cells in the gastrointestinal tract may also have important local or hormonal influence on nutrient entry. The site or sites at which somatostatin exerts its influence on the entry of ingested nutrients have not been identified, but its many inhibitory actions on rate-limiting gastric, intestinal, and pancreatic functions (*13*), suggest that any one or more of these loci may be involved.

In view of these results it seems reasonable to regard splanchnic somatostatin as a true hormone with a regulatory role in the homeostasis of ingested nutrients.

V. SCHUSDZIARRA
E. ZYZNAR
D. ROUILLER
University of Texas Southwestern Medical School and *Veterans Administration Medical Center, Dallas 75216*

G. BODEN
Temple University Health Science Center Philadelphia, Pennsylvania 19140

J. C. BROWN
University of Vancouver, Vancouver, British Columbia V6T 1W5

A. ARIMURA
Tulane University and *Veterans Administration Medical Center, New Orleans, Louisiana 70146*

R. H. UNGER
University of Texas Southwestern Medical School and *Veterans Administration Medical Center, Dallas*

References and Notes

1. R. Luft, S. Efendic, T. Hokfeldt, O. Johannson, A. Arimura, *Med. Biol.* **52**, 428 (1974); M. P. Dubois, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 1340 (1975); L. Orci, D. Baetens, M. P. Dubois, C. Rufener, *Horm. Metab. Res.* 7, 400 (1975).
2. R. H. Unger and L. Orci, *Diabetes* **26**, 241 (1977).
3. The term "true hormone" is used to designate a secretory product secreted into and carried by the circulation to specific target cells upon which it exerts a specific biological action.
4. V. Schusdziarra, D. Rouiller, V. Harris, J. M. Conlon, R. H. Unger, *Endocrinology* **103**, 2264 (1978); V. Schusdziarra, V. Harris, J. M. Conlon, A. Arimura, R. H. Unger, *J. Clin. Invest.* **62**, 509 (1978).
5. S. Kronheim, M. Berelowitz, B. L. Pimstone, *Diabetes* **27**, 523 (1978); A. Arimura, G. Lundquist, J. Rothman, R. Chang, R. Fernandez-Durango, R. Elde, D. H. Coy, C. Meyers, A. V. Schally, *Metab. Clin. Exp.* **27** (Suppl. 1), 1139 (1978); J. M. Conlon, C. B. Srikant, E. Ipp, V. Schusdziarra, W. Vale, R. H. Unger, *J. Clin. Invest.* **62**, 1187 (1978).
6. V. Harris, J. M. Conlon, C. B. Srikant, K. McCorkle, V. Schusdziarra, E. Ipp, R. H. Unger, *Clin. Chim. Acta* **87**, 275 (1978).
7. A. Arimura, S. Stanuko, D. H. Coy, A. V. Schally, *Proc. Soc. Exp. Biol. Med.* **148**, 784 (1975).
8. V. Schusdziarra, E. Zyznar, D. Rouiller, R. H. Unger, *Endocrinology*, in press.
9. R. H. Unger, E. Ipp, V. Schusdziarra, L. Orci, *Life Sci.* **20**, 2081 (1977).
10. The neutralizing sheep antiserum to somatostatin (Arimura S-773) gave a linear standard curve at a final dilution of 1:70,000 on a logit-log graph in a range of 8 to 4096 pg per tube. Tracer-antibody binding was not suppressed by insulin, glucagon, vasoactive intestinal peptide, bovine pancreatic polypeptide, secretin, substance P, or dGH (NIH D1001A) at doses up to 160 ng; 1 ml of antiserum binds 3.3 μg of somatostatin.
11. M. Eggstein and F. H. Kreutz, *Klin. Wochenschr.* **44**, 262 (1966).
12. V. Schusdziarra, D. Rouiller, A. Arimura, R. H. Unger, *Endocrinology* **103**, 1956 (1978).
13. S. R. Bloom, C. H. Mortimer, M. O. Thorner, G. M. Besser, R. Hall, A. Gomez-Pan, V. M. Roy, R. C. G. Russell, D. H. Coy, A. J. Kastin, A. V. Schally, *Lancet* **1974-II**, 1106 (1974); A. Gomez-Pan, J. D. Reed, M. Albimus, B. Shaw, R. Hall, G. M. Besser, D. H. Coy, A. J. Kastin, A. V. Schally, *ibid.* **1975-I**, 888 (1975); G. Boden, H. I. Jacob, A. Staus, *Gastroenterology* **70**, 961 (1976); H. C. Dollinger, S. Raptis, H. Goebell, E. F. Pfeiffer, in *Stimulus-Secretion Coupling in the Gastrointestinal Tract*, R. M. Case and H. Goebell, Eds. (MTP, Lancaster, England, 1976), p. 403; W. Schlegel, R. F. Harvey, S. Raptis, J. M. Oliver, E. F. Pfeiffer, *Lancet* **1977-II**, 166 (1977); J. Wahren and P. Felig, *ibid.* **1976-II**, 1213 (1976); G. Boden, M. C. Sivitz, O. E. Owen, N. Essa-Koumar, J. H. Landor, *Science* **190**, 163 (1975); W. Creutzfeldt, G. Raubisch, U. R. Folsch, *Dtsch. Med. Wochenschr.* **100**, 1135 (1975); S. R. Bloom, D. N. Ralphs, G. M. Besser, R. Hall, D. H. Coy, A. J. Kastin, A. V. Schally, *Gut* **16**, 834 (1975).
14. R. S. Yalow and S. A. Berson, *Gastroenterology* **58**, 1 (1970); S. Cataland, S. E. Crockett, J. C. Brown, E. L. Mazzaferri, *J. Clin. Endocrinol. Metab.* **39**, 223 (1974); J. R. Dryburgh and J. C. Brown, *Gastroenterology* **68**, 1169 (1975); R. S. Yalow and S. A. Berson, *J. Clin. Invest.* **39**, 1157 (1960); G. R. Faloona and R. H. Unger, in *Methods of Hormone Radioimmunoassay*, B. M. Jaffe and H. R. Behrman, Eds. (Academic Press, New York, 1974); R. M. Wilson and G. Boden, *Endocrinology* **102**, 859 (1978).
15. V. Schusdziarra, V. Harris, A. Arimura, R. H. Unger, *Endocrinology* **102**, 1705 (1979).
16. This work was supported by Veterans Administration institutional research support grant 549-8000-01; NIH grant AM 02700-16, and contract N01-AM-62219; CIBA/Geigy Corporation, Summit, N.J.; Eli Lilly, Indianapolis, Ind.; and Salk Institute-Texas Research Foundation R.H.U. is senior medical investigator, VA Medical Center, Dallas. We thank L. Clendenen, J. Diffie, H. Gibson, M. Lintner, K. McCorkle, L. Peace, D. Sandlin, and C. Smith for expert technical assistance. For secretarial assistance we thank S. Freeman and J. Reese.

16 August 1979; revised 13 November 1979

Laminar Organization of Thalamic Projections to the Rat Neocortex

Abstract. *Nerve fibers transmitting information from the thalamus to the cerebral cortex may be classified according to their major cortical layers of termination. (i) One class consists of inputs from thalamic relay nuclei for vision, audition, and somesthesis to layer IV, layer III, or both. In contrast, autoradiographic studies of projections from other thalamic nuclei reveal strikingly different patterns of termination: (ii) layer VI (or layer V, or both) is the target of fibers from the intralaminar nuclei, and (iii) layer I is the target for fibers from the ventromedial and magnocellular medial geniculate nuclei. (iv) The remaining class is typified by termination both in layer I and in additional layers that depend on the cortical area in which the terminations are found. The data demonstrate that convergent thalamic inputs to a given cortical area are usually not confluent within a layer and provide a new framework for categorizing thalamic nuclei.*

Lorente de Nó provided the classic description of thalamic afferent fibers in the rodent neocortex (*1*). His Golgi material displayed two fundamentally distinct laminar distributions of terminal arborizations: the "specific," which is densely aggregated in cortical layers III and IV, and the "unspecific," which is sparsely distributed throughout all cortical layers but appears predominantly in layers I and VI. Subsequent electrophysiological evidence (*2*) buttressed the consensus (*2, 3*) that within the thalamus there are (i) a layer IV projection system including the "specific" sensory relay nuclei and (ii) a "nonspecific" layer I projection system epitomized by the intralaminar nuclei but also including a number of adjacent nuclei (*4*).

In the three decades that followed the initial categorization of thalamic nuclei into specific and nonspecific domains, very little anatomical evidence has supported the dual thalamic projection system concept. Anterograde fiber-tracing data have shown the existence of the specific projection to layers III and IV arising from several thalamic nuclei (*5*) but have largely failed to support (*6*) the contention that the intralaminar nuclei have projections terminating in layer I (*3*). Instead, layer I projections have been found to arise from a posterior site termed the "central intralaminar nucleus" in the hedgehog and the opossum thalamus (*7*), from the magnocellular medial geniculate nucleus in both the rat and the monkey (*8*), and from the ventromedial nucleus in the rat (*9*).

The autoradiographic studies reported here were designed to examine the cortical projections of individual thalamic nuclei in the rat. The first goal was to determine the laminar distributions of nuclei representing specific and nonspecific domains. After stereotaxic injections of tritiated amino acids into thalamic loci, the animals were allowed to survive for periods of either 1 day or 10 to 12 days. Their brains, perfused in formalin (10 percent in 0.9 percent saline), were sectioned and processed according to usual autoradiographic procedures (*9, 10*). The successful restriction of the anterograde

0036-8075/80/0201-0532$00.50/0

marker to cell bodies contained within individual nuclei required iontophoretic delivery (*9*). Long postoperative survival times (approximately 10 days) and long exposure of the brain sections to the emulsion (1 to 7 months) facilitated the visualization of projections, especially of sparse fibers arising from the intralaminar nuclei. Neurons incorporating and transporting the marker within their axons were determined by microscopic analysis to be those within an area of dense silver grains in the emulsion overlying the sections. (The sections had been exposed to the emulsion in the dark at −12°C for 5 to 7 months.) The regions of uptake and transport were in part determined by comparing projections arising from adjacent regions injected in several animals. Of more than 120 cases of small thalamic isotope injections, the ones illustrated in Fig. 1 epitomize each class in terms of successful restriction of isotope to the nucleus in question and the best representation of its projections.

Instead of a dual thalamic projection system, the autoradiography shows four distinct categories of cortical afferent lamination. Category 1 contains the terminations of the ventroposterior, medial geniculate, lateral geniculate, and mediodorsal cell groups (*11*). The projections in this class are aimed predominantly at the middle cortical layers, which typically include all of layer IV and the deep part of layer III. Termination in layer I, when present, is marked by sparse grains that are much less dense than those in layer III or layer IV. Rostral cortical areas lack a granular layer IV, and here the mediodorsal and gelatinosus nuclei project most densely to layer III and also, although with moderate density, to layer I. The ventroposterior nucleus has a cortical projection that terminates heavily in the layer IV granule cell aggregates of the primary somatosensory area (*12*) and extends superficially in greatly reduced magnitude as far as layer I (Fig. 1a). A second, sparser concentration of grains overlies the superficial half of layer VI. A double banding pattern (layers IV and VI) characterizes the projections of the medial and lateral geniculate nuclei to auditory and visual cortical areas as well, though in each of these areas the layer I grain density indicates a definite but minor third band. The middle layer is the most predominantly labeled in each case.

Category 2 comprises terminations confined to layers VI and V from the intralaminar nuclei, including the parafascicular nucleus. This group issues sparse but widespread neocortical projections, the bulk of which terminate in infragranular layers deep to layer IV (Fig. 1f). Amino acid injected into the rostral portion of the central medial nucleus labels projections aimed at layers VI and, to a lesser degree, layer V of virtually all cortical areas. The central lateral and paracentral nuclei (Fig. 2a) project to deep layers of more limited cortical extent. The parafascicular nucleus projects only to layer VI of the motor area. Where sparse fibers extend as far as layer I, the density is far less than that observed in the infragranular layers.

Categories 3 and 4 are typified by dense projections terminating in layer I. The source nuclei include the ventral anterolateral, lateral dorsal, ventromedial, reuniens, posterior, lateral posterior, and magnocellular medial geniculate nuclei. Most of these are located adjacent to the intralaminar nuclei, and their proximity probably accounts in part for the long-standing popular belief that the intralaminar nuclei project to layer I (*3*). However, the small injections used in this study unequivocally show that when isotope is confined to the intralaminar nuclei (Fig. 2a), sparse fibers are found in layer I, but when it is placed just 500 μm laterally, a heavy layer I projection is labeled. Within the category of projections to layer I is the ventromedial nucleus, the projection of which is directed nearly exclusively to the outer half of layer I of almost the entire neocortex (*9*). The magnocellular medial geniculate region projects to layer I of the auditory areas of the cortex, but shows accumulations of grains in deeper layers suggesting additional termination there.

The remaining layer I-projecting cell groups may be considered as a distinct class because they have in common definite additional termination in layers whose locus and density in each instance depend on the cortical area in which they

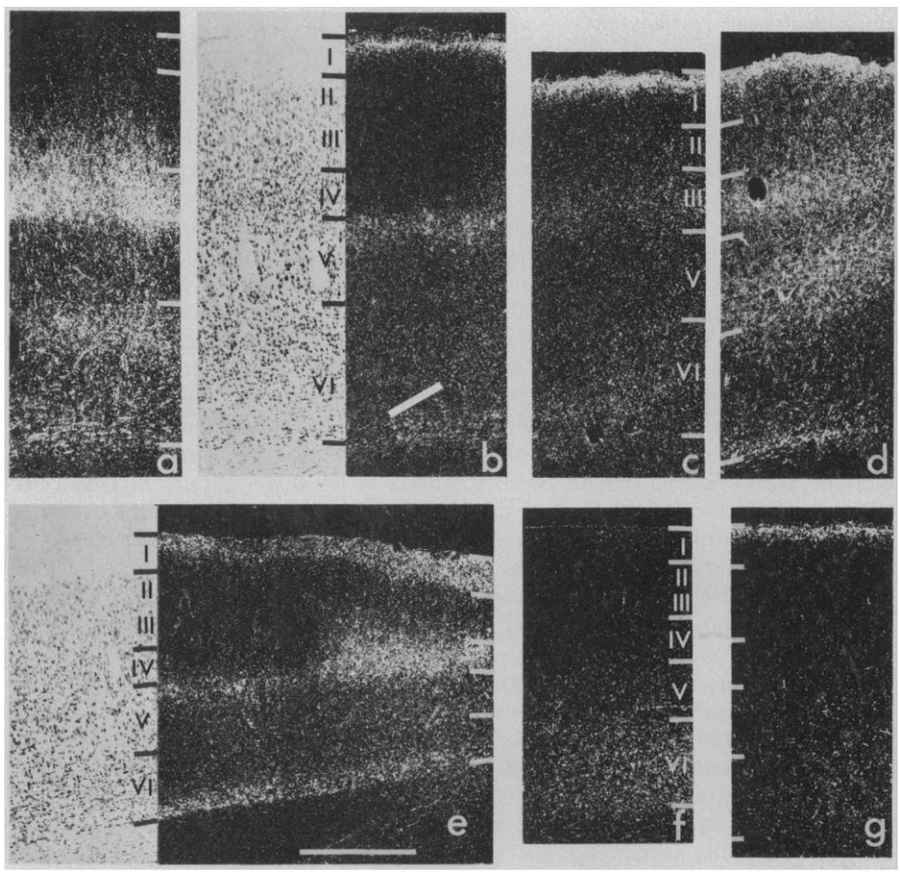

Fig. 1. Dark-field and bright-field microphotographs showing narrow strips of cerebral cortex with autoradiographically labeled thalamocortical afferent fibers and terminals (6- to 7-month exposures). Roman numerals mark cortical layers. All photographs were taken at same magnification (bar, 0.5 mm). (a) Ventroposterior injection labels layers III, IV, and VI in the primary somatosensory area. (b) Posterior injection labels layer I and V in the same cortical area (continued at left in brightfield). (c) The same posterior injection labels I and III in the motor area. (d) Typical trilaminar banding in the motor area after ventral anterolateral injection. (e) Lateral posterior projection to layers I and IV of visual cortical area 18 (right) and to layers I and V of area 17 (left, and continued in brightfield). The intermediate band shifts layers at the border between areas 17 and 18. (f) Intralaminar projections to infragranular layers illustrated here by a central medial projection directed mostly at layer VI of cortical area 17. (g) Anteromedial ventral anterolateral projection to layers I and VI of area 17.

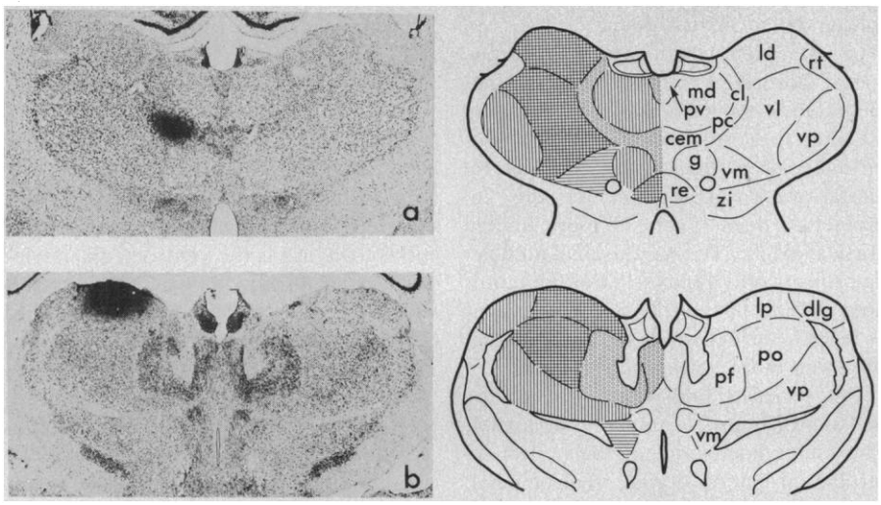

Fig. 2. Photographs (left) and respective projection drawings (right) of two representative thalamic levels. At left are the centers of injection sites in the paracentral nucleus of the intralaminar complex (a) and the lateral posterior nucleus (b) photographed from sections exposed to the emulsion for 5 and 7 months, respectively. At right is the classification scheme. Vertical lines mark cell groups projecting to layer IV or layer III (or both); horizontal lines mark those that project nearly exclusively to layer I; cross-hatching indicates nuclei with area-dependent lamination; circles mark nuclei with projections to area VI or area V (or both). Abbreviations: *cem*, central medial; *cl*, central lateral; *dlg*, dorsal lateral geniculate; *g*, gelatinosus; *ld*, lateral dorsal; *lp*, lateral posterior; *md*, mediodorsal; *pc*, paracentral; *pf*, parafascicular; *po*, posterior; *pv*, paraventricular; *re*, reuniens; *rt*, reticular; *vl*, ventral anterolateral; *vm*, ventromedial; *vp*, ventroposterior; and *zi*, zona incerta.

are found. The ventral anterolateral nucleus is placed within the category of area-dependent lamination because, although it projects to layers I and VI of a wide area of the cortex, including motor and visual areas (areas 4 and 17), it also projects to layers III and V of the motor area (*13*). Isotope confined to the lateral posterior nucleus (Fig. 2b) labels a heavy projection to the peristriate area (area 18), terminating in layers I and IV. The projection also pervades the striate area (area 17), but here it terminates in layer I and the superficial portion of layer V (Va). The intermediate band thus dramatically shifts from layer IV to layer Va at the border between areas 17 and 18 (Fig. 1e). Similarly, the posterior nucleus projections are aimed at layers I and IV of the second somatosensory cortical area and at layers I and Va of the immediately adjacent primary somatosensory area. More rostrally placed injections into the posterior nucleus label fibers to layers I and Va of most of the primary somatosensory area (Fig. 1b) and to layers I and III of the motor area (Fig. 1c). The reuniens and the lateral dorsal nuclei project outside the neocortex, but within their target zones they show area-dependent lamination. The reuniens efferents terminate in the superficial hippocampal layer and in layers I and III of the entorhinal cortex (*14*). The lateral dorsal nucleus projects to layer I of the granular area and to layers I and III of the agranular retrosplenial area.

The thalamic nuclei and their projections, placed in four categories in Fig. 2, have parallels in ontogeny and phylogeny, and the organizational scheme suggests new considerations for electrophysiology and principles of convergent cortical projections. The observations that the intralaminar nuclei may constitute the paleothalamic or "cephalic reticular" core (*15*) show an interesting correspondence with the facts that (i) these nuclei project predominantly to the striatum (*6, 16*), the most rostral end of the brain of lower vertebrates (*17*), and (ii) their laminar termination in the cortex is within the deep layers, which, in ontogeny, are the first to develop (*18*). The thalamic nuclei situated most laterally are the origins of Lorente de Nó's specific projection system. Projections in this category terminate within the middle cortical layers, traditionally considered to be the major cortical input zone (*1*). The cells in layer IV provide by short intracortical connections a complex, sequential, local processing mechanism (*19*) that is bypassed by thalamic inputs terminating in other cortical layers. Interposed between the intralaminar (deep-projecting) and specific (middle-projecting) groups are the superficial (layer I) projecting nuclei, including those categorized here as having area-dependent lamination. The superficial projections spread widely over multiple cortical areas; by their termination on the distal ends of pyramidal cell apical dendrites (*1*), they might be assumed to have a subtle, universal effect on pyramidal cell excitability in the underlying cortical layers. If these nuclei are the ones that modulate cortical neural activity in the fashion described as the recruiting response (*2, 9*), the intralaminar nuclei can be freed from this previously assigned functional role and reconsidered as candidates for different modes of influence on cortical neurons.

Considered as a proportion of thalamic volume, the cell groups projecting to layer I in the rat constitute a sizable fraction (Fig. 2). The existence of homologous and analogous regions in the thalamus of other mammals, including primates, remains to be investigated, but would be predicted on the basis of these results. For the rat at least we can conclude that cortical areas receive convergent thalamic inputs that are usually not confluent within a layer. More fundamentally, the nuclei of the thalamus can be considered to be of four types with respect to the laminar distribution of their projections upon the cortex.

MILES HERKENHAM
Laboratory of Neurophysiology, National Institute of Mental Health, Bethesda, Maryland 20205

References and Notes

1. R. Lorente de Nó, in *Physiology of the Nervous System*, J. F. Fulton, Ed. (Oxford Univ. Press, New York, 1938), p. 274.
2. R. S. Morison and E. W. Dempsey, *Am. J. Physiol.* **135**, 281 (1942); E. W. Dempsey and R. S. Morison, *ibid.*, pp. 293 and 301; J. Hanbery and H. H. Jasper, *J. Neurophysiol.* **16**, 252 (1953); J. Hanbery, C. Ajmone-Marsan, M. Dilworth, *Electroencephalogr. Clin. Neurophysiol.* **6**, 103 (1954).
3. E. G. Jones, *Brain Res.* **85**, 249 (1975); F. F. Ebner, in *Evolution of Brain and Behavior in Vertebrates*, R. B. Masterson, M. E. Bitterman, C. B. G. Campbell, N. Hotton, Eds. (Wiley, New York, 1976), p. 147; H. J. Gould, W. C. Hall, F. F. Ebner, *J. Comp. Neurol.* **177**, 445 (1978); R. G. Carey, D. Fitzpatrick, I. T. Diamond, *ibid.* **186**, 393 (1979).
4. The diffuse or nonspecific thalamic projection system included the thalamic reticular, ventroanterior, ventromedial, reuniens, intralaminar, suprageniculate, and limitans nuclei in the cat [H. H. Jasper, in *Electrical Stimulation of the Brain*, D. E. Sheer, Ed. (Univ. of Texas Press, Austin, 1961), p. 277].
5. S. P. Wise, *Brain Res.* **90**, 139 (1975); C. E. Ribak and A. Peters, *ibid.* **92**, 341 (1975); E. G. Jones, *J. Comp. Neurol.* **160**, 167 (1975); H. J. Gould *et al.*, in (*3*); A. E. Hendrickson, J. R. Wilson, M. P. Ogren, *ibid.* **182**, 123 (1978).
6. W. J. H. Nauta and D. G. Whitlock, in *Brain Mechanisms and Consciousness*, J. F. Delafresnaye, Ed. (Blackwell, Oxford, 1954), p. 81; D. Bowsher, in *The Thalamus*, D. P. Purpura and M. D. Yahr, Eds. (Columbia Univ. Press, New York, 1966), p. 99.
7. H. P. Killackey and F. F. Ebner, *Brain Behav. Evol.* **6**, 141 (1972); *Science* **179**, 283 (1973).
8. D. K. Ryugo and H. P. Killackey, *Brain Res.* **82**, 173 (1974); E. G. Jones and H. Burton, *J. Comp. Neurol.* **168**, 197 (1976).
9. M. Herkenham, *J. Comp. Neurol.* **183**, 487 (1979).
10. W. M. Cowan, D. I. Gottlieb, A. E. Hendrickson, J. L. Price, T. A. Woolsey, *Brain Res.* **37**, 21 (1972).
11. The thalamic terminology and nuclear outlines are based on generally accepted principles of thalamic parcellation in the rat [R. D. Lund and K. E. Webster, *J. Comp. Neurol.* **130**, 301 (1967); H. C. Hughes, *ibid.* **175**, 311 (1977); R. L. M. Faull and J. B. Carman, *ibid.* **178**, 495

(1978); J. P. Donoghue, K. L. Kerman, F. F. Ebner, *ibid.* **183**, 647 (1979); M. Herkenham, in (*9*)].
12. The cortical areas are given general names based on electrophysiological evidence [C. N. Woolsey, in *Biological and Biochemical Bases of Behavior*, H. F. Harlow and C. N. Woolsey, Eds. (Univ. of Wisconsin Press, Madison, 1958), p. 63; C. Welker, *Brain Res.* **26**, 259 (1971); R. D. Hall and E. P. Lindholm, *ibid.* **66**, 23 (1974)] and cytoarchitecture [W. J. S. Krieg, *J. Comp. Neurol.* **84**, 221 (1946); V. S. Caviness, *ibid.* **164**, 247 (1975)].
13. Variously placed small injections show that the ventrolateral and anterior portions of the VL complex have cortical projections that are largely limited to the motor area, whereas the dorsal and medial portions have widespread projections as described. Although cytoarchitectural distinctions are lacking, by analogy to known widespread connections of similarly located portions of the ventrolateral and ventroanterior nuclei in cats and primates, the medial region in the rat may ultimately be shown to be a hodologically distinct area [J. Kievet and H. G. J. M. Kuypers, *Exp. Brain Res.* **29**, 299 (1977); D. L. Kasdon and S. Jacobson, *J. Comp. Neurol.* **177**, 685 (1978); S. H. C. Hendry, E. G. Jones, J. Graham, *ibid.* **185**, 679 (1979)].
14. M. Herkenham, *J. Comp. Neurol.* **177**, 589 (1978).
15. H. H. Jasper, in *Brain Mechanisms and Consciousness*, J. F. Delafresnaye, Ed. (Blackwell, Oxford, 1954), p. 374; W. J. H. Nauta, in *Advances in Neuroendocrinology*, A. V. Nalbandov, Ed. (Univ. of Illinois Press, Urbana, 1963), p. 5.
16. T. P. S. Powell and W. M. Cowan, *J. Anat. (London)* **88**, 307 (1954); E. G. Jones and R. Y. Leavitt, *J. Comp. Neurol.* **154**, 349 (1974).
17. W. J. H. Nauta and H. J. Karten, in *The Neurosciences: Second Study Program*, F. O. Schmitt, Ed. (Rockefeller Univ. Press, New York, 1970), p. 7.
18. J. B. Angevine and R. L. Sidman, *Nature (London)* **192**, 766 (1961); M. Berry and A. W. Rogers, *J. Anat. (London)* **99**, 691 (1965); P. Rakic, *J. Comp. Neurol.* **145**, 61 (1972).
19. D. A. Sholl, *The Organization of the Cerebral Cortex* (Hafner, New York, 1956); E. G. Jones, *J. Comp. Neurol.* **160**, 205 (1975); E. L. White, *ibid.* **181**, 627 (1978).
20. I thank R. Gunn for histological assistance, S. P. Wise for critically reading the manuscript, and E. V. Evarts and W. J. H. Nauta for their continuous encouragement and support.

22 August 1979

Erythrosine (Red No. 3) and Its Nonspecific Biochemical Actions: What Relation to Behavioral Changes?

Abstract. *Biochemical studies have shown that the ability of erythrosine to inhibit dopamine uptake into brain synaptosomal preparations is dependent on the concentration of tissue present in the assay mixture. Thus, the finding that erythrosine inhibits its dopamine uptake (which, if true, would provide a plausible explanation of the Feingold hypothesis of childhood hyperactivity) may simply be an artifact that results from nonspecific interactions with brain membranes. In addition, although erythrosine given parenterally (50 milligrams per kilogram) did not alter locomotor activity of control or 6-hydroxydopamine-treated rats, erythrosine (50 to 300 milligrams per kilogram) attenuated the effect of punishment in a "conflict" paradigm.*

The prevalence of hyperkinesis and learning disabilities among children, and the fact that therapy with stimulant drugs is not entirely satisfactory (*1*) have made the search for alternative forms of treatment for these problems an important issue. Several years ago, Feingold (*2*) reported that removal of synthetic food colors and other ingredients from the diet of hyperactive children could dramatically eliminate the symptoms in about half of them. The impressive results reported by advocates of this treatment have been evaluated by many groups with the general finding that open studies support Feingold's contentions, whereas closed clinical trials and blind challenge studies have been essentially negative (*3*). Little attention has been given to animal or biochemical studies that might define possible mechanisms underlying the proposed behavioral toxicity of the food dyes. The difficulty in selecting a suitable model for study has been one reason for neglecting this area of research.

Recently, Logan and Swanson (*4*) reported that the mixture of eight food dyes commonly used in pediatric clinical studies caused a significant inhibition of neurotransmitter uptake in homogenates of rat brain. In examining this finding further, they reported that one of the dyes in the mixture, erythrosine (Erythrosin B; FD & C Red No. 3) was responsible for this effect, and that it alone inhibited the uptake of all the transmitters tested. The other dyes were ineffective. Lafferman and Silbergeld (*5*) have confirmed that dopamine uptake into crude synaptosomes is significantly inhibited by erythrosine. The suggestion that one dye might be critical in producing the alleged detrimental effects of the food colors coincided with our interest in hyperkinesis, and prompted us to attempt to replicate these findings and to assess their effect on behavior. We now report that the potency of erythrosine in vitro to inhibit neurotransmitter uptake is dependent on the concentration of tissue present in the incubation system. Thus, the findings of Logan and Swanson (*4*), or Lafferman and Silbergeld (*5*), can be essentially confirmed or refuted depending on the experimental conditions chosen. We also report on behavioral studies in rats that suggest that high doses of erythrosine are active in a paradigm also sensitive to drugs that alter hyperkinetic behavior in children (*6*).

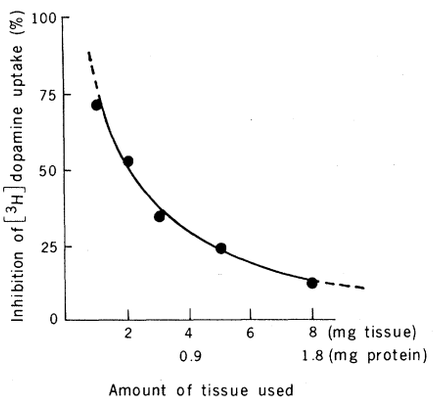

Fig. 1. Ackermann-Potter (*8*) representation of the effects of tissue concentration on [^{3}H]dopamine uptake from a crude synaptosomal preparation from rat striatum. Dopamine uptake was measured essentially by the method of Ferris *et al.* [see (*7*)]. Each data point represents the mean of four separate experiments. The standard error for each data point is never greater than 10 percent of the magnitude of its particular mean.

In our initial experiments (*7*) we failed to confirm the finding reported by others (*4*, *5*) that erythrosine could inhibit the uptake of [^{3}H]norepinephrine or [^{3}H]dopamine into crude synaptosomal preparations of striatum, hypothalamus, or whole brain from Sprague-Dawley rats. In an attempt to resolve this discrepancy, we varied several conditions of the uptake system. These included incubation temperature (0°, 25°, and 37°C), time of incubation with the drug (5, 15, or 30 minutes), presence or absence of light and oxygen, substrate concentration ($10^{-7}M$ and $10^{-8}M$ for norepinephrine, dopamine, or serotonin), erythrosine concentration (0.1 μM to 100 μM), and the concentration of synaptosomal protein present in the medium.

We found that one of the conditions, the concentration of synaptosomal protein present in the incubation medium, influenced significantly the calculated potency of erythrosine as an inhibitor of catecholamine uptake into synaptosomes obtained from all brain areas studied. This is illustrated in Fig. 1, which is a graphical presentation after the suggestions of Ackermann and Potter (*8*) for the case of pseudo-irreversible inhibition. These data clearly show that when the concentration of erythrosine and the concentration of dopamine in the incubation medium are held constant, different degrees of inhibition of dopamine uptake by erythrosine can be achieved by simply varying the concentration of striatal synaptosomal protein present in the medium. The percentage inhibition of dopamine uptake is not directly pro-

0036-8075/80/0201-0535$00.50/0