metric visual field loci. It is especially remarkable that a single region of cortex distinguishes between ipsilateral and contralateral visual fields in its recurrent connections with the LGN, but fails to do so in its associational connections (*15*). The two projections may, however, arise from different populations of cells, as is known to be the case in common cats (*10*).

The difference in the behavior of the two projections suggests that the rules governing the formation of these two sets of connections are different. We suggest that in the formation of associational connections, positional information from the retina is still used, but the sign of the receptive field position—left or right of the vertical midline—is ignored, and only distance from the midline is considered. This relaxation of specificity would not lead to any wrong connections in common cats, since in these animals each hemisphere receives input only from the contralateral half-field. This interpretation must be viewed with some caution, however, since it is based on the assumption that only optic nerve decussation is directly affected by the genetic mutation and that more central visual structures develop according to normal rules. It could be put on a more secure footing if the same misrouting could be produced in common cats, perhaps by fetal surgery.

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9. In the alternative "Midwestern" pattern, the geniculo-cortical projection is not rearranged, but the ipsilateral visual field input to the cortex appears to be functionally supressed (*4*). It has recently been suggested that the Boston and Midwestern patterns can coexist in different regions of the same animal's cortex [M. L. Cooper, G. G. Blasdel, J. D. Pettigrew, *Assoc. Res. Vision Ophthalmol. Abstr.* (1978), 216]. Our study was confined to the cortical representations of the horizontal meridian and inferior visual fields, and in these regions only the Boston pattern was observed.
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11. The 17-18 border was determined during the recording session by locating the maximum excursion into the ipsilateral visual field and was later identified histologically.
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13. We were unable to determine whether the projections from the 17-18 border to area 19 and the Clare-Bishop area were also rearranged because the visuotopic maps of these areas in Siamese cats have not been studied.
14. The demonstration of these anomalous projections from the 17-18 border into area 17 may provide an explanation for a puzzling observation that has occasionally been made in Siamese cats showing the Boston pattern of reorganization. While mapping the contralateral field representation within area 17, Hubel and Wiesel (*6*) noted in some animals a few groups of cells whose receptive fields were located not in the contralateral field but at the mirror-symmetrical locus in the ipsilateral field. Some cells even had "mirror fields," that is, they had one receptive field in each half of the visual field. It was suggested that these cells received a direct geniculate input from neurons in LGN lamina A1 whose axons, for some reason, had not been redirected to the region of the 17-18 border. The present results suggest another interpretation, namely, that the "Boston" rearrangement of the geniculo-cortical projection is complete and that the ipsilateral field input to the mirror-field cells is supplied by the anomalous cortico-cortical projection described above. We should emphasize, however, that mirror fields are uncommon and were not observed in the animals studied here. Furthermore, it is conceivable that a few cells in the medical, abnormal portion of lamina A1 did project to the cortical representation of the contralateral visual field, but the HRP method was insufficiently sensitive to detect them.
15. In the formation of the callosal connections, the tendency is to make a correct choice between ipsilateral and contralateral visual field locations, but some confusion evidently arises.
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β-Adrenergic Regulation of Adenosine 3′,5′-Monophosphate Concentration in Brain Microvessels

Abstract. *Norepinephrine increases the concentration of adenosine 3′,5′-monophosphate (cyclic AMP) in an incubated suspension of brain microvessels. This response can be matched by other drugs that stimulate the β receptors, but the α-adrenergic agonist phenylephrine is without effect; β-adrenergic blockade abolishes the response while α-adrenergic blockade produces no change. The data support the contention that cerebral capillary function is subject to adrenergic neural control.*

Precise control of brain volume, through adjustment of cell water and electrolyte content, is important for the normal function of the brain not only because it is confined in the rigid and indistensible environment of the skull (*1*) but also because changes in cell volume may affect important functional relationships between cells (*2*). This volume homeostasis must be achieved in the face of the fluctuating osmotic and hydrostatic forces imposed by the incoming blood supply while respecting functionally critical ionic gradients within the brain.

The capillary endothelium, the primary barrier between blood and brains has several features common to membranes known to regulate water and electrolyte permeabilities, such as trout gill, toad urinary bladder, frog skin, rabbit gallbladder, and mammalian distal nephron (*3*), and may have an important role in regulation of brain volume and environment. There is some indication that brain vascular function is under neural influence; especially notable is the change in water permeability of the brain vasculature in response to adrenergic stimulation or centrally administered vasopressin (*3*). New techniques available for the preparation of very pure microvascular tissue from brain tissue now allow direct study of the pharmacology of microvessels in vitro to determine how capillary function might be modulated. In the experiments reported here we measured the effects of neurotransmitters and vasopressin on adenosine 3′,5′-monophosphate (cyclic AMP) concentrations in isolated microvessels of brain. The cyclic AMP system has been closely linked to some neurotransmitter receptors, and there is ample evidence relating this substance to hormone-induced changes in water and electrolyte permeability in other tissues (*4*).

Brain microvessels were prepared from male Sprague-Dawley rats (120 to 250 g) by the method of Goldstein *et al.* (*5*). We examined each preparation of microvessels by phase-contrast and dark-field microscopy to determine the nature and proportion of cell types present. We observed virtually no contamination by neuronal elements. The difference between smooth muscle and endothelial cells was clear in dark field, and we used only those preparations in which muscular vessels were estimated to constitute less than 5 percent of the isolated tissue.

In each experiment, tissue isolated from cerebral cortices of four to six rats was pooled and suspended in Krebs-Ringer bicarbonate buffer previously equilibrated with 95 percent O_2 and 5 percent CO_2. Portions (250 μl) of this tissue suspension containing approximately 30 μg of protein were incubated at

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37°C for 5 minutes, or for the periods of time designated, in the presence or absence of the test agents. After incubation, 25 μl of 50 percent trichloroacetic acid was added to each sample to inactivate the tissue and precipitate protein. Supernatant fluid and protein were separated by centrifugation. After removal of trichloroacetic acid by washing with ether, cyclic AMP in the supernatant fluid was measured by radioimmunoassay (*6*). Protein was measured by the method of Lowry *et al.* (*7*).

Basal levels of cyclic AMP in cerebral microvessels were 2 to 3.5 pmole per milligram of protein. Norepinephrine (100 μ*M*) increased cyclic AMP concentrations two- to eightfold. The accumulation of cyclic AMP was rapid, reaching a maximum in 5 minutes, and the elevation persisted for at least 10 minutes (Fig. 1A). Half-maximal effect (ED_{50}) was produced by 5 to 10 μ*M* norepinephrine. Other β-adrenergic agonists, isoproterenol and epinephrine, at concentrations of 100 μ*M*, increased the concentration of cyclic AMP to the same degree as an equivalent concentration of norepinephrine, but the α-adrenergic agonist phenylephrine (100 μ*M*) had no effect. Dopamine, serotonin, histamine, and acetylcholine at 100 μ*M*, and 20 μg (per milliliter) of angiotensin II and substance P were also ineffective. We did observe, however, that adenosine increased cyclic AMP by 76 percent, and this effect was additive with that of norepinephrine. Although vasopressin at a concentration of 0.02 I.U./ml has been reported to produce maximal accumulation of cyclic AMP in other tissues (*8*), neither this concentration nor 2.0 I.U./ml had any effect on cyclic AMP concentration in the cerebral microvessels.

Consistent with the finding that only β-adrenergic agonists increased the concentration of cyclic AMP, the β-adrenergic blocking agent propranolol (100 μ*M*) completely prevented the norepinephrine-induced increase in cyclic AMP, whereas 100 μ*M* phentolamine, an α-adrenergic antagonist, did not inhibit the action of norepinephrine.

To our knowledge there has been only one other report of the cyclic AMP system in brain microvessels. Joo *et al.* (*9*) studied adenylate cyclase activity in a relatively crude capillary-enriched tissue fraction from rat cerebral cortex and found significant stimulation by histamine but not by norepinephrine. However, their broken-cell preparation could not localize the histamine reactivity to vascular or neuronal elements and, further, the tissue homogenization could easily have disrupted any coupling between adrenergic receptors and adenylate cyclase.

Table 1. Accumulation of cyclic AMP in rat brain microvessels in response to adrenergic agonists (100 μ*M*). The data (presented as means ± standard error) were analyzed by Student's *t*-test. The number of samples in each group is given in parentheses.

Agonist	Cyclic AMP (pmole/mg protein)
Control	2.26 ± 0.22 (*N* = 4)
Norepinephrine	13.23 ± 1.20* (*N* = 4)
Epinephrine	9.49 ± 0.65* (*N* = 4)
Isoproterenol	11.04 ± 1.25* (*N* = 4)
Phenylephrine	2.33 ± 0.16 (*N* = 6)

*$P \leq .001$.

In two additional studies investigators measured norepinephrine-stimulated accumulation of cyclic AMP in vascular endothelial tissue derived from sources outside the brain (*10, 11*). Buonassisi and Venter (*10*) studied endothelium cultured from rabbit aorta and found a norepinephrine-stimulated cyclic AMP response which could be fully blocked by propranolol. Wagner *et al.* (*11*), however, discovered both α and β responses in capillaries isolated from rat epididymal fat pads. Both groups reported accumulation of cyclic AMP in response to a variety of other substances, such as serotonin, histamine, and vasopressin, which we tested without success. Although methodological differences might explain this difference in responsiveness to substances other than norepinephrine, it is perhaps a reflection of a difference between brain microvascular endothelium and other vascular endothelia.

The demonstration of an anatomical association between central noradrenergic fiber varicosities and brain capillary endothelium suggests that these vessels are innervated (*3*). This suggestion is supported by the observation that stimu-

Table 2. Effects of adrenergic blocking agents (100 μ*M*) on the response of cyclic AMP in rat brain microvessels to norepinephrine (100 μ*M*). The data (presented as means ± standard error) were analyzed by Student's *t*-test and are expressed as picomoles of cyclic AMP per milligram of protein. The number of samples in each group is given in parentheses.

Antagonist	Agonist	
	None	Norepinephrine
None	3.05 ± 0.16 (*N* = 16)	6.13 ± 0.62* (*N* = 12)
Phentolamine	2.92 ± 0.18 (*N* = 12)	5.70 ± 0.49* (*N* = 12)
Propranolol	2.10 ± 0.17 (*N* = 12)	3.00 ± 0.11 (*N* = 14)

*$P < .001$.

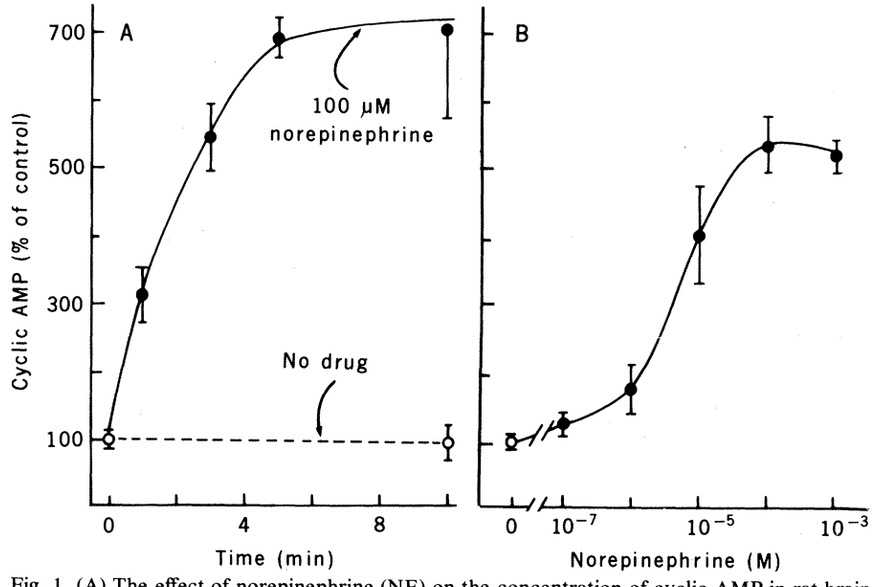

Fig. 1. (A) The effect of norepinephrine (NE) on the concentration of cyclic AMP in rat brain microvessels as a function of incubation time. The control value for cyclic AMP was 3.35 ± 0.47 pmole per milligram of protein. Both control points (open circles) represent the mean (± standard error of the mean) of three samples, whereas each experimental point (closed circles) represents four samples. (B) The dose-response curve for rat brain microvessels incubated for 5 minutes with various concentrations of norepinephrine. The control value was 2.73 ± 0.31 pmole per milligram of protein. The response of microvascular cyclic AMP to NE becomes significant at 1 μ*M* ($P < .1$, Student's *t*-test). Each point represents the mean (± standard error of the mean) of four samples.

lation of the locus coeruleus, the primary source of adrenergic neurons in the central nervous system, can reversibly change the permeability of the brain vasculature to water. Taken together, these functional and anatomical observations lead to the hypothesis that brain capillaries are under neural control. The capillaries might function in a manner similar to other membranes known to regulate water and electrolyte permeability and, as such, contribute to fluid and electrolyte homeostasis of the brain. Our data further support this hypothesis.

Vasopressin reversibly increases brain capillary water permeability when injected into the brain ventricle of the rhesus monkey (*3*). In our study, vasopressin did not affect the cyclic AMP system of brain microvessels in vitro. It is possible that vasopressin acts directly at the capillary by some mechanism unique to brain, which does not involve cyclic AMP. However, the demonstration by Tanaka *et al.* (*12*) that intraventricular vasopressin alters brain norepinephrine turnover in a number of areas, plus the discovery by Swanson (*13*) of an anatomical interconnection between the noradrenergic system and the vasopressin and oxytocin systems in brain, argue for noradrenergic mediation of the vasopressin effect in vivo.

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Iron Deficiency Prevents Liver Toxicity of 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin

Abstract. *The compound 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) causes hepatocellular damage and porphyria in C57Bl/6J mice, among a wide range of toxic effects. We compared the effect of TCDD toxicity in iron-deficient mice with that in mice receiving a normal diet. Porphyria did not develop in the iron-deficient animals, and these animals were also protected from hepatocellular damage and certain other toxic effects of TCDD.*

The compound 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a potential contaminant of 2,4,5-trichlorophenoxyacetic acid, is extremely toxic to living systems. Pathologic changes in the liver, thymus, skin, and immune system have been reported, and, in addition, TCDD is teratogenic (*1*). This compound causes porphyria cutanea tarda (PCT) in humans and an analogous disturbance of heme synthesis in rats and mice (*2*). More common causes of PCT are alcohol and estrogen therapy. Essentially, this disease comprises light-induced skin damage due to sensitization of the skin by increased concentrations of circulating porphyrin. This porphyrin is predominantly uroporphyrin and a seven-carboxyl porphyrin; it originates in the liver where conversion of uroporphyrinogen to coproporphyrinogen is partially blocked by a decreased activity of uroporphyrinogen decarboxylase (UD). The mechanism whereby TCDD or other hepatotoxins decrease UD is unknown (*3*). As PCT in man may be treated by repeated venesections to reduce body stores of iron (*4*), we designed an experiment to determine whether iron deficiency would protect mice from developing porphyria during exposure to TCDD.

We found that iron deficiency did indeed prevent porphyria, and no decrease in UD was observed in iron-deficient, TCDD-treated mice. However, we found that iron deficiency also protected mice against skin disease caused by TCDD and against liver damage. This was severe in TCDD-treated animals on a normal diet but absent from those animals previously rendered iron-deficient. These results have significance for our understanding of the mechanism of toxicity due to TCDD and related compounds and possibly also for the treatment of TCDD poisoning in man.

We obtained C57Bl/6J mice aged 6 weeks from Jackson Laboratories and fed half either a synthetic iron-deficient diet (*5*) or laboratory chow (*6*). The mice in the group being rendered iron-deficient were anesthetized with ether, and 0.2 to 0.25 ml of blood was withdrawn from the cavernous sinus of each animal twice weekly for 4 weeks; at the end of 4 weeks the hemoglobin concentration had dropped to 5.5 g/dl (*7*). Two of 17 animals died during this preliminary procedure. The iron-deficient diet was maintained; the groups of animals on iron-deficient and regular diets were each subdivided into two groups, treatment and control, and all four groups

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