*OLFACTORY BULB REMOVAL: EFFECTS ON BRAIN NOREPINEPHRINE**

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Abstract.—Removal of one olfactory bulb causes marked changes in the norepinephrine contents of several brain regions. The brainstem catecholamine level is higher on the side of the lesion than on the control side, whereas telencephalic norepinephrine is lower ipsilateral to the lesion. The apparent decline in telencephalic norepinephrine is associated with a parallel decrease in the ability of this region to take up and retain ^{3}H-norepinephrine injected into the lateral cerebral ventricle. Within the ipsilateral olfactory tubercle, there is also a significant decrease in the activity of the enzyme phenylethanolamine-*N*-methyl transferase, which catalyzes the conversion of norepinephrine to epinephrine. The results of this study suggest that measurement of changes in the uptake of ^{3}H-norepinephrine injected into the cerebral ventricle can be used as a technique for mapping central adrenergic pathways.

Certain brain lesions can alter the levels of biogenic amines in remote areas of the brain. For example, transection of the medial forebrain bundle is associated with a decrease in the norepinephrine content of the ipsilateral telencephalon.[1, 2] Two mechanisms have been proposed as explanations for this decrease: The lesions may destroy adrenergic axons with subsequent degeneration of their norepinephrine-containing nerve endings, or they may interrupt a physiologic input to other neurons whose ability to synthesize or store the amine requires this input (i.e., a transsynaptic effect).[3, 4]

We report here that another brain lesion, the removal or transection of the olfactory bulb in rats, is also associated with distant changes in brain norepinephrine content. The mechanism by which this lesion decreases telencephalic norepinephrine has been studied by examination of its effect on the ability of the telencephalon to take up and retain ^{3}H-norepinephrine placed in the lateral cerebral ventricles.

Recent experiments in our laboratory have shown that the activity of the enzyme phenylethanolamine-*N*-methyl transferase, which catalyzes the conversion of norepinephrine to epinephrine, is relatively high in the olfactory bulb and tubercle of the rat.[5] Hence, we have also examined the effect of removing the olfactory bulb on activity of this enzyme in the olfactory tubercle.

Materials and Methods.—Thirty female Sprague-Dawley rats weighing 200–250 gm were housed in a room maintained at 20°C and illuminated from 0600 to 1800 hours with Vita-Lite (Duro-Test Manufacturing Co., North Bergen, N.J.), a fluorescent light source which simulates the natural solar spectrum. Three animals were kept in each cage and given free access to Purina Chow and water. In 20 rats the left olfactory bulb was separated from the rest of the brain by transection at the olfactory peduncle;[6] 27 days after surgery 6 μc of ^{3}H-norepinephrine (6 mc/μM, New England Nuclear Co., Boston,

Mass.) in a total volume of 30 μl were injected into the lateral ventricle of the right hemisphere.[7] The animals were decapitated 24 hr later, and the brains were quickly removed. Each brain was dissected into five regions as described before,[5] except that the hippocampus and the adjoining amygdalo-piriform complex were not included in the part identified as telencephalon. The samples were frozen on dry ice and stored at −20°C until assayed. Each sample was weighed, homogenized in 1 ml of cold water, and centrifuged at 100,000 × *g* for 25 min. A 100-μl aliquot of the supernatant fluid was assayed for PNM transferase activity.[5, 8] The remainder of the sample, including the sediment, was mixed with 4 ml of 0.4 *N* $HClO_4$ and centrifuged again at 100,000 × *g* for 25 min. The catecholamines and ^{3}H-catecholamines present in the resulting supernatant fluid were extracted with alumina[9] and eluted with 0.2 *N* HCl. The ^{3}H-catecholamines in an aliquot of the eluate were mixed with a naphthalene-dioxane phosphor and assayed in a liquid scintillation spectrophotometer. The unlabeled norepinephrine in another aliquot was assayed fluorimetrically.[10]

Results.—Removal of the olfactory bulb was associated with a relative increase in the norepinephrine content of the brainstem ipsilateral to the lesion (Table 1). A similar tendency toward an increase was also noted in the hypothalamus on the side of the lesion; however, this change was not statistically significant. Telencephalic norepinephrine, on the other hand, showed a decrease on the operated side compared to the control side. The amount of ^{3}H-norepinephrine retained by the telencephalon 24 hours after its injection into the lateral ventricle was also depressed on the side of the lesion as compared to the unoperated side.

Small amounts of PNM transferase are found in mammalian brain; enzyme activity is highest in the olfactory bulb and tubercle.[5] The removal of an olfactory bulb was associated with a significant decline in the PNM transferase activity of the tubercle: enzyme activity fell from 35.2 ± 3.6 to 28.1 ± 3.0 mμmoles/gm of tissue ($P < 0.05$). The ability of the olfactory tubercle to store ^{3}H-norepinephrine placed in the lateral cerebral ventricle decreased by 30 per cent on the lesioned side; this decrease, however, was not statistically significant (Table 1).

Discussion.—Recent anatomical studies of the olfactory system of the rat have demonstrated that the overwhelming majority of the fibers from the ol-

TABLE 1. *Changes in the storage of norepinephrine and ^{3}H-norepinephrine in rat brain after unilateral destruction of an olfactory bulb.*

Region	Norepinephrine (ng/gm)		^{3}H-norepinephrine in normal and lesioned sides (% of normal side)	
	Normal	Lesioned	Normal	Lesioned
Brainstem	743.6 ± 16.1	866.6 ± 23.8†	100.0 ± 15.0	89.7 ± 18.7
Hypothalamus	1166.9 ± 80.0	1244.9 ± 64.0	100.0 ± 13.3	99.2 ± 15.7
Telencephalon	120.6 ± 5.2	79.1 ± 6.1†	100.0 ± 9.6	65.0 ± 12.0*
Olfactory tubercle	—	—	100.0 ± 17.0	70.0 ± 11.0

Groups of rats were subjected to the destruction or transection of a single olfactory bulb. ^{3}H-norepinephrine (6 μc) was placed in the right lateral ventricle. On the following day the rats were killed, and various brain regions were assayed for norepinephrine and ^{3}H-norepinephrine content. There was not sufficient endogenous norepinephrine in individual olfactory tubercles to permit their assay. Data are presented as the mean ± standard error of the mean.

* $P < 0.05$.
† $P < 0.001$.

factory bulb remain uncrossed and terminate profusely in the superficial layers of the olfactory peduncle, the olfactory tubercle, the prepiriform and periamygdaloid cortices, and the corticomedial amygdaloid nuclei.[6, 11, 12] Since a significant number of olfactory bulb fibers seem to reach as far caudally as the ventral entorhinal area, practically all basal regions of the cerebral hemisphere in the rat represent primary olfactory cortex. It therefore seems reasonable to expect any effect of olfactory bulb removal to be most marked in the basal telencephalic regions ipsilateral to the lesion. The decrease in telencephalic norepinephrine level ipsilateral to the lesion was accompanied by a parallel decrease in its retention of ^{3}H-norepinephrine. Therefore, the fall in endogenous norepinephrine content may have resulted from a loss of adrenergic nerve endings within those parts of the primary olfactory cortex included in the telencephalic sample.

The higher norepinephrine level in the brainstem on the side of the lesion can be explained if it is assumed that the olfactory bulb exerts an "inhibitory" influence on norepinephrine storage in this region (i.e., its input tends to decrease the steady-state norepinephrine concentration). However, no direct connections between the olfactory bulb and the brainstem seem to exist in the rat. Any such influence must thus be mediated via multisynaptic pathways. Although no detailed study has been done on the distribution of fibers from various parts of the olfactory cortex, several anatomical and physiological studies have shown a close relationship between the olfactory system and the hypothalamus.[6, 11–18] There is also a massive monosynaptic projection from the olfactory cortex to the mediodorsal nucleus of the thalamus,[19, 20] a structure included in the brainstem sample. Considering the many neuronal circuits between the diencephalon and the upper brainstem, there are a variety of multisynaptic pathways through which olfactory bulb removal might affect the activity and monoamine content of adrenergic neurons in the brainstem. However, there is no reason to expect that olfactory bulb removal would cause a loss of adrenergic nerve endings within the brainstem. This is consistent with our finding that the uptake and retention of ^{3}H-norepinephrine in this area remain unchanged.

The activity of PNM transferase showed a small decrease in the olfactory tubercle on the lesioned side. The ability of the tubercle to retain ^{3}H-norepinephrine fell proportionally, although the decline was not statistically significant. These data indicate that only a small fraction of the PNM transferase in the olfactory tubercle may be located in axons originating from the olfactory bulb. Alternatively, the small decline observed in the activity of this enzyme could also have resulted from removal of a "stimulatory" transsynaptic input.

These studies provide evidence that a technique used widely to locate adrenergic nerve endings in the periphery, i.e., the loss of their ability to take up and retain circulating ^{3}H-norepinephrine after transection of their axons, can also be used to trace adrenergic tracts in the brain.[21] Because very little circulating norepinephrine crosses into the brain, the ^{3}H-catecholamine must be administered via injection into the cerebrospinal fluid. Because the proportion of injected material taken up by the various brain regions varies somewhat among animals, the experimental lesions should be unilateral so that each animal can serve as its own control. Our data suggest that this technique can also be helpful

in differentiating the changes in brain norepinephrine content that are caused by a loss of adrenergic nerve endings from those caused by transsynaptic effects.

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