References and Notes

1. M. A. Ondetti, N. J. Williams, E. F. Sabo, J. Pluščec, E. R. Weaver, O. Kocy, *Biochemistry* **10**, 4033 (1971); abbreviations used are as follows: < Glu, pyroglutamic acid; Trp, tryptophan; Pro, proline; Arg, arginine, Gln, glutamine; Ile, isoleucine.
2. B. Rubin, E. H. O'Keefe, D. G. Kotler, D. A. DeMaio, D. W. Cushman, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **34**, 770 (1975); E. H. O'Keefe, D. G. Kotler, M. H. Waugh, B. Rubin, *ibid.* **31**, 511 (1972); H. S. Cheung and D. W. Cushman, *Biochim. Biophys. Acta* **293**, 451 (1973).
3. S. L. Engel, T. R. Schaeffer, B. I. Gold, B. Rubin, *Proc. Soc. Exp. Biol. Med.* **140**, 240 (1972); S. L. Engel, T. R. Schaeffer, M. H. Waugh, B. Rubin, *ibid.* **143**, 483 (1973).
4. H. Gavras, H. R. Brunner, J. H. Laragh, J. E. Sealey, I. Gavras, R. A. Vukovich, *N. Engl. J. Med.* **291**, 817 (1974); H. Gavras, H. R. Brunner, J. H. Laragh, I. Gavras, R. A. Vukovich, *Clin. Sci. Mol. Med.* **48**, 57S (1975); J. G. Johnson, W. D. Black, R. A. Vukovich, F. E. Hatch, Jr., B. I. Friedman, C. F. Blackwell, A. N. Shenouda, L. Share, R. E. Shade, S. R. Acchiardo, E. E. Muirhead, *ibid.*, p. 53S.
5. D. W. Cushman and H. S. Cheung, *Biochem. Pharmacol.* **20**, 1637 (1971).
6. Since angiotensin-converting enzyme is also an active bradykinin-inactivating enzyme [see E. G. Erdös, *Circ. Res.* **36**, 247 (1975)], even the most specific inhibitors of this enzyme have two primary biological activities: inhibition of the effects of angiotensin I, and augmentation or prolongation of the effects of bradykinin.
7. D. W. Cushman, J. Pluščec, N. J. Williams, E. R. Weaver, E. F. Sabo, O. Kocy, H. S. Cheung, M. A. Ondetti, *Experientia* **29**, 1032 (1973); D. W. Cushman and H. S. Cheung, in *Hypertension '72*, J. Genest and E. Koiw, Eds. (Springer, Berlin, 1972), p. 532; Y. E. Elisseeva, V. N. Orekhovich, L. V. Pavlikhina, L. P. Alexeenko, *Clin. Chim. Acta.* **31**, 413 (1971).
8. M. Das and R. L. Soffer, *J. Biol. Chem.* **250**, 6762 (1975).
9. F. Quiocho and W. N. Lipscomb, *Adv. Protein Chem.* **25**, 1 (1971).
10. L. D. Byers and R. Wolfenden, *Biochemistry* **12**, 2070 (1973).
11. J. W. Bunting and C. D. Myers, *Can. J. Chem.* **52**, 2053 (1974).
12. R. J. Laffan, A. Peterson, S. W. Hitch, J. Jeunelot, *Cardiovasc. Res.* **6**, 319 (1972); A. Bianchi, D. B. Evans, M. Cobb, M. T. Peschka, T. R. Schaeffer, R. J. Laffan, *Eur. J. Pharmacol.* **23**, 90 (1973).
13. H. Gavras, H. R. Brunner, H. Thurston, J. H. Laragh, *Science* **188**, 1316 (1975); J. R. Douglas, Jr., E. M. Johnson, Jr., J. F. Heist, G. R. Marshall, P. Needleman, *J. Pharmacol. Exp. Ther.* **196**, 35 (1976).

14. We thank E. F. Sabo, H. S. Cheung, J. Baumann, D. DeMaio, J. P. High, D. G. Kotler, E. H. O'Keefe, T. R. Schaeffer, and M. H. Waugh for technical assistance; Drs. M. E. Goldberg, R. J. Laffan, and F. L. Weisenborn for suggestions and advice; and Dr. Z. P. Horovitz for encouragement and support throughout the course of our studies.

6 December 1976; revised 1 February 1977

Major Innervation of Newborn Rat Cortex by Monoaminergic Neurons

Abstract. *A major monoaminergic innervation in infant rat neocortex, predominantly in layer IV, has been demonstrated by ultrastructural and biochemical studies after the administration of exogenous catecholamine precursors and congeners. One-third of all cortical synapses have an uptake-storage mechanism for catecholamines. In newborn cortex, the storage capacity for catecholamines is tenfold greater than the endogenous levels, and the uptake-storage mechanism matures earlier than the ability to synthesize neurotransmitter.*

Noradrenergic neurons with cell bodies in the pons give rise to a widespread innervation of the cerebral cortex (*1*). Dopaminergic neurons with cell bodies in the midbrain contribute an additional innervation to restricted areas of the neocortex (*2*). Despite the extensive projection of these catecholaminergic axons, it is estimated that they form less than 1 percent of the total synaptic contacts in the adult cerebral cortex (*3*).

In the neocortex of perinatal rats, the density of aminergic terminals, as revealed by histofluorescence methods, appears to be extremely low (*4*), and the levels of catecholamine synthesizing enzymes and endogenous amines in neocortex remain relatively low throughout the newborn period (*5, 6*). Yet the major catecholaminergic neurons are formed and undergo differentiation at early stages of fetal development (*7*), and their axons reach the neocortex by 1 week before birth (*8*). Either there is a long delay between the arrival of aminergic axons in the cortex and the subsequent formation of synapses, or immature catecholaminergic axons innervate the cortex at birth but are deficient at that age in their ability to synthesize or transport endogenous catecholamines. To address this problem, we have attempted to characterize the monoaminergic innervation of immature cortex by an approach not dependent upon the presence of endogenous neurotransmitters. The in vivo uptake of exogenous catecholamine precursors and congeners by the cortex was studied by electron microscopic and biochemical techniques.

Monoaminergic terminals can be identified at the ultrastructural level by the presence of small (40- to 50-nm) granular vesicles (SGV's), which are the storage sites for the amines (*9*). The demonstration of SGV's in the central nervous system is enhanced by exposure of tissue to the catecholamine congener 5-hydroxydopamine (5-OHDA) (*10*). This "false" neurotransmitter is selectively taken up and concentrated in the synaptic vesicles of monoaminergic nerve terminals, where, after aldehyde fixation, it forms an electron-opaque precipitate. This indirect method permits the ultrastructural analysis of those synaptic terminals that have an uptake-storage mechanism specific for monoamines (*10, 11*). Since the immature blood-brain barrier is permeable (*12*), systemic administration of 5-OHDA was used to study the distribution of monoaminergic terminals in the neocortex of infant rats (*13*).

The morphology and distribution of synapses in the lateral neocortex of infant rats (from birth to 7 days old) were analyzed with the electron microscope [for details see (*13–15*)]. Interneuronal appositions with characteristic vesicles (30 to 50 nm in diameter) adjacent to an area of membrane specialization were designated as synapses. The density of synapses in newborn cortex is extremely low compared with that in the adult. In rats from birth to 7 days old, treated with 5-OHDA, 30 percent of all synaptic terminals in the lateral neocortex contain SGV's; these vesicles are round, 40 to 50 nm in diameter, and contain a dense, eccentric, electron-opaque granule (Fig. 1). In control animals (untreated), all synaptic terminals contain clear (that is, empty) vesicles. In rats that have been treated with reserpine (10 mg per kilogram of body weight) 12 hours before 5-OHDA, no SGV's can be demonstrated. Hence the vesicular accumulation of 5-OHDA is reserpine-sensitive and probably is restricted to monoaminergic terminals.

By 1 week of age, the six cytoarchitectonic layers characteristic of mature cortex can be recognized. The synapses—still relatively sparse—are concentrated in strata that are parallel to the surface (*15*). In 5-OHDA-treated rats, the radial distribution of SGV synapses is characteristic and highly reproducible: in layer IV (the deep third of the cortical plate) more than 70 percent of the synaptic terminals contain SGV's (*13*). In other cortical layers, SGV synapses are sparse, except in the marginal zone where 10 to 30 percent of the boutons contain SGV's. Many of the SGV boutons form synapses de passage as is characteristic of central catecholaminergic neurons, and in layer IV many are on proximal dendrites. The use of 5-OHDA reveals, in immature cortex, a previously unrecognized set of axon terminals deficient in endogenous transmitter but with the capacity to take up and store this catecholamine congener. We attempted to confirm and characterize this uptake-storage capacity by application of biochemical methods.

Rats of various ages were treated with the catecholamine precursor L-dopa. Subsequently, dopamine and norepinephrine levels in neocortex were measured by a sensitive radiometric enzymat-

ic assay (*6*). Since L-dopa can be decarboxylated in nonaminergic cells with a resulting diffuse or unspecific increase in dopamine levels (*16*), that portion of catecholamines which was localized in a reserpine-sensitive storage site was determined. Rats first treated with reserpine were compared to rats not treated with reserpine with respect to the amount of catecholamines that accumulated in the lateral neocortex after the administration of L-dopa; the difference between the amine levels in the two treatment groups (Table 1) indicates the specific storage capacity for monoamines.

Two hours after systemic administration of L-dopa to newborn rats, the neocortical concentrations of norepinephrine and dopamine increase 4- and 45-fold, respectively (*17*). In newborns treated with reserpine before L-dopa, the concentrations of norepinephrine and dopamine increase only 1.5- and 20-fold, respectively. Thus, a major proportion (60 percent) of the increase in catecholamines induced by L-dopa is localized in a reserpine-sensitive site. These data reveal a storage compartment specific for catecholamines with a capacity of 1362 pg per milligram of tissue, which is ten times greater than the endogenous stores (Table 1). Similarly, in the 6-day-old rat, L-dopa treatment uncovers a reserpine-sensitive storage capacity of 796 pg per milligram of tissue, which is nearly eight times greater than the basal stores.

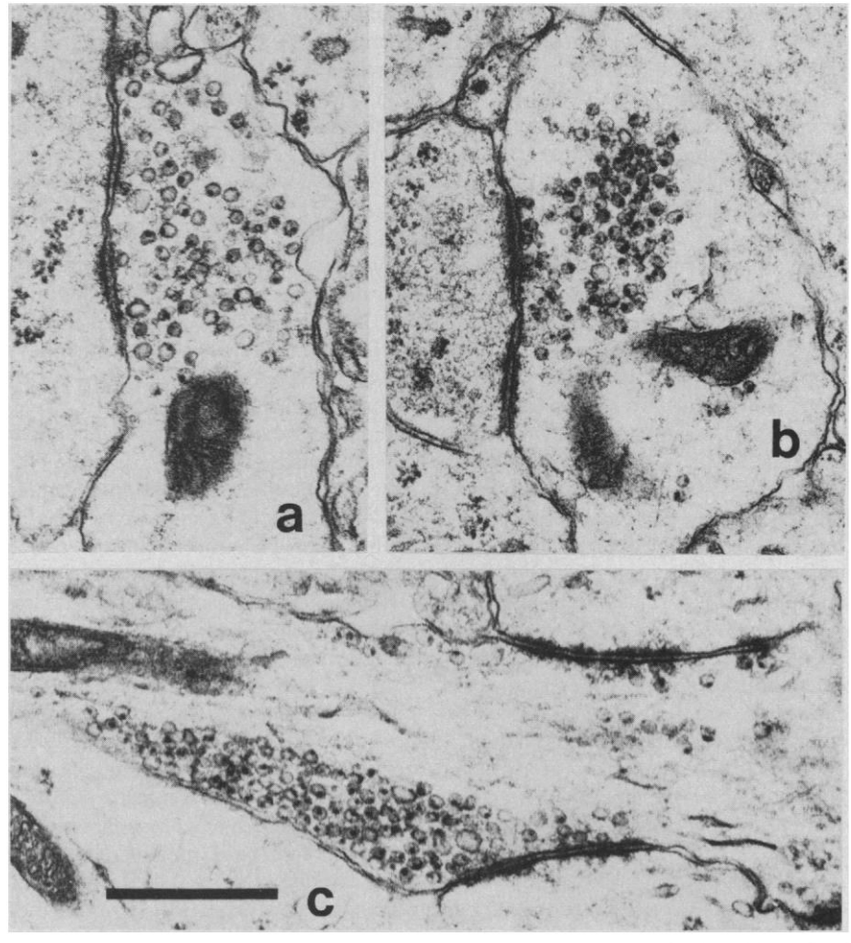

Fig. 1. Electron micrographs of aminergic synapses in lateral neocortex of 6-day-old rat. Small granular vesicles are seen after administration of 5-OHDA. These are true synapses with asymmetric membrane specializations. Axon in (c) forms two synapses de passage. Aldehyde-osmium fixation; bar = 500 nm.

Table 1. Effects of pharmacologic treatments on the levels of catecholamines in parietal cortex of the rat. Sprague-Dawley rats were administered reserpine (2.5 mg/kg) or an equivalent volume of vehicle by subcutaneous injection 6 to 8 hours before being killed. Two hours before being killed, they received L-dopa (100 mg/kg) by subcutaneous injection. In one experiment, adult rats were treated with the peripheral decarboxylase inhibitor RO-4063 (50 mg/kg) 30 minutes before administration of L-dopa (250 mg/kg). In another experiment, 3-day-old rats were administered 5-OHDA (5 mg per animal). The rats were killed by decapitation, and the lateral cortex was dissected at 2°C with care to exclude the tail of the caudate. Cortices were homogenized in 50 to 100 volumes of 0.1*N* perchloric acid, and the catecholamines in the extract were determined by the radiometric-enzymatic assay of Coyle and Henry (*6*). The levels of apparent 5-OHDA in the extracts from animals treated with this agent were determined by the radiometric-enzymatic assay with internal and external standards of authentic 5-OHDA to correct for recovery. Values are expressed as picograms of catecholamine per milligram (wet tissue) lateral cortex with standard errors of the mean indicated; each value is a mean derived from at least five animals.

Age	Treatment	Catecholamine (pg/mg)			
		Dopamine	Norepinephrine	Total catecholamine	Reserpine-sensitive catecholamines (A − B)
1 day	None	37 ± 10	106 ± 38	142 ± 42	
	A. L-Dopa (100 mg/kg)	1854 ± 206*	413 ± 103†	2267 ± 280*	
	B. L-Dopa (100 mg/kg) + reserpine (2.5 mg/kg)	754 ± 56	151 ± 30	905 ± 81	1362
6 days	None	41 ± 13	74 ± 10	115 ± 20	
	A. L-Dopa (100 mg/kg)	1010 ± 129*	195 ± 17*	1205 ± 145*	
	B. L-Dopa (100 mg/kg) + reserpine (2.5 mg/kg)	362 ± 47	47 ± 7	409 ± 42	796
Adult	None	104 ± 21	209 ± 37	313 ± 35	
	A. L-Dopa (100 mg/kg)	126 ± 26†	188 ± 52†	314 ± 39*	
	B. L-Dopa (100 mg/kg) + reserpine (2.5 mg/kg)	40 ± 9	21 ± 9	61 ± 8	253
Adult	A. RO-4063 (50 mg/kg) + L-dopa (250 mg/kg)	887 ± 95	254 ± 32*	1141 ± 124	
	B. RO-4063 (50 mg/kg) + L-dopa (250 mg/kg) + reserpine (2.5 mg/kg)	746 ± 73	94 ± 4	840 ± 79	301
3 days	None			122 ± 56	
	A. 5-OHDA (5 mg per rat)			1330 ± 74*	
	B. 5-OHDA (5 mg per rat) + reserpine (2.5 mg/kg)			684 ± 106	646

*A versus B: $P < .01$ (Student's t-test). †A versus B: $P < .05$.

Identical treatment in the adult rat does not significantly increase the cortical levels of catecholamines. In newborn rats, the activities of dopa decarboxylase and catecholamine catabolizing enzymes outside the brain are much lower and the blood-brain barrier is much less effective than in the adult; these differences may explain the lack of effect of L-dopa (100 mg/kg) on the catecholamine levels in adult neocortex. To better control for such ontogenetic differences, adult rats were first treated with a peripheral decarboxylase inhibitor and higher doses of L-dopa (250 mg/kg). This latter treatment regimen considerably increases the catecholamine levels in the adult cortex but the increase is in a reserpine-resistant (that is, nonspecific) compartment. Thus, in the adult neocortex, the reserpine-sensitive binding sites for catecholamines are normally fully occupied by endogenous amines; treatment with high doses of L-dopa does not reveal any latent additional capacity for specific catecholamine storage. These precursor loading studies indicate that immature neocortex can take up and store catecholamines in a reserpine-sensitive compartment with a capacity (1362 pg/mg) that exceeds the endogenous stores (142 pg/mg) by eight- to tenfold and even exceeds the adult storage capacity (253 pg/mg) by fivefold.

Since 5-OHDA, the marker used in the electron microscopy studies, is a catechol, it is a substrate for catechol-*O*-methyltransferase and can be measured by the enzymatic radiometric assay for catecholamines (*18*). Two hours after a single subcutaneous injection of 5 mg of 5-OHDA in 3-day-old rats, catecholamine levels in neocortex increase tenfold. Prior treatment with reserpine reduces the apparent accumulation of 5-OHDA by 50 percent, revealing a specific storage capacity for 5-OHDA in lateral cortex of nearly 700 pg/mg (more than fivefold greater than the basal catecholamine levels). Thus, the biochemical results corroborate the electron microscopic observations that in the immature neocortex significant quantities of 5-OHDA can be stored in a reserpine-sensitive compartment that, in untreated rats, is only partially occupied.

The density of all types of synapses in the newborn cortex is extremely low as compared with that in adults (*15*). Despite the paucity of synapses, our morphologic and biochemical results demonstrate that monoaminergic terminals make a major contribution to the innervation of immature rat neocortex (*19*). The monoaminergic innervation may be one of the earliest cortical inputs established. Its density is subsequently diminished by the formation of more numerous synapses of other types; in addition, immature aminergic axons may form synapses that are temporary. The relatively dense monoaminergic innervation to layer IV of the immature cortex is probably a unique but transient feature of young cortex since monoaminergic synapses in mature cortex are reported to be sparse (*3*), especially below layer I. The early differentiation of the monoaminergic neurons, which have axonal projections reaching the cortex before birth, points to the catecholaminergic nuclei in the brainstem as a likely source of this innervation; a possible contribution from the indoleamine nuclei must also be considered. The possibility that immature neurons, which are not monoaminergic, may take up 5-OHDA and synthesize and store catecholamines in a reserpine-sensitive site has not been excluded (*19a*).

In this study we have demonstrated a correspondence between the high proportion of SGV synapses in newborn cortex and the substantial uptake-storage capacity for monoamines. The relatively low levels of catecholamines in young neurons has hampered heretofore the demonstration of their terminals in immature neocortex by standard histofluorescence techniques. This low concentration of catecholamines in immature cortex reflects the low activity of the biosynthetic enzymes for the neurotransmitters (*5*), but does not reflect the storage capacity or the full extent of the catecholamine innervation. We propose that the maturation of the uptake-storage mechanism of monoamine terminals precedes and is independent of the mechanism for neurotransmitter synthesis (*20*) and that the presence of neurotransmitter in axon terminals is not a necessary condition for synaptogenesis.

The functional significance of this early monoaminergic input to the immature neocortex is yet to be defined. Amphetamine and L-dopa, the actions of which are dependent on the presence of presynaptic catecholaminergic terminals, produce characteristic behavioral responses in the newborn rat, which suggests that functional catecholaminergic synapses and receptors are present at this stage of development (*21*). Because of the high proportion of SGV synapses in layer IV of the cortex, monoaminergic neurons may exert a potent regulatory influence on the neurons that receive the thalamic input (*22*) to the immature cortex. Thus, cortical activity in the infant may be more responsive to aminergic input from the brainstem than to specific thalamocortical inputs. This transient innervation may also influence neuronal differentiation. Transsynaptic activation of dopamine or norepinephrine receptors (or both), which are specific adenylate cyclases (*23*), may regulate the intracellular concentration of adenosine 3′,5′-monophosphate, which modulates several aspects of neuronal differentiation (*24*). Alternatively, in light of the low levels of neurotransmitters in these terminals, the monoaminergic processes may play a role not primarily related to neurotransmission but rather may exert a trophic influence on dendrite growth (*25*) or act as "pioneer" synapses that may induce postsynaptic membrane specialization (*26*).

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References and Notes

1. K. Fuxe, *Acta Physiol. Scand.* **64** (Suppl. 247), 39 (1965); U. Ungerstedt, *Acta Physiol. Scand. Suppl.* **367**, 1 (1971); K. Fuxe, B. Hamberger, T. Hökfelt, *Brain Res.* **8**, 125 (1968).
2. K. Fuxe, T. Hökfelt, O. Johansson, G. Jonsson, P. Lidbrink, A. Ljungdahl, *Brain Res.* **82**, 349 (1974); O. Lindvall, A. Björklund, R. Y. Moore, U. Stenevi, *ibid.* **81**, 325 (1974); B. Berger, J. P. Tassin, G. Blanc, M. A. Mayne, A. M. Thierry, *ibid.*, p. 332.
3. F. E. Bloom, *Int. Rev. Neurobiol.* **13**, 27 (1970); T. Hökfelt, *Z. Zellforsch. Mikrosk. Anat.* **91**, 1 (1968); Y. Lapierre, A. Beaudet, N. Demianczuk, L. Descarries, *Brain Res.* **63**, 175 (1973); T. Hökfelt and Å. Ljungdahl, *Adv. Biochem. Psychopharmacol.* **6**, 1 (1972).
4. L. Loizou, *Brain Res.* **40**, 395 (1972).
5. J. T. Coyle and J. Axelrod, *J. Neurochem.* **19**, 449 (1972); *ibid.*, p. 1117; W. Porcher and A. Heller, *ibid.*, p. 1917; F. Lamprecht and J. T. Coyle, *Brain Res.* **41**, 503 (1972).
6. J. T. Coyle and D. Henry, *J. Neurochem* **21**, 61 (1973).
7. J. T. Coyle, in *The Neurosciences, Third Study Program*, F. O. Schmitt and F. G. Worden, Eds. (MIT Press, Cambridge, Mass., 1974), pp. 877–884; J. M. Lauder and F. E. Bloom, *J. Comp. Neurol.* **155**, 469 (1974).
8. L. Olson and Å. Seiger, *Z. Anat. Entwicklungsgesch.* **137**, 301 (1972); Å. Seiger and L. Olson, *ibid.* **140**, 281 (1973).
9. F. E. Bloom, *J. Histochem. Cytochem.* **21**, 333 (1973); T. Hökfelt, *Z. Zellforsch. Mikrosk. Anat.* **91**, 1 (1968); D. E. Wolfe, L. T. Potter, K. C. Richardson, J. Axelrod, *Science* **138**, 440 (1962).
10. J. Tranzer and H. Thoenen, *Experientia* **23**, 743 (1967); J. G. Richards and J. P. Tranzer, *Brain Res.* **17**, 463 (1970).
11. K. Ajika and T. Hökfelt, *Brain Res.* **57**, 97 (1973); S. C. Landis and F. E. Bloom, *ibid.* **96**, 299 (1975).
12. L. A. Loizou, *Br. J. Pharmacol.* **40**, 800 (1970); C. Sachs, *J. Neurochem.* **20**, 1753 (1973); B. Singh and J. DeChamplain, *Brain Res.* **48**, 432 (1972); J. C. Dupin, L. Descarries, J. deChamplain, *ibid.* **103**, 588 (1976).
13. M. E. Molliver and D. A. Kristt, *Neurosci. Lett.* **1**, 305 (1975).
14. M. E. Molliver and H. Van der Loos, *Ergeb. Anat. Entwicklungsgesch.* **42**, 1 (1970).
15. D. A. Kristt and M. E. Molliver, *Brain Res.* **108**, 180 (1976).
16. J. A. Romero, L. D. Lytle, L. A. Ordonez, R. J. Wurtman, *J. Pharmacol. Exp. Ther.* **184**, 67 (1973).

17. These increases in catecholamine levels in immature rat brain after treatment with L-dopa are consistent with previous reports: C. Kellogg and P. Lundborg, *Psychopharmacologia* **25**, 187 (1972); J. T. Coyle, in *Dynamics of Degeneration and Growth in Neurons*, K. Fuxe, L. Olson, and Y. Zotterman, Eds. (Pergamon, Oxford, England, 1974), pp. 425–434.
18. H. C. Goldberg and C. A. Marsden, *Pharmacol. Rev.* **27**, 135 (1975).
19. Similarly, histofluorescent "terminals" were visualized in smear preparations of human fetal cortex after incubation in α-methylnorepinephrine [L. Olson, L. O. Boreus, A. Seiger, *Z. Anat. Entwicklungsgesch.* **139**, 259 (1973)].
19a. *Note added in proof:* That this possibility merits consideration is supported by recent studies demonstrating that sympathetic neurons in culture may synthesize, take up, and store different neutrotransmitters at successive stages of development, dependent upon the culture conditions [M. Johnson, D. Ross, M. Meyers, R. Bunge, *Neurosci. Abstr.* **II**, 766 (1976); S. Landis, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 4220 (1976); L. Reichert, P. Patterson, L. Chun, *Neurosci. Abstr.* **II**, 225 (1976)]. However, preliminary results from our laboratory reveal that the SGV synapses in immature neocortex are not derived from intrinsic or thalamic neurons since they are significantly decreased after brainstem lesions of the ascending noradrenergic axons (N. Zecevic and M. E. Molliver, in preparation).
20. Similarly, in fetal rabbit striatum a large uptake-storage capacity for catecholamines was demonstrated despite low levels of endogenously synthesized dopamine [R. E. Barrett, L. Coté, V. M. Tennyson, C. Mytilineou, *J. Neuropathol. Exp. Neurol.* **31**, 166 (1972)]. Likewise, in immature rat hippocampus, the high affinity uptake for norepinephrine develops ahead of the capacity to synthesize endogenous norepinephrine [R. Smith and R. Y. Moore, *Neurosci. Abstr.* **II**, 228 (1976)].
21. H. C. Fibiger, L. D. Lytle, B. A. Campbell, *J. Comp. Physiol. Psychol.* **72**, 384 (1970); M. Dewyngaert and C. Kellogg, *Brain Res.* **73**, 175 (1974); E. G. McGeer, H. C. Fibiger, V. Wickson, *ibid.* **32**, 433 (1971).
22. S. P. Wise, *Brain Res.* **90**, 139 (1975).
23. J. W. Kebabian, G. L. Petzold, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2145 (1972); S. Kakiuchi and T. W. Rall, *Mol. Pharmacol.* **4**, 367 (1968); T. W. Rall and A. G. Gilman, *Neurosci. Res. Program Bull.* **8**, 221 (1970).
24. K. N. Prasad, S. Kamer, K. Gilmer, A. Vernadakis, *Biochem. Biophys. Res. Commun.* **50**, 973 (1973); R. Simantov and L. Sachs, *Eur. J. Biochem.* **30**, 123 (1972); F. J. Roisen, R. A. Murphy, W. G. Braden, *Science* **177**, 809 (1972); B. K. Schrier and D. L. Shapiro, *Exp. Cell Res.* **80**, 459 (1973).
25. T. Maeda, M. Tohyama, N. Shimizu, *Brain Res.* **70**, 515 (1974).
26. In the immature cortex, aminergic synapses have specialized membrane appositions, whereas in the adult central nervous system, monoaminergic boutons are only rarely observed with junctional specializations [L. Descarries, A. Beaudet, K. C. Watkins, *Brain Res.* **100**, 563 (1975); V. M. Tennyson, R. Heikkila, C. Mytilineou, L. Coté, G. Cohen, *ibid.* **82**, 341 (1974)]. Thus, membrane specializations may be induced by aminergic boutons in immature cortex and later become postsynaptic to other types of terminals.
27. Supported by PHS grants MH-26654, DA-00266, NS-08153, NS-10920, and NS-11034 and by United Cerebral Palsy grant R244-71. We thank R. Zaczek and P. Taylor for expert technical assistance and D. Kristt for collaboration on preliminary morphologic studies.

22 July 1976

Trigeminal Substrates of Intracranial Self-Stimulation in the Brainstem

Abstract. *Intracranial self-stimulation was elicited by electrodes located in the trigeminal motor nucleus of the rat. Rebound jaw movements were also elicited at positive self-stimulation placements, but control experiments revealed that the lever pressing was not a motor artifact. It is suggested that modulation of trigeminal motoneurons may serve as an important reinforcement mechanism in the brainstem.*

Intracranial self-stimulation (ICSS) at sites in the pontine region of the brainstem has been attributed to excitation of noradrenergic neurons located in the locus coeruleus (LC) (*1–3*). This hypothesis has been challenged recently by a number of experiments in which both whole brain depletion of noradrenaline (*4*) and localized neurotoxic (*5*) and electrolytic (*6*) lesions of the noradrenergic dorsal tegmental bundle ascending from the LC failed to disrupt ICSS in the LC. In the original mapping study that implicated the LC in ICSS, the investigators emphasized that repetitive jaw movements were an excellent indicator of positive placements and cautioned that one could not rule out the involvement of the trigeminal system in pontine ICSS (*1*). These observations are supported by the preponderance of oral rather than locomotor behavior elicited by electrical stimulation in the dorsal pontine area (*7*). We have now confirmed a trigeminal substrate of brain stimulation reward by showing that both ICSS and repetitive oral behavior are elicited from electrode placements in and immediately dorsal to the motor nucleus of the trigeminal nerve (Mot V).

The mesencephalic nucleus of the trigeminal nerve (Mes V) is situated lateral to and intermingled with the LC in the dorsal pontine tegmentum (*8*). This nucleus is comprised of neurons that innervate the masticatory muscles and whose central axons establish bilateral (*9*), monosynaptic (*10*), excitatory (*11*) connections with masticatory motoneurons in Mot V. An analysis of histological data from several studies of ICSS in the dorsal pons reveals many positive placements in Mes V (*1, 3, 5, 12*). Furthermore, placements in the LC are in such close proximity to this nucleus that its neurons could easily be influenced by current spread. If Mes V mediates ICSS and stimulation-bound oral behavior, then electrical stimulation of its terminal projections in Mot V should also provide positive reinforcement. Accordingly, we mapped the region of the brainstem in which Mot V is located for ICSS and stimulation-bound behavior (SBB).

Male Wistar rats, in which small-diameter (125 μm) bipolar electrodes had been stereotaxically implanted were tested for ICSS in five Plexiglas Skinner boxes (*13*). Animals were trained in daily 30-minute sessions to bar-press for brain stimulation. During the first 15 minutes of six daily test sessions, the experimenter "shaped" the bar-pressing response by initially reinforcing orientation to the manipulandum and later by rewarding contacts with the bar. In the second 15-minute period, spontaneous bar-presses were recorded. Those rats attaining a criterion of at least 50 spontaneous responses in 15 minutes on test day 7 were classified as having a positive ICSS placement. On completion of behavioral testing, each brain was prepared for histological analysis (*14*). Of the 66 placements, 29 supported ICSS; positive placements were located throughout and dorsal to Mot V (Fig. 1). Negative placements were found ventral to Mot V, laterally in the main sensory trigeminal nucleus, and medially in a region that would coincide with the ascending trajectory of both the noradrenergic central tegmental tract (*15*) and the adrenergic axon bundle (*16*). The mean of the responses at positive sites (per 15-minute period) was 148, and the current intensities employed ranged from 15 to 50 μa ($\bar{X}$ = 35 μa). In contrast, the mean rate obtained on test day 7 by animals with ineffective placements was 6, and by an unstimulated control group, 8.

If the trigeminal system plays an important role in brainstem ICSS, it is important to consider its relationship with other ICSS sites in the pons and medulla. The connection between Mes V and Mot V has already been emphasized. Preliminary unpublished observations appear to rule out involvement of the LC, as bilateral destruction of this nucleus by electrolytic lesions had no effect on ICSS in Mot V. Another important site for ICSS is located caudal to Mot V, in a region ventral to the solitary nucleus (*17*); this region is also innervated by the tract of Mes V (*10*). Stimulation of this area would produce antidromic excitation of trigeminal motoneurons; not surprisingly, jaw movements are an excellent predictor of ICSS at this site. Positive ICSS placements dorsal to Mot V (Fig. 1) may reflect activation of the intertrigeminal commissural pathway (*18*), which projects bilaterally to Mot V and the parvicellular reticular formation.

As a control for the possibility that bar-pressing was an artifact of biting elicited by stimulation of Mot V, the pattern