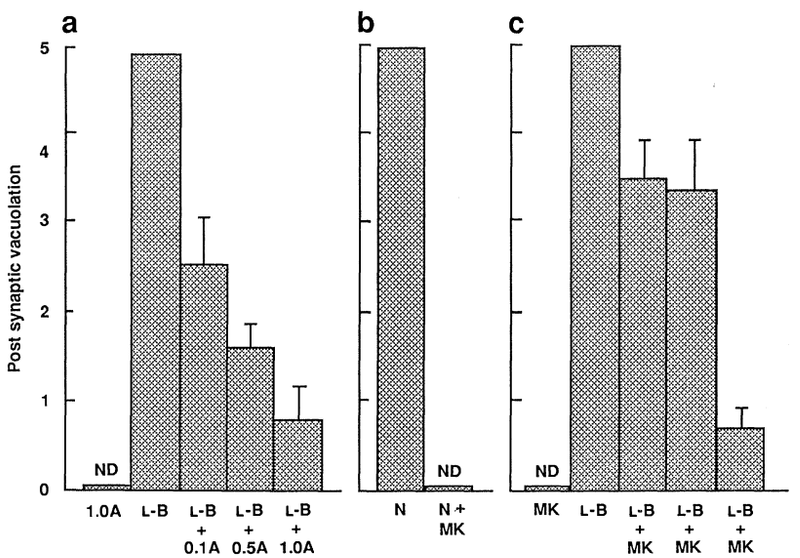

Fig. 3. (**a**) Concentration-dependent inhibition of acute (3.2 m*M*) L-BMAA–induced neuronotoxicity (postsynaptic vacuolation) observed after pretreatment with AP7 (A) (0.1 to 1.0 m*M*). [Preliminary studies with L-BMAA (L-B) demonstrated concentration-dependent increases in postsynaptic vacuolation from 0.16 to 3.2 m*M* (EC_{100} = 1.6 m*M*)]; (**b**) 1.56 m*M* NMDA (N)-induced postsynaptic vacuolation was blocked by pretreatment with MK801 (MK) or AP7 (A) (1 m*M*); (**c**) 1.6 m*M* L-BMAA–induced neuronal changes were attenuated by MK801 in a concentration-dependent manner (0.1 to 10 μ*M*); ND, no vacuolation. Five to ten explants were used to collect data for each concentration point in the L-BMAA, AP7, and NMDA studies, and three to ten explants per data point were utilized in the L-BMAA plus MK801 experiments.

Our demonstration of a primate motor-system toxin in *C. circinalis* suggests that oral and percutaneous exposure to cycad seed in Chamorro food and medicine may play a significant role in the etiology of ALS-PD in the Marianas Islands. This proposal is consistent with (i) the restriction of the disease to Chamorros and others who have shared their culture (*22*), (ii) the reliance by Chamorros of Guam and Rota on cycad seed during World War II (*7, 23*) and the high prevalence of clinical (*2*) and subclinical neurodegenerative disease after the war (*23*), (iii) the historically low incidence of ALS-PD on Saipan where little cycad was available (*24*), (iv) the direct relation between the declining incidence and increased age at onset of Guam ALS and PD, and the disappearing practice of cycad consumption (*8, 25*), and (v) the induction of a degenerative motor-system disease in animals fed components or products of *Cycas* spp. (*9, 14*).

Uncertainties in linking the experimental observation of L-BMAA–induced primate motor-system disease to Guam ALS-PD include (i) the time lapse (up to three decades or more) sometimes observed between exposure to the Chamorro environment of Guam and disease onset (*22, 26*), (ii) the usual occurrence of the disorder in the second half of life (*25*), and (iii) the presence of extrapyramidal and Alzheimer-type neuropathology in the human disease (*27*). However, our primates received relatively large amounts of L-BMAA over a short period of time, the motor disorder evolved with great rapidity, and the neuropathology was studied weeks to months—rather than years to decades—after initial exposure to the neurotoxin and commencement of the pathologi-

cal process. Furthermore, primates were not exposed to other peculiar environmental factors that have been proposed as causally related to Guam ALS-PD (*28*), to the combined effects of toxic damage and decades of age-related attrition of susceptible neurons (*29*), or to other cycad toxins.

Because human neurological disorders comparable to ALS-PD are reported to occur frequently elsewhere in the distribution of *Cycas* spp. (*30*) (Fig. 2), it will be important to determine whether early exposure to "slow toxins" in plants such as cycads played an etiologic role in these conditions. If cycads in isolation or in combination with other factors are responsible for western Pacific ALS-PD, an explanation must be provided for the differential clinical expression of this stereotypic neurodegenerative disorder. In this regard, it is noteworthy that signs of motor-neuron dysfunction developed early after a high dose of L-BMAA, whereas signs suggestive of extrapyramidal compromise and behavioral change surfaced later in animals receiving smaller daily doses of the toxin. Because Guam ALS tends to develop in younger subjects whereas PD generally affects older individuals (*25*), the differential clinical expression of motor-system disease in humans and primates might be related to differences in the rate (or degree) of intoxication.

L-BMAA bears a chemical and neuropharmacological relationship to β-*N*-oxalylamino-L-alanine (L-BOAA), another "unusual" plant-derived nonprotein amino acid that induces corticomotoneuronal (but not extrapyramidal) deficits in primates after repeated oral administration (*31*). L-BOAA [undetectable in *C. circinalis* seed, Bell *et al.* (*12*)] is etiologically implicated in human lathyrism, an upper-motor-neuron disease (spastic paraparesis) caused by heavy consumption of the chickling pea (*Lathyrus sativus*) or a related species (*31*). This amino acid—a potent stereospecific glutamate agonist—shares with L-BMAA the property of eliciting convulsions in rodents (*12, 32*) and inducing the appearance of selective postsynaptic dendritic edema and dark shrunken neurons in exposed regions of the CNS, a pattern of neuropathology associated with excitotoxic amino acids (*32*). Postsynaptic vacuolation also develops rapidly (seconds to minutes) and in a dose-dependent manner in mouse "motor" cortex explants treated with the L-isomers of BMAA (EC_{100} = 1.6 m*M*) or BOAA (EC_{100} = 28 μ*M*), or with other glutamate-receptor agonists including *N*-methyl-D-aspartate (NMDA), quisqualate, and kainate (*33*). In this system, the acute toxic action of L-BOAA is largely mediated by quisqualate or kainate receptors (or both) (*33*), whereas that of L-BMAA is substantially attenuated (but not obliterated) by AP7 or MK801 (Fig. 3), selective antagonists for the NMDA receptor and the associated ion channel, respectively (*34*). Because L-BMAA lacks the characteristic dicarboxylic acid structure of a glutamate analogue, its acute seizuregenic and neuronotoxic properties are probably attributable to a metabolite or to some other indirect mechanism. The receptor species associated with the acute neuronotoxicity of L-BOAA and L-BMAA may also be involved in the motor-system disorders that develop in primates treated with subconvulsive doses of these agents. Results of preliminary dose-finding experiments, in which eight male cynomolgus monkeys received daily, for up to 6 months, L-BMAA (100 to 250 mg/kg by mouth) with or without a prior daily gavage of MK801 (250 to 500 μg/kg), encourage formal studies to test rigorously the possible protective role of this orally active NMDA antagonist (*34*).

The broader importance of our observations lies in the demonstration that two chemically related convulsant amino acids (L-BMAA and L-BOAA) of exogenous origin are linked to human motor-system diseases (Guam ALS-PD [cycadism?] and lathyrism, respectively). Others (*35*) have proposed that motor-system diseases (Huntington's chorea, Parkinson's disease, and olivopontocerebellar atrophy) and Alzheimer's disease may be related to the toxic potential of endogenous excitatory amino acids.

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7. *Cycas* (Greek) *circinalis* (fernlike), L., Sp. Pl. 1188, 1753. Vernacular names: *fadan* or *fadang* (Marianas), *fadane* (Chamorro), *fadang* (Guam), *federico* (Guam, Spanish name). C. Gaudichaud [*Bot. Voy. Uranie* (1829), pp. 432 and 1826] first recorded use of *Cycas* for food among aboriginal inhabitants of Guam [F. R. Fosberg and M.-H. Sachet, in *Flora of Micronesia,* 1. *Gymnospermae* (Smithsonian Institution Press, Washington, DC, 1975), p. 7]. By 1900, cycad consumption was much less common, except when maize was scarce or in times of famine after hurricane [W. E. Safford, *Contrib. U.S. Natl. Herb.* **9**, 1 (1905)]. During the Japanese occupation of Guam (1941 to 1944), when rice was hard to obtain, cycad seed represented a major source of food for indigenous residents of Guam, including Umatac, the village with the highest reported incidence of ALS and PD (*2, 3*). Cycad flour, used to make *tortillas* (bread), *atole* (beverage), and soup, is prepared from the endosperm of ripe (green-brown) seeds; this is removed from the seed integument, halved, quartered, sliced or crushed, soaked for days or weeks in fresh water that is changed periodically (for example, daily) to remove unidentified acutely poisonous substances, dried in the sun, and ground to powder [Bell *et al.* (*12*) stated that BMAA was present in dried meal prepared from seeds]. In addition, the fresh seed integument was used as a chew "to relieve thirst" and, after drying, as a confection. Cycad seed also enjoyed common use as a medicine during World War II: freshly grated cycad seed, or the juice thereof, was applied as a poultice to open wounds, leg ulcers, abscesses, and boils (*6*). In 1875, Don Felipe de la Corte [*Guam Rec.* **3**, 171 (1926)] noted that prolonged use of *fadang* flour makes people very excitable, and M. G. Whiting [*Econ. Bot.* **17**, 271 (1963)] reported that some residents of Guam attributed *lytiko* (incapacitating paralysis) to the handling and consumption of cycad plant material. Improperly washed *Cycas* seed has caused delayed-onset seizures in humans [I. Hirono, H. Kachi, T. Kato, *Acta Pathol. Jpn.* **20**, 327 (1970)].
8. D. M. Reed, D. Labarthe, R. Stallones, *Am. J. Epidemiol.* **92**, 94 (1970). See also Kurland and Molgaard, in *Human Motor Neuron Diseases*, L. P. Rowland, Ed. (Raven, New York, 1982), p. 171 (L. T. Kurland responding to question from P. S. Spencer). Cycad flour products are now considered a specialty favored by middle-aged and elderly traditional Chamorros living in rural communities. Preference for traditional Chamorro food was the only one of 23 selected variables found to be significantly associated with an increased risk for PD [D. M. Reed, D. Labarthe, K. M. Chen, R. Stallones, *Am. J. Epidemiol.* **125**, 92 (1987)].
9. Various genera and species (including *C. circinalis*) are implicated. Acute cycadism in grazing animals is associated with muscle twitching and tremor; delayed effects (after a few weeks) are characterized by paresis or paralysis of the hind limbs, with scantily defined tractal degeneration in the posterior-lateral and ventromedial columns of the spinal cord [M. M. Mason and T. N. Fredrickson, in *Conferences on the Toxicity of Cycads (Fourth)*, M. G. Whiting, Ed. (Department of Health, Education, and Welfare, Washington, DC, 1965), pp. 144–165]. Reports (1880s through 1930s) of cycad-induced locomotor disorders in cattle and sheep are reviewed by M. G. Whiting (*7*). See also W. T. K. Hall and M. D. McGavin, *Pathol. Vet.* **5**, 26 (1968); P. T. Hooper, in *Effects of Poisonous Plants on Livestock*, R. F. Keeler,

K. R. Van Kampen, L. F. James, Eds. (Academic Press, New York, 1978), pp. 337–347; P. T. Hooper, in *Handbook of Natural Toxins*, R. Keeler and A. T. Tu, Eds. (Dekker, New York, 1983), vol. 1, pp. 463–471; P. S. Spencer and D. K. Dastur, in *Neurological Sciences—An Overview of Current Problems*, section VI, *Tropical Neurology and Neurotoxicology*, D. K. Dastur, M. Shahani, E. P. Bharucha, Eds. (Interprint, New Delhi, in press)].

10. G. L. Laqueur, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **23**, 1386 (1964); H. Matsumoto and F. M. Strong, *Arch. Biochem.* **101**, 299 (1963); M. G. Yang *et al.*, *J. Nutr.* **90**, 153 (1966).
11. A. Vega and E. A. Bell, *Phytochemistry* **6**, 759 (1967).
12. Natural or synthetic D,L-BMAA injected intraperitoneally caused generalized convulsions in young Sprague-Dawley rats (onset at 6 to 8 hours after injection) and 2- to 6-month-old mice, and, in 1- to 3-day-old R-X-S–strain chicks, unsteadiness and falling (28 to 136 minutes), head circling and retraction, "running backwards" (40 to 210 minutes), and, at high dosage, violent spasms [E. A. Bell, A. Vega, P. B. Nunn, in "Fifth Conference on Cycad Toxicity" (unpublished transcript of Proceedings, University of Miami, Miami, 24 and 25 April 1967), p. X1-1; F. I. Polsky, P. B. Nunn, E. A. Bell, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **31**, 1473 (1972)]. In laboratory species, cycasin and its aglycone variably caused microcephaly, cerebellar microplasia, retinal abnormalities, and altered levels of hypothalamic and pituitary hormones [I. Hirono, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **31**, 1493 (1972); M. Jones, M. Yang, O. Mickelsen, *ibid.*, p. 1508; A. Hirano and M. Jones, *ibid.*, p. 1517; V. L. Sanger, M. Yang, O. Mickelsen, *ibid.*, p. 1524; Y. Malevski *et al.*, *ibid.*, p. 1530; A. Rabe and R. K. Haddad, *ibid.*, p. 1536].
13. K. Kondo, in *Amyotrophic Lateral Sclerosis*, T. Tsubaki and Y. Toyokura, Eds. (University Park Press, Baltimore, 1979), pp. 61–103; H. Matsumoto, in *CRC Handbook of Naturally Occurring Food Toxicants,* M. Rechcigl, Jr., Ed. (CRC Press, Boca Raton, FL, 1983), pp. 43–61; P. S. Spencer and H. H. Schaumburg, in *Human Motor Neuron Diseases,* L. P. Rowland, Ed. (Raven, New York, 1982), pp. 249–266.
14. D. K. Dastur, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **23**, 1368 (1964).
15. Animals (1.4 to 2.3 kg starting body weight) daily received a freshly prepared aqueous solution (1.5 to 3.0 ml) containing L-BMAA·HCl (166 mg/ml, neutralized with $NaHCO_3$) as follows: 100 mg/kg per day for 12 weeks (group 1, $n = 4$); 250 mg/kg per day for 2 weeks plus 125 mg/kg per day for 5 to 7 weeks (group 2, $n = 4$); 200 mg/kg per day for 6 to 10 weeks (group 3, $n = 2$), plus 250 to 350 mg/kg per day for 7 weeks (group 4, $n = 1$); or 300 to 315 mg/kg per day for 2.5 to 3 weeks (group 5, $n = 3$), plus 200 mg/kg per day for 6 weeks (group 6, $n = 1$). Treated and weight-matched control (group 7, $n = 5$) animals were provided with Purina Monkey Chow (St. Louis, MO) and water ad libitum. Dosed animals that became anorexic received daily a slurry of the same food administered by gavage. Care was in accordance with institutional guidelines. L-BMAA was prepared as described [A. Vega, E. A. Bell, P. B. Nunn, *Phytochemistry* **7**, 1885 (1968)]. Twice recrystallized L-BMAA·HCl was homogeneous electrophoretically (*p*H 3.6) and chromatographically in butanol–acetic acid–water (1:1:1 by volume) after staining with 1% (w/v) ninhydrin in acetone. Melting point was 177°C (with decomposition). Infrared and mass spectrometric analyses were consistent with those of the pure, authentic compound. The specific rotation, $[\alpha]_D^{25}$, in 5*M* HCl was (+) 36.8° (approximately 1% 5*M* HCl). Typical elemental abundances of carbon, hydrogen, nitrogen, and chloride were 30.91, 7.21, 18.25, and 23.59%, respectively (calculated for $C_4H_{11}O_2$·HCl: 31.06, 7.11, 18.12, and 22.97%).
16. Central and peripheral motor and sensory pathways of animals (groups 1 through 7) were examined noninvasively in a temperature-controlled setting by methods used in the neurophysiological examination of human subjects. We used a recently introduced method to examine motor pathways regulating the function of hand and foot after unilateral scalp stimulation of the motor cortex or spinal cord (at spinal vertebrae C_6 or L_1), a procedure that induces no abnormal clinical signs and no detectable pathological damage of underlying (cortical) neurons (A. C. Ludolph, J. Hugon, P. S. Spencer, *Electroencephalogr. Clin. Neurophysiol.*, in press).
17. Deeply anesthetized animals (groups 2 through 7) underwent intraaortic perfusion with 4% paraformaldehyde (20 seconds) followed by 5% glutaraldehyde (10 minutes), each in phosphate buffer (*p*H 7.4). No more than 10 seconds elapsed between opening the thorax and commencing aortic perfusion. Selected regions of brain and spinal cord were excised, immersed in Dalton's 2% chrome osmium solution, and prepared for transmission light and electron microscope examination.
18. S. Gilman, in *Primate Models of Neurological Disorders,* B. S. Meldrum and C. D. Marsden, Eds. (Raven, New York, 1975), pp. 55–76; P. S. Spencer *et al.*, *Lancet* **1986-II**, 1066 (1986).
19. After 6 to 8 weeks of L-BMAA treatment, animals (group 2) received a single oral infusion (1 mg/kg) of L-dopa containing a peripheral decarboxylase inhibitor (Madopar). Guam PD patients treated with L-dopa show a selective improvement in extrapyramidal function [J. A. Schur, T. N. Chase, J. A. Brody, *Neurology* **21**, 1236 (1971)].
20. Ultrastructural study revealed motor neurons with abnormal chromatin patterns (rare), numerous nuclear pores, peripheral (subplasmalemmal) clusters of polysomes and ribosome-studded endoplasmic reticulum, numerous mitochondria and lipofuscin, disorganized skeins of neurofilaments and neurotubules, clumps of electron-dense material, multivesicular bodies, and other single membrane–bound cytoplasmic vacuoles. Isolated nigral neurites of one animal were swollen with "amylaceous" material associated with clusters of abnormal filaments and Hirano-like bodies.
21. J. W. Langston, L. S. Forno, C. S. Rebert, I. Irwin, *Brain Res.* **292**, 390 (1984); R. S. Burns, S. P. Markey, J. M. Phillips, C. C. Chiueh, *Can. J. Neurol. Sci.* **11**, 166 (1984).
22. Twenty Filipinos who had shared the same environment as Guamanians for 15 to 26 years developed ALS and eight showed PD or parkinsonism [R. M. Garruto, D. C. Gajdusek, K.-M. Chen, *Ann. Neurol.* **10**, 341 (1981)]. One ex-Marine who stayed in Guam after World War II developed ALS, and a second was diagnosed as having Alzheimer's disease [see K.-M. Chen and Y. Yase (*2*)]. By contrast, 2 million U.S. Armed Forces veterans who passed through Guam and the northern Marianas showed no increased incidence of ALS or PD [J. A. Brody, A. Hirano, R. M. Scott, *Neurology* **21**, 528 (1971)] nor did some 10,000 U.S. construction workers stationed in Guam from 1945 to 1954 [J. A. Brody, A. H. Edgar, M. M. Gellaspe, *J. Am. Med. Assoc.* **240**, 551 (1979)].
23. Eighty-six percent of Guam ALS patients and 100% of PD patients, as compared with 83% of clinically unaffected controls, responded affirmatively when asked if they had "ever frequently eaten cycad" [see D. M. Reed and J. A. Brody (*3*)]. Sixty-nine Guamanians of Chamorro descent who had no known ALS or PD, and who died of unrelated causes, showed neurofibrillary pathology in cerebral cortex, substantia nigra, and hippocampus, features characteristic of ALS-PD. By contrast, the brains of six Caucasians who had spent many of their adult years on Guam were unremarkable [F. H. Anderson, E. P. Richardson, H. Okazaki, J. A. Brody, *Brain* **102**, 65 (1979)]. Further studies of 302 control brains of Chamorros aged 35 years and older who died of nonneurological causes showed that over 70% had significant numbers of neurofibrillary tangles, and, of these, 15% were indistinguishable from definitive PD cases [see K.-M. Chen and Y. Yase (*2*)].
24. According to M. G. Whiting (*6*), German settlers replaced the cycad forests of Saipan with sugar cane plantations in the early part of this century. Cycads were therefore relatively unavailable on this island, and cycad starch was sent to Saipan Chamorros by relatives living on Guam. ALS-PD prevalence data for Saipan are available [L. T. Kurland and D. W. Mulder, *Neurology* **4**, 355 (1954); R. T. Yanagihara, R. M. Garruto, D. C. Gajdusek, *Ann. Neurol.* **13**, 84 (1983)].
25. Although motor-neuron disease once affected Chamorro subjects as young as 20 years old [see L. T. Kurland and D. W. Mulder (*24*)], during the period 1959 to 1979 the mean age of onset (and mean duration) for Guam ALS changed from approximately 47.6 to 51.9 years (5.5 to 3.4 years) for males and 42.1 to 52.5 years (8 to 3.9) for females. For PD, the corresponding figures are 55.5 to 59.5 years (4.5 to 5.6 years) for males and 50.9 to 58.9 years for females [P. Rodgers-Johnson *et al.*, *Neurology* **36**, 7 (1986)].
26. Some Chamorro migrants (who "occasionally received gifts of dried cycad") developed ALS from 1 to 34 years after leaving Guam. On the basis of the age of migration, the minimum exposure to the Guam environment was the first 18 years of life [R. M. Garruto *et al.* (*2*)].
27. A. Hirano, N. Malamud, L. T. Kurland, *Brain* **84**, 662 (1961); N. Malamud and L. T. Kurland, *Arch. Neurol.* **5**, 401 (1961); see also F. H. Anderson *et al.* (*23*).
28. It has been suggested that unusually low concentrations of calcium, magnesium, and zinc and a relative excess of aluminum in Guam drinking water [but see W. J. Zolan and L. Ellis-Neill, *Technical Report 64*, Water and Energy Research Institute of the Western Pacific (University of Guam, Agaña, 1986)] lead to aberrant mineral metabolism and secondary hyperparathyroidism, with the deposition of aluminum and other elements in vulnerable neurons and the formation of calcium hydroxyapatite in CNS tissues of patients with ALS-PD [Y. Yase, *Lancet* **1972-II**, 292 (1972); in *Amyotrophic Lateral Sclerosis*, T. Tsubaki and Y. Toyokura, Eds. (University of Tokyo Park, Tokyo, 1979), pp. 307–318; *Neurotoxicology* **1**, 101 (1980); D. P. Perl and A. R. Brody, *Science* **208**, 297 (1980); see R. Yanagihara *et al.* (*3*); D. P. Perl, D. C. Gajdusek, R. M. Garruto, R. T. Yanagihara, C. J. Gibbs, Jr., *Science* **217**, 1053 (1982); see R. M. Garruto *et al.* (*3*); R. M. Garruto, C. Swyt, R. Yanagihara, C. E. Fiori, D. C. Gajdusek, *N. Engl. J. Med.* **315**, 711 (1986)]; K.-M. Chen and Y. Yase, Eds., *Amyotrophic Lateral Sclerosis in Asia and Oceania* (National Taiwan University, Taiwan, 1984).
29. D. B. Calne, A. Eisen, E. McGeer, P. S. Spencer, *Lancet* **1986-II**, 1967 (1986).
30. ALS-PD occurs in high incidence in Western Pacific foci other than Guam and Rota, including the Kii Peninsula of Japan [K. Kimura *et al.*, *Dis. Nerv. Syst.* **24**, 155 (1963); H. Shiraki, in *Motor Neuron Diseases, Research of Amyotrophic Lateral Sclerosis and Related Disorders,* F. H. Norris and L. T. Kurland, Eds. (Grune and Stratton, New York, 1969), pp. 80–84; Y. Uebayashi, in *Amyotrophic Lateral Sclerosis in Asia and Oceania*, K.-M. Chen and Y. Yase, Eds. (National Taiwan University, Taiwan, 1984), pp. 43–64] and southern West New Guinea (Irian Jaya), Indonesia [D. C. Gajdusek and A. M. Salazar, *Neurology* **32**, 107 (1982)]. Before 1955, these three foci of ALS-PD fell within the known distribution of living *Cycas* spp. [*Encyclopedia Americana*, vol. 8, p. 356 (1955)], all of which contain L-BMAA [F. I. Polsky, P. B. Nunn, E. A. Bell. *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **31**, 1473 (1972)]. *Cycas* spp. now have a more restricted distribution, which excludes the Kii peninsula [R. W. Read and M. L. Solt, *Lyonia* **2**, 37 (1986)], although *C. revoluta* is grown there as an ornamental and its BMAA-containing seed is used occasionally in Chinese medicine [unpublished observations, North Muro (June 1987)] (Fig. 2).
31. A. Kessler, *Monatsschr. Psychiatr. Neurol.* **113**, 76 (1947); P. S. Spencer, H. H. Schaumburg, D. F. Cohn, P. K. Seth, in *Research Progress in Motor Neurone Disease,* F. C. Rose, Ed. (Pitman, London, 1984), pp. 312–327; see P. S. Spencer *et al.* (*18*); A. C. Ludolph *et al.*, *Brain* **110**, 149 (1987). Certain species of *Lathyrus* contain β-(γ-L-glutamyl)aminopropionitrile, a nonneurotoxic agent that disrupts collagen metabolism and produces multiple osteochondromata in laboratory animals [H. Selye, *Rev. Can. Biol.* **16**, 1 (1957)], an experimental condition known as osteolathyrism that might also accompany human (neuro)lathyrism induced by *L. sativus* [D. F. Cohn, in *Lathyrus and Lathyrism*, A. K. Kaul and D. Combs, Eds. (Third World Medical Research Foundation, New York, 1986), pp. 315–317]. The high incidence of multiple osteochondromata in the Chamorro population [R. S. Krooth, M. T. Macklin, T. H. Hilbish, *Am. J. Hum. Genet.* **13**, 340 (1961)], together with the disorganization of skin collagen in Guam ALS and PD patients [H. M. Fullmer, H. D. Diedler, R. S. Krooth, L. T. Kurland, *Neurology* **10**, 717 (1960)], suggested an etiologic relation between lathyrism and Guam ALS [L. T. Kurland, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **23**, 1337 (1964)]. The evolution of these ideas with respect to the role of cycads and other toxic plants has been chronicled recently [see P. S. Spencer (*6*)].
32. S. L. N. Rao, P. R. Adiga, P. S. Sarma, *Biochemistry* **14**, 5218 (1964); V. V. S. Murti, T. R. Seshadri, T. A. Venkitasubramanian, *Phytochemistry* **3**, 73 (1964); J. C. Watkins, D. R. Curtis, T. J. Biscoe, *Nature (London)* **211**, 637 (1966); J. W. Olney, C. H. Misra, V. Rhee, *ibid.* **264**, 659 (1976); E. A. Bell, *ibid.* **203**, 378 (1964); J. W. Olney, in *Experimental Clinical Neurotoxicology,* P. S. Spencer and H.

H. Schaumburg, Eds. (Williams & Wilkins, Baltimore, 1980), pp. 272–294; S. Pearson and P. B. Nunn, *Brain Res.* **206**, 178 (1981); R. A. Chase, S. Pearson, P. B. Nunn, P. L. Lantos, *Neurosci. Lett.* **55**, 89 (1985). Administration of L-BOAA intracerebroventricularly to neonatal mice elicits a spectrum of time-dependent behavioral states including arm and leg extension (3.5 ± 1.6 minutes, SE), convulsive-jumping fits (6.7 ± 2.5 minutes), and resting tremor and head flip (54.8 ± 22.3 minutes), each of which increases in duration in direct relation to the administered dose (ED_{100} = 50 μg, intracerebroventricularly). Comparable administration of the less potent compound L-BMAA induces a transitory hyperexcitable state (9 ± 6 minutes) followed by a long-lasting whole-body shake and wobble (90 ± 7 minutes) (ED_{100} = 1000 μg intracerebroventricularly) [S. M. Ross and P. S. Spencer, *Synapse* **1**, 248 (1987)].
33. P. S. Spencer, S. M. Crain, M. B. Bornstein, E. R. Peterson, T. Van de Water, *Food Chem. Toxicol.* **24**, 539 (1986); P. B. Nunn, M. Seelig, J. C. Zagoren, P. S. Spencer, *Brain Res.* **410**, 375 (1987); P. S. Spencer *et al.*, in *Selective Neuronal Death,* G. Bock and M. O'Connor, Eds. (Ciba Foundation Symposium, Wiley, Chichester, 1987), pp. 221–238; P. S. Spencer, S. M. Ross, P. B. Nunn, D. N. Roy, M. Seelig, in *Model Systems in Neurotoxicology—Alternative Approaches to Animal Testing,* A. Shahar, Ed. (Liss, New York, in press); S. M. Ross, M. Seelig, P. S. Spencer, *Brain Res.*, in press.
34. Cross sections of motor cortex from 2-day-old Swiss albino mice were explanted onto collagen-coated cover slips, incorporated into Maximow depression-slide assemblies, and maintained in the lying-drop position at 34° to 35°C. Cultures were fed twice weekly with nutrient fluid consisting of Eagle's minimum essential medium. Tissue maintained in this manner matures in 3 weeks. Individual mature explants were exposed for 15 to 30 minutes to L-BMAA or NMDA as positive controls, or to varying concentrations of NMDA glutamate-receptor antagonists (AP7, MK801) followed 15 minutes later by L-BMAA or NMDA [AP7: 2-amino-7-phosphonoheptanoic acid (Cambridge Research Biochemicals, Atlantic Beach, NY); J. C. Watkins and R. H. Evans, *Annu. Rev. Pharmacol. Toxicol.* **21**, 165 (1981); MK801: (+)-5-methyl-10,11-dihydro-5H-dibenzo (α,δ)-cyclohepten-5,10-imine maleate (Merck, Sharp and Dohme Research Laboratories, Harlow, Essex, United Kingdom), a potent and selective NMDA open-channel antagonist that acts in a noncompetitive and agonist-dependent manner, possibly by direct interaction with a phencyclidine recognition site associated with the NMDA receptor. The drug is orally active with ready access to the primate brain. B. V. Clineschmidt, G. E. Martin, P. R. Bunting, *Drug Dev. Res.* **2**, 123 (1982); E. H. F. Wong *et al. Proc. Natl. Acad. Sci. U.S.A.* **83**, 7104 (1986); P. A. Loo, A. F. Braunwalder, M. Williams, M. A. Sills, *Eur. J. Pharmacol.* **135**, 261 (1987)]. Explants were fixed for epoxy embedding, and 1-μm-thick sections were examined under double-blind conditions, two independent investigators separately rating the cultures according to location and severity of (postsynaptic) vacuolation (confirmed by transmission electron microscopy). Degree of vacuolation was scored on a scale of 0 to 5 (no change to severe damage) for each explant. At the end of each study, arithmetic means were obtained from the two ratings of each individual explant and the code was revealed.
35. A. Plaitakis, S. Berl, M. D. Yahr, *Science* **216**, 193 (1982); B. S. Meldrum, *Clin. Sci.* **68**, 113 (1985); R. Schwarz *et al.*, *Life Sci.* **35**, 19 (1984); M. F. Beal *et al.*, *Nature (London)* **321**, 168 (1986).
36. D. L. Price and K. R. Porter, *J. Cell Biol.* **53**, 24 (1972).
37. J. E. Smith, *Trans. Linn. Soc. (London)* **6**, 312 (1802).
38. J. Banks, *Journal of Cook's First Voyage* (1770), pp. 299, 313, and 421.
39. J. Grey, *Journal of Two Expeditions of Discovery in N.W. Australia* (1841), vol. ii, pp. 61 and 295.
40. We thank L. Tedesco, L. Baboukis, M. Fenton, and Y. Kress for technical help; the Third World Medical Research Foundation for manpower assistance; and E. A. Bell, J. Brody, D. Calne, K.-M. Chen, D. Dastur, R. Fosberg, L. L. Iversen, L. T. Kurland, H. Lowndes, M. D. McGavin, J. Morton, M. Ohta, V. Palmer, B. Schoenberg (to whom this report is dedicated), J. Steele, M. G. Whiting, and G. Woodruff for discussion, assistance, and material. Supported by NIH grant NS-19611; the Muscular Dystrophy Association; Merck, Sharpe and Dohme Research Laboratories, United Kingdom; Fondation pour la Recherche Médicale, France (J.H.); Deutsche Forschungsgemeinschaft, West Germany (A.C.L.); and grants from the Wellcome Trust and Royal Society (United Kingdom) (P.B.N.).

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Transient Morphological Features of Identified Ganglion Cells in Living Fetal and Neonatal Retina

ARY S. RAMOA,* GREGOR CAMPBELL, CARLA J. SHATZ*

The function and morphology of retinal ganglion cells in the adult mammalian visual system has been well studied, but little is known about how the adult state is achieved. To address this question, the morphological changes that retinal ganglion cells undergo during development were studied. Ganglion cells were first identified by retrograde labeling with rhodamine latex microspheres deposited in retinorecipient targets in fetal and early postnatal cats. The structure of ganglion cells was then revealed by intracellular injection of Lucifer yellow in living retinas removed and maintained in vitro. As early as 2 weeks before birth, a morphologically diverse assortment of ganglion cells is present, some of which resemble the α, β, and γ classes found in the adult. However, in contrast to the adult, developing ganglion cells exhibit several transient features, including excessive axonal and dendritic branching and exuberant somatic and dendritic spines. These morphological features indicate that there is a transient network of connectivity that could play an important role in the final determination of retinal ganglion cell form and function.

IN THE ADULT MAMMALIAN CENTRAL nervous system there is an enormous diversity of cell types and connections. For example, the retina of the adult cat contains a variety of retinal ganglion cell types that are well characterized on the basis of their form, function, and unique pattern of connections (*1, 2*). At least three distinct classes have been extensively studied and can be distinguished from each other on morphological grounds: the α, β, and γ cells. Alpha cells have the largest cell bodies and long, sparsely branched dendrites; β cells have medium-sized cell bodies and short, extensively branched dendrites; and γ cells, although possibly heterogeneous both morphologically and physiologically, have small cell bodies and long, relatively unbranched dendrites (*1, 2*). Although the exact size and shape of ganglion cells vary with their distance from the central retina, at each retinal locus cells of all three classes can be identified (*1*). What developmental events lead to the establishment of these ganglion cell classes? We have begun to answer this question by studying the morphological development of ganglion cells during fetal and neonatal life to determine if and when morphological remodeling of ganglion cells plays a role in the acquisition of adult morphology.

The morphology of ganglion cells was revealed by intracellular injection of Lucifer yellow in living fetal and neonatal cat retinas maintained in vitro. We studied 187 ganglion cells in 16 retinas from 14 animals between embryonic day 36 (E36) and birth (E65), and between postnatal day 3 (P3) and the adult. Fetal animals were anesthetized via the maternal circulation: mother cats received a mixture of halothane and nitrous oxide [see (*3*) for complete details]. Postnatal animals were anesthetized with Nembutal (intraperitoneal, 30 mg per kilogram of body weight) and were subsequently killed with an overdose of Nembutal. Eyes were removed and immediately placed in cold Ringer solution (*4*). Retinas were dissected free from the pigment epithelium and lens and placed in a tissue-slice chamber mounted on the stage of a compound microscope so that ganglion cells could be injected intracellularly with Lucifer yellow.

Retinal ganglion cells were identified by the retrograde transport of rhodamine-labeled latex microspheres (*5, 6*) that had been injected into the central targets of the retinal projection. To do so in the fetal animals, the head was exteriorized by cesarean section,

Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

*To whom correspondence should be addressed.