TELENCEPHALIC DISTRIBUTION OF TERMINALS OF BRAINSTEM NOREPINEPHRINE NEURONS*

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DURING the past 10 years there have been remarkable advances in the understanding of the organization and function of central monoamine-containing neuron systems. The first evidence associating monoamines with a specific group of central neurons was a demonstration that destruction of the medial forebrain bundle, or areas contributing axons to that pathway in the lateral hypothalamus, produced significant decreases in brain serotonin and norepinephrine in the rat (cf. HELLER *et al.*, 1968; HELLER and MOORE, 1965; MOORE, 1970). Subsequent studies showed that the loss of amines and their synthetic enzymes following medial forebrain bundle lesions occur uniformly throughout the telencephalon but that degenerating axons and terminals cannot be demonstrated by conventional neuroanatomical methods in all affected telencephalic areas (MOORE and HELLER, 1967; HELLER and MOORE, 1968). This discrepancy between the loss of amines and enzymes and the apparent distribution of anterograde axonal degeneration following the lesion led to introduction of the concept that the amine and enzyme changes occur, at least in part, as transsynaptic neurochemical effects within otherwise intact neurons. In contrast to this, studies employing the Falck–Hillarp histochemical method indicate that all monoamine-producing neuron cell bodies are located in the diencephalon and brainstem with axons and terminals distributed throughout the neuraxis (ANDEN *et al.*, 1966; FUXE *et al.*, 1970; UNGERSTEDT, 1971). Ungerstedt's (1971) work, in particular, has suggested that the nucleus locus coeruleus of the pontine tegmentum gives rise to most, if not all, of the norepinephrine terminals innervating telencephalic structures. The studies to be presented here were undertaken, first, to determine if evidence could be obtained for the existence of norepinephrine-producing neurons in denervated cerebral cortex and, second, to attempt providing evidence for a direct projection from the norepinephrine neurons of locus coeruleus throughout the telencephalon.

In the first study the auditory cortex of the cat was isolated from subcortical and other cortical areas with its blood supply intact and subsequently analyzed for norepinephrine content and the capacity to produce norepinephrine from intravenously administered, labelled tyrosine. If norepinephrine-producing neurons are present, both cell bodies and axons, in cortex, they should be preserved after isolation of the cortex and should continue to have the capacity to produce norepinephrine (cf. MOORE, 1970). In isolated cat auditory cortex, however, there is no assayable norepinephrine present and none is produced from labelled tyrosine even though the tyrosine enters cortex (Table 1). The greater tyrosine content of denervated cortex probably reflects the loss of white matter in these samples. Thus, no evidence is obtained from this study for the presence of norepinephrine-producing cells in cat neocortex.

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TABLE 1. NOREPINEPHRINE BIOSYNTHESIS FROM LABELLED TYROSINE IN THE ISOLATED CAT CEREBRAL CORTEX

Sample analysed	Tyrosine		Norepinephrine	
	(μg/g tissue)	(dis/min per n mole)	(μg/g tissue)	(dis/min per n mole)
Control Cortex	2.70	825	0.21	69
Isolated	4.65	665	—	—

Two cats were subjected to bilateral superior cervical ganglionectomy followed by complete unilateral isolation of auditory cortex (all cortex from the superasylvian gyrus to the temporal pole). Thirty days later each received an intraveneous injection of tyrosine-^{14}C and after one hour the auditory cortex from each side was removed and analysed for tyrosine and norepinephrine content and specific activity (ZIGMOND and WURTMAN, 1970). No fluorimetrically identifiable norepinephrine was present in isolated cortex nor were any counts above background present in the eluate off alumina.

In the second study the effect of restricted unilateral lesions in the locus coeruleus on telencephalic and diencephalic amines was analyzed. As can be seen in Table 2, localized locus coeruleus destruction produces a nearly total unilateral depletion of telencephalic norepinephrine without significantly affecting thalamic or hypothalamic norepinephrine levels. Similar lesions also were found to markedly diminish the formation of labelled norepinephrine from tyrosine in the telencephalon without affecting diencephalic synthesis (MOORE, unpublished observations). These observations, in accord with those of UNGERSTEDT (1971), support the concept of a direct axonal projection from the locus coeruleus to the entire telencephalon. Further corroboration for this is obtained from the third study in which tritiated leucine was injected into the locus coeruleus. The available evidence indicates that the amino acid will be incorporated into protein in the cell bodies of the nucleus and transported to terminals of the axons arising from the cells (COWAN *et al.*, 1972; MOORE and LENN, 1972). After injection of the locus coeruleus, labelled protein is transported to all areas of the telencephalon (Table 3). Because of the temporal parameters of the experiment, the labelled material would be transported within the rapid phase of axonal transport predominantly to axon terminals and without significant transneuronal transport (COWAN *et al.*, 1972; GRAFSTEIN, 1971). As with the lesion effects on norepinephrine levels, the transport of labelled material takes place largely unilaterally and throughout

TABLE 2. LOCUS COERULEUS LESION—EFFECT ON REGIONAL NOREPINEPHRINE LEVELS IN RAT BRAIN

Region analysed	Norepinephrine content (μg/g $\pm$ S.E.)		Percentage difference	P
	Control side	Lesion side		
Telencephalon	0·200 $\pm$ 0·017	0·007 $\pm$ 0·001	−97	$< 0{\cdot}001$
Thalamus	0·071 $\pm$ 0·063	0·562 $\pm$ 0·072	−2	N.S.
Hypothalamus	2·415 $\pm$ 0·382	2·140 $\pm$ 0·396	−11	N.S.

Unilateral locus coeruleus lesions were produced using a radiofrequency lesion maker. Animals were sacrificed 60 days later and brain samples were analysed for norepinephrine content by a modification of the method of Anton and Sayre (1962). P values were obtained using a two-tailed t-test for differences; N.S. refers to differences with $P > 0{\cdot}05$.

TABLE 3. RAPID AXONAL TRANSPORT OF LABELLED PROTEIN TO TELENCEPHALON FOLLOWING LOCUS COERULEUS INJECTION OF LABELLED AMINO ACID

Sample counted	Telencephalic region (dis/min per/mg tissue ± S.E.)							
	Olfactory bulb	Septum	Amygdala	Hippo-campus	Caudate-putamen	Frontal cortex	Temporal cortex	Occipital cortex
Injected side	91 ± 14	411 ± 43	212 ± 43	121 ± 24	170 ± 22	134 ± 28	82 ± 5	102 ± 5
Control side	48 ± 4	185 ± 23	82 ± 20	61 ± 8	66 ± 11	50 ± 5	45 ± 4	62 ± 12

Unilateral injections of tritated leucine (25 μCi in 1 μl saline were placed stereotaxically in the locus coeruleus. Animals were sacrificed 48 hr later, brains dissected and telencephalic regions counted. Site of injection was verified autoradiographically. Unilateral injections of an identical amount of tritiated leucine into the restiform body gave a homogeneous background bilaterally in all telencephalic structures with a mean of approximately 50 dis/min per/mg tissue.

the telencephalon. These data, then, are all in support of the view that norepinephrine in telencephalic structures is exclusively located in axons and terminals arising from neuronal cell bodies of the ipsilateral locus coeruleus.

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