compositions in the two groups were very similar; this strongly suggests that the tubercle exudate is the wax material that eventually covers the surface to waterproof the beetle. The type of protein present and the chemical nature of the lipid-protein interaction were not determined. The protein fraction is probably essential for the morphological changes observed at the various humidity extremes, in view of the hydrophobic nature of the hydrocarbons and most of the other components of the lipid fraction.

The adaptive value of the color phases exhibited by *C. verrucosa* in response to humidity is uncertain. The wax filament network that accumulates at low humidity occurs at a time when the potential for transpiration is high. The boundary layer of air between the cuticle surface and the inner surface of the wax filaments, plus the meshwork of filaments itself, will increase the resistance to diffusion of water. A similar accumulation of wax filaments on sorghum leaves is believed to be responsible for this species' tolerance of drought (*6*). Increased diffusion resistance was tested for in *C. verrucosa* by comparing the rates of water loss of blue and black beetles at 40°C and 0 RH over a 6-hour period (*7*). The mean water loss of blue beetles (0.109 ± 0.032 mg cm^{-2} $hour^{-1}$) was slightly lower than that of black beetles (0.140 ± 0.026 mg cm^{-2} $hour^{-1}$) ($P < .01$; Student $t = 4.43$; 18 d.f.). This difference can be attributed largely to evaporation of adsorbed cuticular moisture in black beetles (*8*).

The blue (light) color created by the wax meshwork should also increase reflection of radiation and thus reduce the heat load on individuals directly exposed to sunlight. Namib Desert tenebrionid beetles with white elytra exhibited higher reflectance and lower body temperatures than closely related all-black species (*9*); similar results were obtained when *Eleodes armata* (black) from the Sonoran Desert were compared with individuals with their elytra painted white (*10*). Attempts to experimentally verify a similar relationship in *C. verrucosa* by comparing heating rates of blue and black beetles under artificial radiation were unsuccessful. Evaporation of water from the cuticle of black beetles cooled them sufficiently to keep their heating rates slightly below those of blue beetles in each test run. These results, however, are rather meaningless in view of the nature of the black phase, which is unlikely to occur under actual desert conditions. Blue phase beetles, which are commonly encountered in the field, probably derive some thermoregulatory benefits from increased reflection of radiation, but this is also of questionable significance because of the species' predominantly nocturnal surface activity. In any case, these adaptive benefits are secondary to the low cuticular transpiration rates that result from the production and deposition of surface lipids by this unique wax-secreting system.

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7. Both groups ($N = 10$ in each) were hydrated and placed in a flow-through system during the measurement period.
8. An attempt was made to lessen the effect of evaporation of adsorbed cuticular water on total water loss by placing black beetles in a desiccator for approximately 2 hours before the initial weighing.
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Methylazoxymethanol Treatment of Fetal Rats Results in Abnormally Dense Noradrenergic Innervation of Neocortex

Abstract. *A single injection of methylazoxymethanol in pregnant rats at 15 days of gestation results in severe cortical atrophy in the offspring. In the adult offspring, the neurochemical markers for the cortical γ-aminobutyric acid–containing neurons are severely reduced, whereas the noradrenergic markers are minimally altered. Immunohistofluorescence microscopy demonstrates a marked increase in the density of noradrenergic axons which have an abnormal pattern of distribution in the atrophic cortex. The results suggest that the central noradrenergic neurons determine the number of axons to be formed early in brain development, but local factors in the terminal field regulate the ultimate distribution of the noradrenergic axons.*

Cell divisions of functionally and morphologically distinct neuronal groups in the central nervous system occur in temporally discrete bursts (*1*). When two neuronal types differ significantly with respect to their time of "birth," the selective destruction of one group with sparing of the other may follow brief fetal or neonatal exposure to agents toxic to dividing cells (*2*). After this type of developmentally incurred brain damage, novel synaptic relationships among the remaining neurons probably account for some neurologic and behavioral abnormalities (*3*). In this report, we demonstrate that a single treatment of the rat fetus with methylazoxymethanol, a nucleic acid alkylating agent, results in an abnormally dense noradrenergic innervation of the atrophic neocortex in adulthood.

The cerebral cortex receives widespread innervation from noradrenergic neurons with cell bodies localized in the locus coeruleus. The cells that form the locus coeruleus undergo a period of intense mitotic activity between 11 and 13 days of gestation in the rat, after which cell division ceases; neurochemical and histofluorescence microscopic studies indicate that noradrenergic axons reach the primordial neocortex by 15 days of gestation (*4, 5*). In contrast, the neurons with cell bodies intrinsic to the rat neocortex undergo terminal cell divisions during days 14 to 20 of gestation with the smaller and more superficially located neurons dividing last (*6*).

In the present experiments we destroyed selectively, in fetal rats, the cortical neurons which divide on day 15 of gestation by injecting pregnant dams with a single dose of methylazoxymethanol acetate (MAM), the aglycone of cycasin. This compound is a potent alkylating agent that kills dividing cells by methylating the purine bases of the nucleic acids; the cytotoxic effects of MAM are confined to a brief period from 2 to 24 hours after injection, with maximum effects occurring at 12 hours (*7*). Previous studies have shown that a single injection of MAM to rats during the last week of gestation results in offspring with microcephaly (*8*).

Pregnant Sprague-Dawley rats that were sperm-positive on a definite date were housed in separate cages after 13 days of gestation. On day 15 of gestation, the rats received intraperitoneal injections of either MAM (20 mg/kg, diluted to a concentration of 10 mg/ml in 0.9

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percent NaCl; Aldrich Chemical) or an equivalent volume of diluent. The litters were born on day 22 of gestation and were reduced to ten or fewer pups with runts being discarded. Offspring of the MAM-treated mothers did not differ significantly from controls with respect to size at 7 weeks of age [controls, 153 ± 6 g (mean ± standard error); MAM-treated, 165 ± 5 g; $N = 12$ in each group] or the incidence of external anomalies.

All rats exposed to MAM prenatally had strikingly smaller forebrains at 7 weeks of age which left the colliculi exposed by the deficient cortical expansion. Forebrain weight was reduced to 47 percent of control (MAM-treated, 573 ± 19 mg; control, 1207 ± 16; $N =$ 10 in each group; $P < .001$). In contrast, the appearance and weight of the pons, medulla, and cerebellum were unchanged. The size and weight of unilateral neocortical slabs dissected according to white matter landmarks (*9*) were considerably reduced in the treated animals (MAM-treated, 15 ± 2 mg; control, 45 ± 3 mg; $N = 10$ in each group; $P < .001$). Nissl-stained sections through the lateral neocortex revealed marked thinning and cytoarchitectonic alteration of the cortex with an increased thickness in the apparent layer I, virtual absence of neurons in layers II and III, and some disruption of pyramidal cell orientation in the remaining cell layers.

To assess the effects of MAM treatment on a defined population of neurons in the cerebral cortex, we examined neurochemical markers for the γ-aminobutyric acid (GABA)-containing neurons [glutamate decarboxylase (E.C. 4.1.1.15) and endogenous GABA] intrinsic to the cortex and for the noradrenergic axons [tyrosine hydroxylase (E.C. 1.14.16.2) and endogenous norepi-

Table 1. Female rats were injected with MAM (20 mg/kg body weight) on day 15 of gestation. The neurochemical markers for GABA-containing neurons and noradrenergic axons in lateral neocortex were determined in the offspring when they were 7 to 10 weeks old. Control dams were treated with diluent alone and their litters were reared under identical conditions. Each value (mean ± standard error) was the result of at least five determinations in duplicate. Animals in each experiment were from at least three different litters.

Group	Tyrosine hydroxylase	Norepinephrine	Glutamate decarboxylase	γ-Aminobutyric acid
	Specific activity or concentration			
	(pmole hour^{-1} mg protein^{-1})	(ng/mg tissue)	(pmole hour^{-1} mg protein^{-1})	(nmole/ mg tissue)
Control	12.5 ± 0.9	0.44 ± 0.04	35.8 ± 1	1.07 ± 0.09
Treated	54.5 ± 5.6*	1.03 ± 0.03*	31.4 ± 1†	1.17 ± 0.12
Percentage change	+336	+133	−12	+9
	Total activity or amount			
	(pmole hour^{-1} per slab)	(ng per slab)	(nmole hour^{-1} per slab)	(nmole per slab)
Control	44.3 ± 4.2	31.5 ± 2.4	127 ± 7.7	62.7 ± 7.1
Treated	51.3 ± 9	24.5 ± 1.2†	29.6 ± 4.5*	26 ± 4.9‡
Percentage change	+16	−22	−77	−59

*$P < .001$. †$P < .05$. ‡$P < .01$ (for control versus treated rats, Student's t-test).

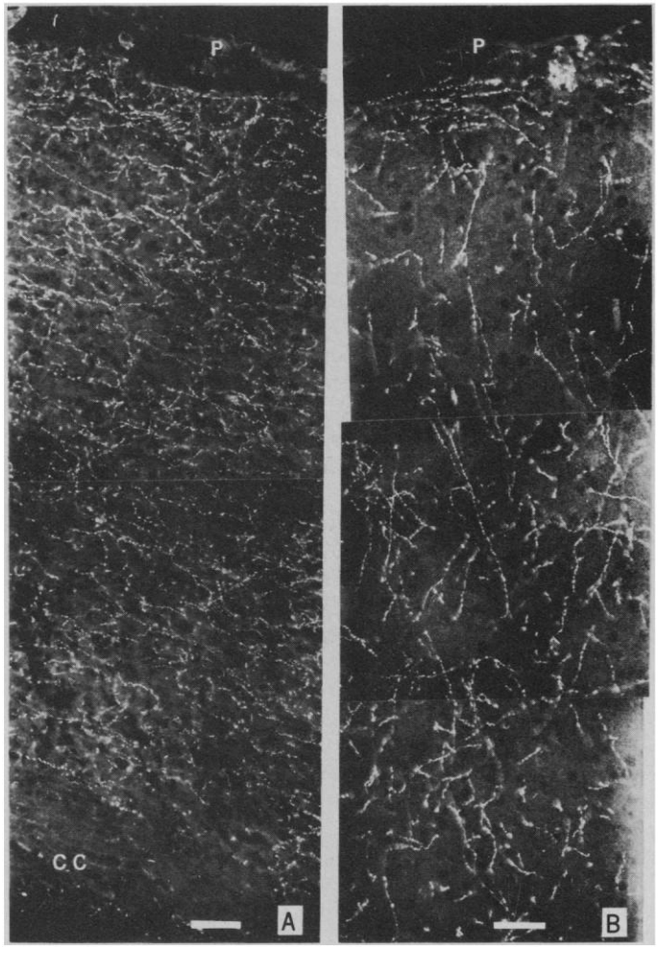

Fig. 1. Photomicrographs of noradrenergic axons in the lateral cortex of (A) MAM-treated and (B) control rats as revealed by immunohistofluorescence microscopy with antiserum to rat dopamine β-hydroxylase (*10, 12*). Both photomontages, which are typical of the results obtained from three rats in each treatment group, are presented at the same magnification (scale bar, 50 μm) to allow for comparison of the relative density of noradrenergic axons; as a result, only the upper 40 percent of the cortex from a control rat is shown, whereas the entire thickness of the atrophic cortex from a MAM-treated rat occupies the same area. The sections in (A) show the dense plexus of noradrenergic axons; the pattern of innervation is constant throughout all cortical layers from the pial surface (*P*) to the corpus callosum (*CC*) with short, randomly oriented axon segments. In contrast, the cortex from the control rat (B) has a distinctly lower density of noradrenergic axons, which run parallel to the pial surface (*P*) in layer I and have a radial orientation in the deeper layers.

nephrine] innervating the cortex (*10*). The lateral cortex from the MAM-treated rats contained considerably higher concentrations of the catecholaminergic neurochemical markers than cortex from the controls (Table 1). The specific activity of tyrosine hydroxylase was four times higher in the MAM-treated rats than in the controls and the concentration of its end product, norepinephrine, was more than two times higher. In contrast, the specific activity of glutamate decarboxylase was reduced slightly, but significantly, by 12 percent and the concentration of endogenous GABA was not significantly altered. Although the concentrations of neurochemical markers are relevant to understanding the local relationships within the damaged neocortex, expressing the results in terms of total amount per cortical slab highlights the absolute deficiency in the neuronal number produced by MAM treatment. When expressed in this manner, the MAM-treated neocortex exhibited severe deficits in the presynaptic markers for the GABA-containing neurons, with a 77 percent reduction in glutamate decarboxylase activity and a 59 percent reduction in the amount of GABA. In contrast, the total activity of tyrosine hydroxylase per cortex was not significantly altered and the amount of endogenous norepinephrine was only slightly reduced. These results strongly suggest that the MAM-treated cortex possesses a normal complement of catecholaminergic axons whose density is increased as a result of the deficiencies in other cortical neuronal populations.

To examine more directly the altered noradrenergic innervation of MAM-lesioned cortex, we visualized the noradrenergic axons by means of immunohistofluorescence microscopy, using a homologous antibody to dopamine β-hydroxylase (E.C. 1.14.17.1) (*11*), an antigenic marker for the noradrenergic neurons (*12, 13*). The lateral cortex from the MAM-treated animals (Fig. 1) exhibited a much higher density of dopamine β-hydroxylase–containing axons than cortex from the same area in control rats. In coronal sections of cortex from control rats, the noradrenergic innervation follows the laminar organization of the cortex with fibers running parallel to the pial surface in layer I and a predominance of long, radially oriented fibers in the deeper layers (*14*). In contrast, in the MAM-lesioned cortex, noradrenergic axons form a dense plexus of randomly oriented segments of fibers with no laminar organization apparent. These immunohistochemical studies concur with the neurochemical data in demonstrating a relative noradrenergic hyperinnervation of the cerebral cortex rendered atrophic by a fetal insult.

This example of noradrenergic hyperinnervation was produced by elimination of neurons intrinsic to the cerebral cortex at early stages of their formation. A similar increase in noradrenergic axon density has been described in the cerebellum of certain mutant mice in which the granule cell population fails to develop (*15*); however, the cerebellar dysgenesis occurs after birth whereas the pharmacologic lesion used in the present study takes place at much earlier stages of brain differentiation. Perinatal treatment of rats with 6-hydroxydopamine, a toxin specific for catecholaminergic axons, can markedly attenuate the development of noradrenergic innervation to the rostral regions of the brain but results in a compensatory hyperplasia of noradrenergic fibers in caudal regions (*16*). Thus, the present neurochemical and immunohistofluorescence observations, as well as previous studies, suggest that central noradrenergic neurons are programmed early in development for the total number of their axons to be formed in a manner that is relatively independent of the regions innervated. Nevertheless, our results indicate that the postsynaptic neurons play an essential role in regulating the pattern of axon distribution in the terminal field. This conclusion is supported by the observation that irises implanted into brain develop a pattern of innervation from central noradrenergic neurons typical for iris and not for brain tissue (*17*). The maintenance of a normal number of noradrenergic axons in the atrophic cortex with an altered pattern of innervation makes it likely that unusual functional relationships may exist between the noradrenergic axons and the residual cortical neurons.

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10. For enzyme assays, fresh cortex slabs were homogenized by sonication in 20 volumes of 50 m*M* tris-HCl, *p*H 7.4, containing 0.2 percent Triton X-100. Homogenates were centrifuged at 10,000 rev/min for 15 minutes and portions of supernatant were assayed. Glutamate decarboxylase was assayed by a modification of the method of S. H. Wilson, J. L. Schrier, J. Farber, E. J. Tompson, R. N. Rosenberg, A. J. Blume, and M. W. Nirenberg [*J. Biol. Chem.* **247**, 3159 (1972)] with L-[^{14}C]glutamic acid being used as the substrate. Tyrosine hydroxylase activity was measured as described by Coyle and Axelrod (*4*) with the exception that DL-methyl-5,6,7,8-tetrahydropterine hydrochloride was used as the cofactor. Tissue slabs for norepinephrine and GABA determinations were quick-frozen on Dry Ice. Frozen tissue was homogenized in 20 volumes of either 0.1*N* perchloric acid for norepinephrine determination or formic acid and acetone (15 : 85 by volume) for GABA assay. Homogenates were centrifuged at 1500 rev/min for 5 minutes. Norepinephrine was measured in the supernatant by the method of J. T. Coyle and D. Henry [*J. Neurochem.* **21**, 61 (1973)]. The supernatants of homogenates for GABA assay were dried in a vacuum centrifuge and then assayed by the enzymatic fluorometric method of L. T. Graham and M. H. Aprison [*Anal. Biochem.* **15**, 487 (1966)]. Protein was measured by the method of O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall [*J. Biol. Chem.* **193**, 265 (1951)] with bovine serum albumin used as the standard.
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